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Forensics Application Guidebook

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Application News

No.C98

Liquid Chromatography Mass Spectrometry

Analysis of Steroids and NSAIDs Using the Shimadzu LCMS-8050 Triple Quadrupole Mass Spectrometer

With performance enhancing drug use considered contrary to fair play, along with the adverse effects they may have on the health and social welfare of athletes, sports doping testing is increasing and has been conducted according to the provisions of WADA (World Anti-Doping Agency).
Drugs that are registered as prohibited substances mainly fall into the categories of anabolic steroids (AAS) used primarily for building muscle strength, steroidal anti-inflammatory drugs for their anti-inflammatory and immunosuppressive effects, and narcotic and designer drugs. Also, non-steroidal anti-inflammatory drugs (NSAIDs) are drugs used to treat pain and inflammation as well as fever, and although they are not specified as prohibited drugs, their abuse by athletes is being viewed as a problem due to their side effects.
Since doping tests provide information for making critical decisions that actually affect athletes' lives, accuracy at the time of testing, as well fairness, are necessary. In this Application News, we introduce an accurate identification method for typical steroidal and non-steroidal anti-inflammatory drugs using multiple reference ion ratios, in addition to an example of high-sensitivity measurement.

MRM Analysis of Standards and Matrix-Matched Calibration Curves

We conducted MRM measurement of a mixed standard solution consisting of 14 typical steroids and non-steroidal anti-inflammatory drugs. Fig. 1 shows the MRM chromatograms obtained using the mixed standard solution (each component at 50 ng/mL), and Fig. 2 shows MRM chromatograms obtained from analysis of typical compounds at concentrations near their respective LOQs. Table 1 shows minimum and maximum concentrations used for generating the respective calibration curves. The lower limits of quantitation ranged from 10 to 100 pg/mL (20 – 200 fg on column), and excellent linearity was obtained over a wide range of more than 3 orders of magnitude for each substance.

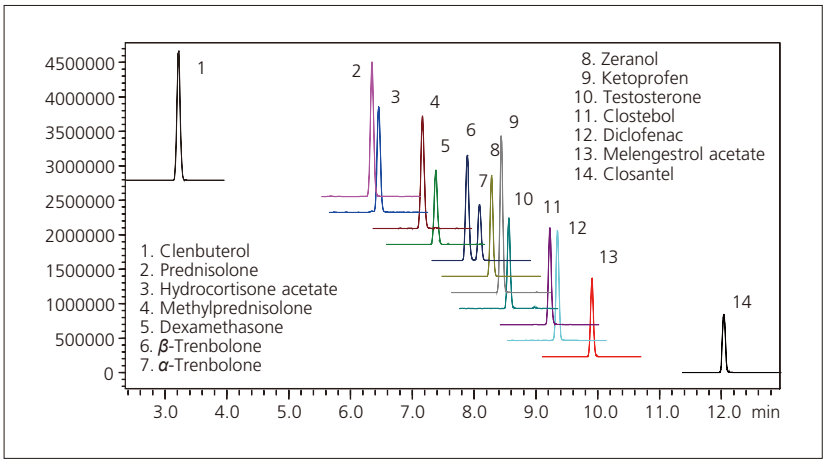


Fig. 1 Chromatograms of Steroids and NSAIDs

Table 1 Calibration Curve Min. /Max. Concentrations

Compounds	Min. Conc.	Max. Conc.
Clenbuterol	0.01	10
Prednisolone	0.05	20
Hydrocortisone acetate	0.1	50
Methylprednisolone	0.5	50
Dexamethasone	0.5	50
α-, β-Trenbolone	0.1	50
Zeranol	0.1	50
Ketoprofen	0.05	50
Testosterone	0.05	10
Clostebol	0.05	50
Diclofenac	0.01	50
Melengestrol acetate	0.05	50
Closantel	0.01	10

(Unit: ng/mL)

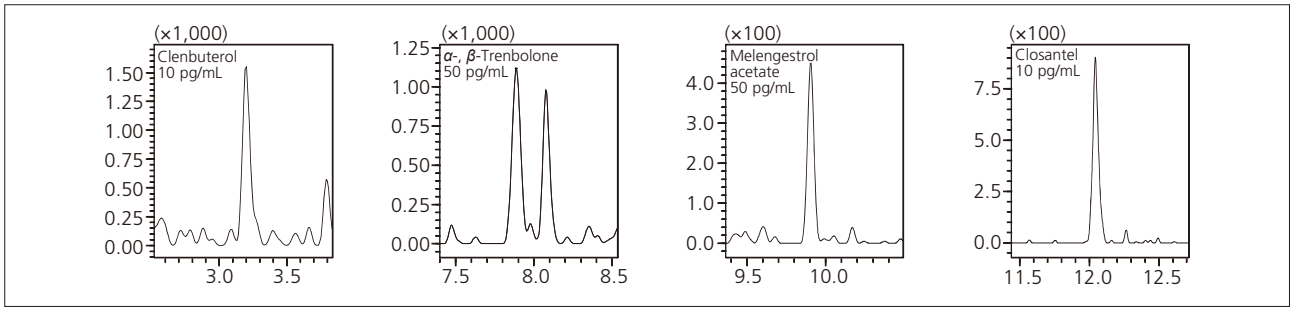


Fig. 2 MRM Chromatograms Near the LOQ of Typical Compounds

Peak Determination Using Multiple Reference Ions

When using multiple reference ions to conduct high-accuracy identification, the process of selecting and making the associated entries becomes complicated.
As of Labsolutions Ver. 5.65, however, this selection and entry process for qualifier MRM transitions now provides for automatic selection and entry as reference ions.

< Examples of New Features >

(1) Multiple reference ions are automatically entered (desired transitions can be selected and changed using drop-down menu).
(2) The ion ratio of the STD is automatically set as the reference value.
(3) A different allowable width of relative ion ratio can be set for each reference ion.
(4) The identification range (%) is automatically calculated from the ion ratio, allowable width and reference ion mode.

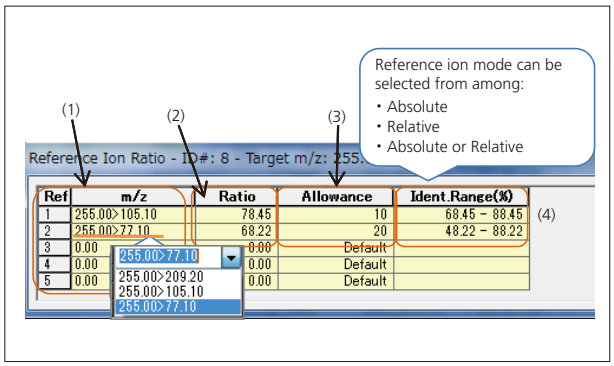
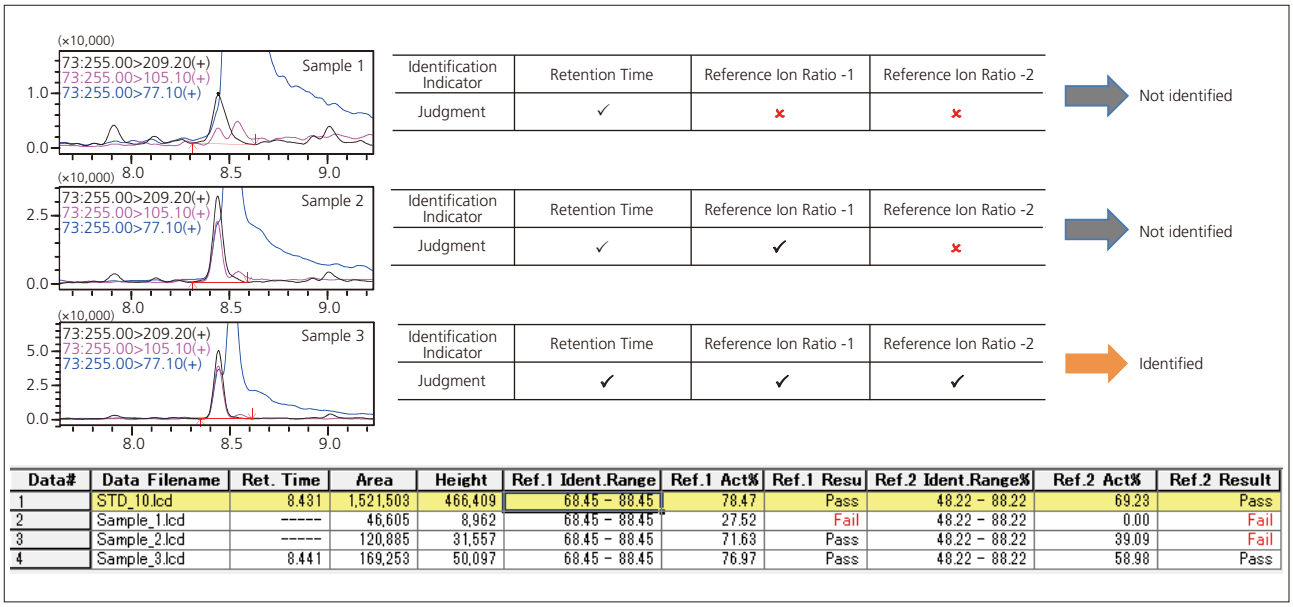


Fig. 3 Reference Ion Setting Window



Data#	Data Filename	Ret. Time	Area	Height	Ref.1 Ident.Range	Ref.1 Act%	Ref.1 Resu	Ref.2 Ident.Range%	Ref.2 Act%	Ref.2 Result
1	STD_10.lcd	8.431	1,521,503	466,409	68.45 – 88.45	78.47	Pass	48.22 – 88.22	69.23	Pass
2	Sample_1.lcd	-----	46,605	8,962	68.45 – 88.45	27.52	Fail	48.22 – 88.22	0.00	Fail
3	Sample_2.lcd	-----	120,885	31,557	68.45 – 88.45	71.63	Pass	48.22 – 88.22	39.09	Fail
4	Sample_3.lcd	8.441	169,253	50,097	68.45 – 88.45	76.97	Pass	48.22 – 88.22	58.98	Pass

Fig. 4 Example of Peak Determination Using Multiple Reference Ions

Table 2 Analytical Conditions

Column	: Shim-pack XR-ODS Ⅱ (2.0 × 75 mm, 2.2 µm)
Mobile Phase A	: 0.1 % Formic acid – Water
Mobile Phase B	: Acetonitrile
Time Program	: 1 %B (0 min) → 15 %B (1 min) → 40 %B (6 min) → 100 %B (10 - 13 min) → 1 %B (13.01 - 16 min) (12.01 - 15 min)
Flowrate	: 0.2 mL/min.
Injection Volume	: 2 µL
Oven Temperature	: 40 °C
Ionization Mode	: ESI (Positive / Negative)
Probe Voltage	: +4.5 kV / -3.5 kV
Neburizing Gas Flow	: 3.0 L/min.
Drying Gas Flow	: 10.0 L/min.
Heating Gas Flow	: 10.0 L/min.
Interface Temperature	: 400 °C
DL Temperature	: 200 °C
Block Heater Temperature	: 400 °C

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Application News

Liquid Chromatography Mass Spectrometry

Analysis of doping agents using ultrafast LC-MS/MS with scheduled MRM

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■ Introduction

Faster, higher, further - doping accompanies sports already for many centuries. But as it was not possible to detect the illegal substances at that time, the first doping case was discovered in 1812 - only because the culprit was caught in the act.

In general, doping refers to the use of banned performance-enhancing drugs, or the use of banned methods to improve performance. But doping not always means improvement of performance. In horse racing, for example, terms such as negative doping, which is doping to defeat, are an issue.

In the past the attitude "Allowed is, what is not found" predominated. Nowadays improved analytical methods allow the detection of even the slightest traces of doping agents in blood and urine. Thus, the analytical possibilities of the different labs are crucial for the detection of a substance.

Here we show the advantage of an ultrafast MS technique with excellent sensitivity when analyzing horse doping agents.

LCMS-8050 triple quadrupole mass spectrometer

■ Materials and Methods

Real urine samples from a horse doping laboratory were tested after sample pretreatment for various corticosteroids and other small molecules. The samples were analyzed using the high sensitivity triple quadrupole mass spectrometer LCMS-8050 coupled to a NEXERA X2 UHPLC (Shimadzu, Japan) operating in scheduled MRM mode with fast polarity switching (5 msec) for the detection of positively and negatively charged ions in one run. To corroborate the data quality of the ultrafast scheduled MRM analysis two different screening methods containing MRM transitions for either 13 components (resulting in 26 MRMs) or 127 components (resulting in 254 MRMs) were compared. In addition the repeatability of MRM only experiments compared to MRM experiments including a synchronized survey scan (data dependent product ion scan) were investigated.

■ Analytical Conditions

UHPLC

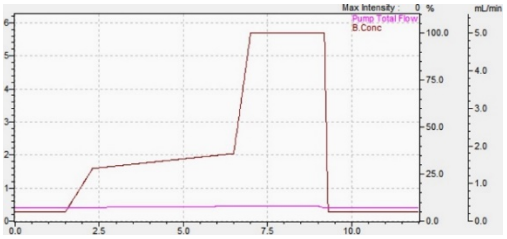
LC system:	Nexera X2 (Shimadzu, Japan)
Analysis Column:	Acquity C.(2.1 mm I.D. x 100 mm L., 1.7 µm)
Mobile Phase A:	Water + 0.1 % Formic acid
Mobile Phase B:	Acetonitrile + 0.1 % Formic acid
Column Temperature:	25 °C
Injection Volume:	10 µL

MS

MS system:	LCMS-8050 (Shimadzu, Japan)
Ionization:	HESI (positive/negative)
Nebulizing Gas Flow:	3.00 L/min (N_2)
Drying Gas Flow:	5.00 L/min (N_2)
Heating Gas Flow:	15.0 L/min (Air)
DL Temperature:	250 °C
Block Temperature :	450 °C
Interface Temperature :	300 °C

LC Gradient

Time (min)	Flow rate (mL/min)	%B
0.00	0.35	5
1.50	0.35	5
2.30	0.35	28
6.50	0.35	36
7.00	0.35	100
7.20	0.40	100
9.00	0.40	100
9.20	0.35	100
9.30	0.35	5
12.00	0.35	5



■ Results

Data comparison

The chromatograms to evaluate the data quality when using ultrafast scheduled MRM methods were obtained from a urine extract containing 1 pg/mL of 13 different components. Comparison between acquisition with 13 events (26 MRM) and 127 events (254 MRM) show that even at high speed with 1ms dwell time and ultra fast polarity switching the sensitivity is at the same level (Fig. 1, Fig. 2).

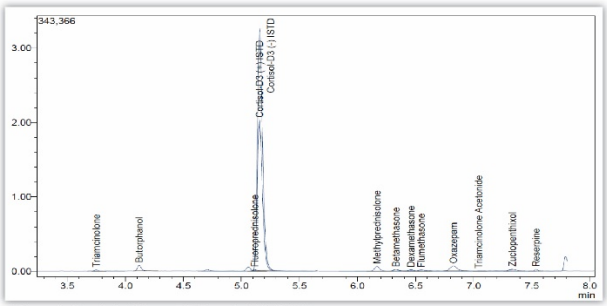


Figure 1. 1 pg/mL Urine – 13 Events / 26 MRMs

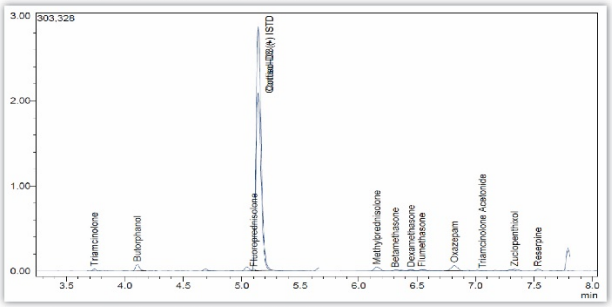


Figure 2. 1 pg/mL Urine – 127 Events / 254 MRMs

The detailed assessment of a less intense peak (e.g. Triamcinolone) proves the consistent high quality of data despite a strongly raised number of MRMs in the method (Fig. 3)

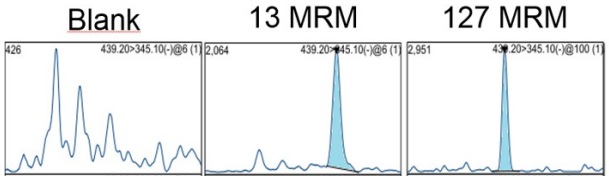


Figure 3: Data comparison 13 MRMs vs 127 MRMs

Repeatability

A standard solution equivalent to an extracted sample at 2 pg/mL was injected 6-fold. An acquisition method using MRM mode only was compared with an acquisition mode combining MRM mode with a synchronized survey product ion scan at 30000 Da/sec.

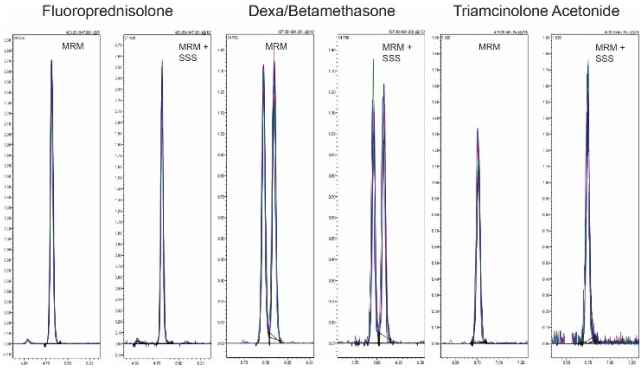


Figure 4: Examples for repeatability MRM only and MRM + synchronized survey scan.

Compound	Peak area %RSD MRM only	Peak area %RSD MRM + Survey scan
Cortisol (neg)	2.2 %	2.7 %
Cortisol (pos)	5.1 %	7.5 %
Butorphanol	0.8 %	0.6 %
Reserpine	3.1 %	3.8 %
Oxazepam	2.0 %	3.3 %
Zuclopenthixol	2.0 %	2.3 %
Bethamethasone	2.5 %	4.3 %
Dexamethasone	2.1 %	3.0 %
Flumethasone	3.5 %	4.5 %
Methylprednisolone	1.7 %	2.9 %
Fluoroprednisolone	2.4 %	4.5 %
Triamcinolone Acetonide	4.1 %	3.6 %
Triamcinolone	9.3 %	4.3 %

■ Conclusion

Independent from the number of MRMs or simultaneously performed synchronized survey scans the LCMS-8050 coupled to a Nexera X2 system provides excellent sensitivity with high data quality in scheduled MRM mode with ultra fast polarity switching (5 msec) for the detection of positively and negatively charged analytes in one run.

ULTRA FAST MASS SPECTROMETRY


LCMS-8040


LCMS-8045


LCMS-8050


LCMS-8060


LCMS-2020


LCMS-IT-TOF

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Application News

No.C142A

Liquid Chromatography Mass Spectrometry

Screening Analysis of Highly Polar Doping Agents in Urine Using 2DLC/MS/MS

The use of performance-enhancing drugs, or "doping," has been recognised for decades and since 1999 the World Anti-Doping Agency (WADA) has governed and harmonized the worldwide sports drug testing efforts. However, these needs are changing and the continuing. discovery of new doping strategies with naturally occurring substances, such as androgenic steroids, pro-hormones and related metabolites, peptide hormones, as well as the emergence of designer drugs and the manipulation of blood and blood components results in sports drug testing methods which are capable of a range of tests. In this application news, we report the simultaneous analysis of highly polar doping agents including meldonium and adrenergic agents such as synephrine, norfenefrine, etilefrine, oxilofrine and octopamine using 2D LC/MS/MS.

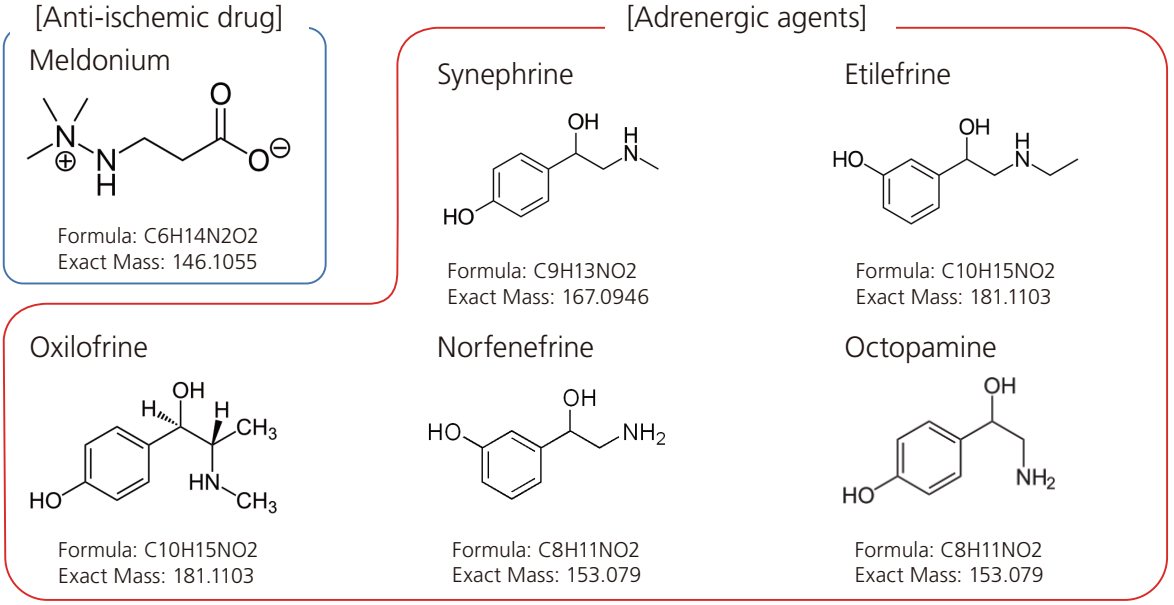

Fig. 1 Structures of 6 Compounds

Table 1 Analytical Conditions

[LC] NexeraX2 System		[MS] LCMS-8060	
Analytical Column	: Nucleodur HILIC (100 mm L. × 2 mm I.D., 1.8 µm)	Ionization	: ESI (+/-)
Trapping Column	: Nucleodur HILIC (20 mm L. × 2 mm I.D., 3 µm)	Nebulizing Gas Flow	: 3.0 L/min.
Mobile Phase	: A: H_2O + 5 % buffer,	Drying Gas Flow	: 15.0 L/min.
	B: Acetonitrile + 5 % buffer,	Heating Gas Flow	: 15.0 L/min.
	C: Acetonitrile + 5 % buffer	HB Temp.	: 500 ˚C
	(buffer: 200 mM Ammonium Acetate + 0.15 % glacial acetic acid)	DL Temp.	: 300 ˚C
		Interface Temp.	: 400 ˚C
Column Oven Temp.	: 40 ˚C		
Injection Volume	: 30 µL		

MRM parameter:

#	Name	Polarity	Q1	Q3 Qualifier 1	Q3 Qualifier 2	Ret. Time (min)	CE Qualifier 1	CE Qualifier 1
1	Meldonium	+	147.20	58.25	59.25	8.18	-27	-18
2	Etilefrine	+	182.30	135.25	91.25	5.34	-20	-27
3	Norfenefrine	+	154.20	91.25	65.25	6.01	-21	-35
4	Octopamine	+	154.20	91.25	119.20	6.00	-21	-15
5	Oxilofrine	+	182.30	149.25	105.25	5.69	-20	-22
6	Synefrine	+	168.20	135.20	107.25	5.87	-20	-31
7	Meldonium-d3	+	150.20	62.25	60.25	8.18	-18	-30
8	Etilefrine sulphate	+	262.20	164.15		5.19	-19	
9	Synefrine sulphate	+	248.20	150.25	135.20	5.68	-15	-30
10	Norfenefrine sulphate	+	234.20	136.20	91.20	5.62	-18	-35
11	Etilefrine sulphate_neg	-	260.20	180.20	121.10	5.19	18	39
12	Oxilofrine sulphate_neg	-	260.20	77.10	178.20	5.49	26	12
13	Synefrine sulphate_neg	-	246.20	148.20	106.10	5.70	20	30
14	Norfenefrine sulphate_neg	-	232.20	152.20	121.15	5.69	17	36
15	Octopamine sulphate_neg	-	232.20	134.15	107.10	5.81	22	30

#7 : Internal Standard
#8 ～ 15 : Confirmation of Sulpho-conjugate

Compound list including MRM transitions for unchanged parent drug molecules and corresponding sulfonated metabolites. Rapid polarity switching was used during the analysis to confirm peak identification.

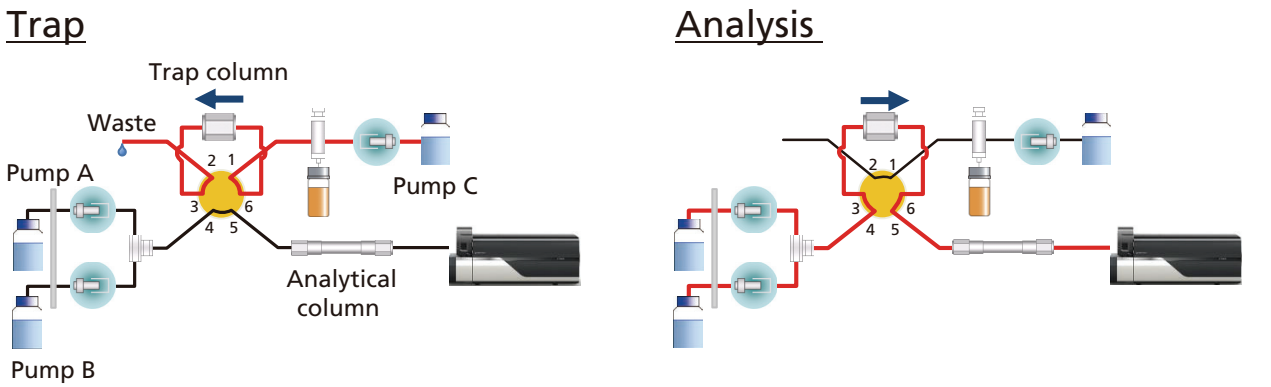

Fig. 2 Flow Diagram of 2D-HILIC System

Diluted urine samples were injected directly onto the 2D HILIC system using a HILIC trapping column for clean-up and pre-concentration followed by an effective HILIC analytical separation.

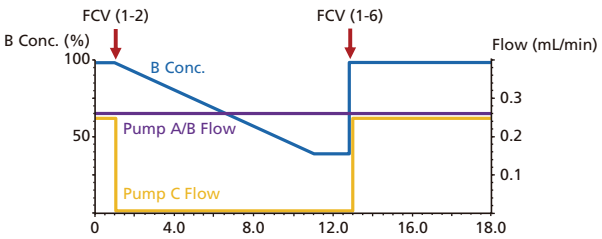

Fig. 3 Flow Rate and Gradient Program

Sample Preparation of Urine Sample

1. Centrifuge urine samples at 3,000 rpm for 10 min at room temperature.
2. Transfer 60 µL supernatant to new tube and add 10 µL IS solution (*) and 140 µL acetonitrile, mix the solution by vortex mixing.
3. Centrifuge at 13,000 rpm for 5 min.
4. Transfer 180 µL supernatant to vial.
   (*) Meldonium-d3 in 200 mM Ammonium Acetate

Calibration Curves

Fig. 4 shows calibration curves of 6 compounds spiked into urine. Meldonium was included in the World Anti-Doping Agency (WADA) Prohibited List on 1 January 2016, the guidance for meldonium in urine samples collected after 30 September 2016 applies normal results management to samples above a concentration of 100 ng/mL. In this method, the urine calibration range between 1 to 200 ng/mL resulted in a linear response for all compounds with regression coefficients r2 > 0.997.

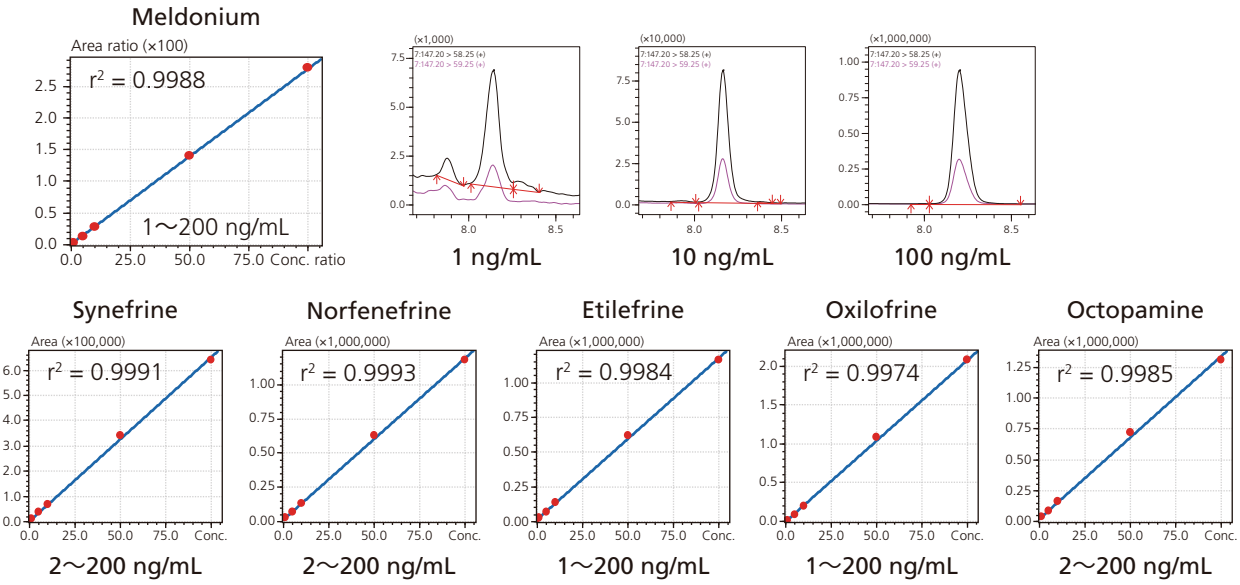

Fig. 4 Calibration Curves and MRM Chromatograms of 6 Compounds

Analysis of Synephrine, Etilefrine and Oxilofrineine in Urine

Each urine samples were collected from volunteers being separately administered with synephrine, etilefrine and oxilofrine were analyzed using 2D-HILIC System. In all samples, both the unchanged form and sulphated metabolites were detected.

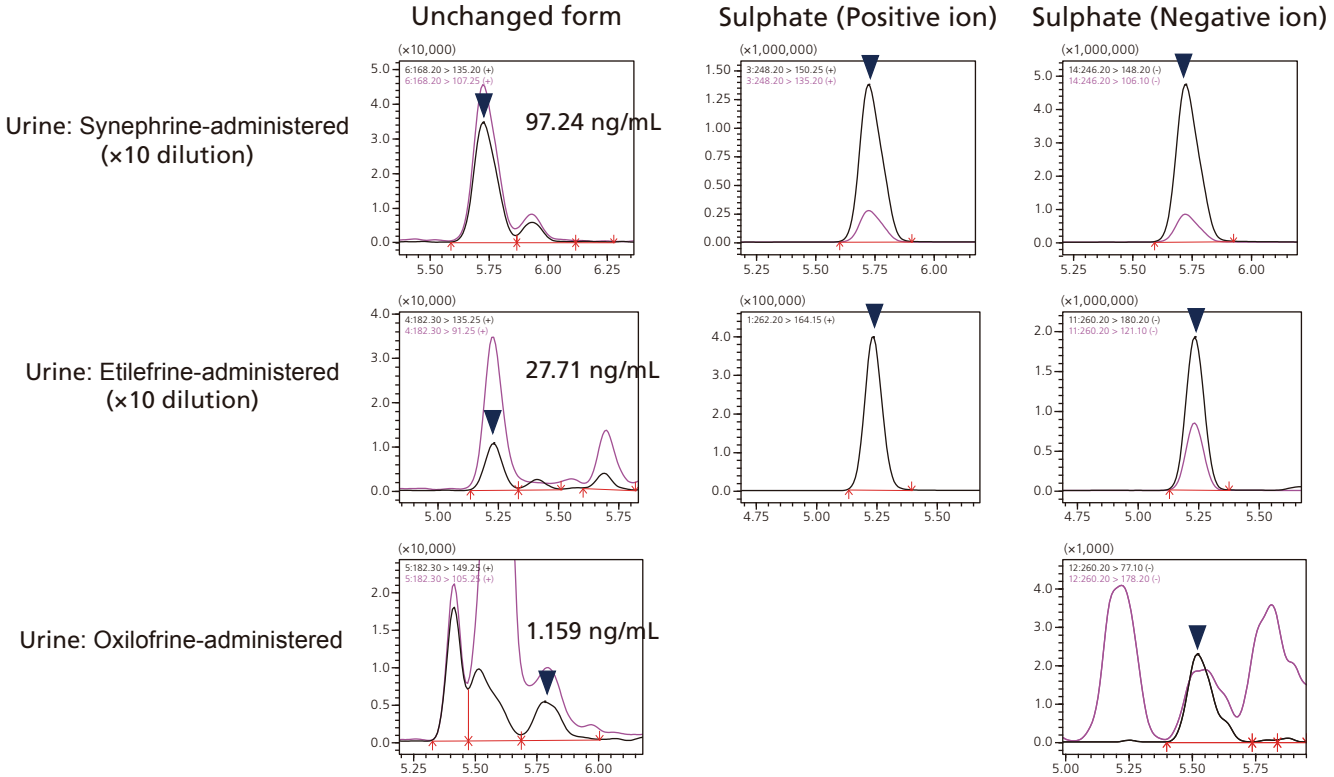

Fig. 5 Results of Urine: Synephrine, Etilefrine and Oxilofrine were separately administered

Distinguishing Norfenefrine and Octopamine in Urine

Norfenefrine is a positional isomer of octopamine resulting in the same retention time and MRM transitions for the unchanged parent drug molecule. However, by detecting the corresponding sulphate metabolite using rapid polarity switching enabled a positive identification.

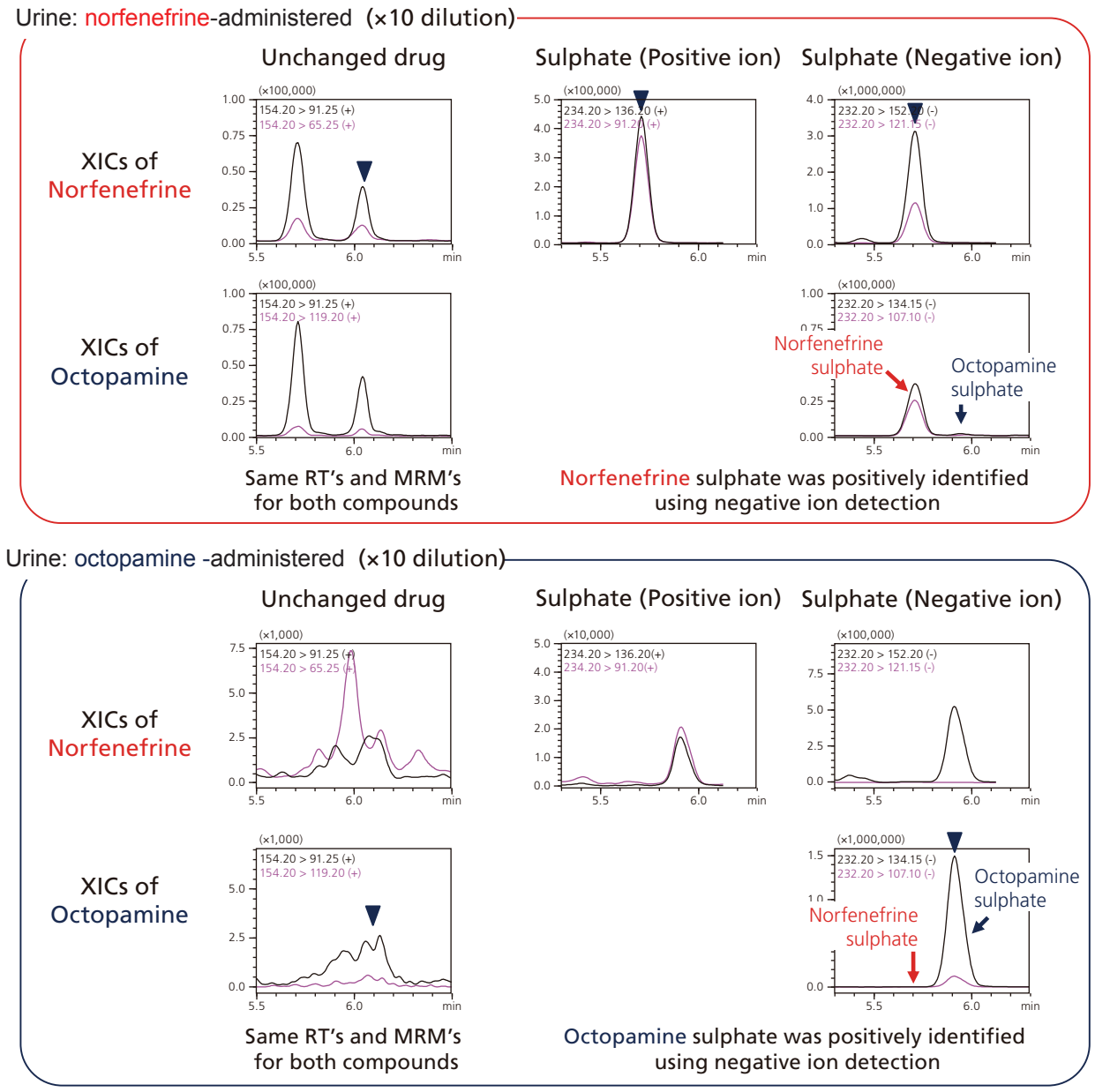

Fig. 6 Results of Urine: Norfenefrine and Octopamine were separately administered

The sample used for this analysis was provided by Anti-Doping Laboratory, LSI Medience Corporation, Tokyo, Japan

References: Anal Bioanal. Chem. (2015), 407, 5354-5379
Drug Test. Analysis (2015), 7, 973–979

Notes: • The products mentioned in this article have not received approval for use as medical devices based on the Pharmaceutical and Medica Device Act.
• The analytical methods mentioned in this article cannot be used for diagnostic purposes, for Research Use Only (RUO).

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Application News

No. M243A

Gas Chromatograph Mass Spectrometer

Screening Techniques in Doping Analysis by GC/MS

■ Introduction

Sports doping is not only contradictory to the concept of fair play, but it has a negative impact on the health of athletes as well as society in general. For these reasons, drug doping testing is conducted based on regulations imposed by the World Anti-Doping Agency (WADA).

Table 1 lists the sports doping screening techniques. The quadrupole GC/MS is used for analysis of difficult-to-volatilize drugs (Screening Method No.2), diuretics (No.5), and β-blocker agents (No.7).

This Application News introduces an example of the analysis of a difficult-to-volatilize drug (Screening Method No.2) obtained with the cooperation of MITSUBISHI KAGAKU BIO-CLINICAL LABORATORIES, INC., officially recognized as a WADA testing agency.

Table 1: Classification of Screening Methods in Sport Doping Analysis

Screening No.	Classification	Drug Example	Analytical Instrument
1	Volatile drugs	Amphetamine	GC-NPD
2	Difficult to volatilize drugs	Cocaine metabolites	GC/MS (Scan)
3	Thermally decomposed substances	Dexamethasone	Q-TOF LC/MS
4	Designer steroids	Testosterone	GC/MS (SIM)
	Anabolic steroids	Stanozolol	GC/HRMS (SIM)
5	Diuretics	Furosemide	GC/MS (SIM)
6	Steroid hormones	Androstenedione	GC/C/IRMS
7	β-blocker agents	Metoprolol	GC/MS (Scan)
8	Peptide hormones	EPO, hCG	EIA, immunoblotting

■ Analytical Procedures

The pretreatment flow chart and GC/MS analytical conditions for Screening Method No.2 are shown in Figure 1 and Table 2, respectively. In the pretreatment procedure, 6 M of hydrochloric acid was added to 5 mL of urine, and this was heated for 30 minutes at 105 °C to conduct hydrolysis. After washing with diethyl ether, 2-methyl-2-propanol and internal standards were added to the liquid phase, and after adjusting the pH to 9.6+/-0.1, extraction was conducted with diethyl ether. The extract was dried under a stream of nitrogen gas, and after adding methyl orange/acetonitrile/TFA solution, MSTFA was added until the solution turned yellow, after which the solution was heated for 5 minutes at 80 °C. Then, MBTFA was added, and the solution was heated for 10 minutes at 80 °C to conduct N-TFA-O-TMS derivatization.

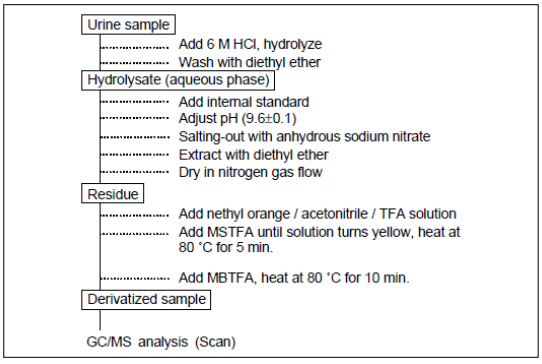

Figure 1: Pretreatment Flow for Screening Method No. 2

Table 2: Analytical Conditions

Model	GCMS-QP2010		
Workstation	GCMSsolution Ver2.5		
Column	DB-5 (15 m × 0.25 mm I.D. df=0.25 (um)		
-GC-		**-MS-**	
Inj. Temp.	280°C	**Interface Temp.**	300°C
Column Temp.	100°C (1 min)-16 °C/min-300°C (2 min)	**Ion Source Temp.**	200°C
Carrier Gas	He (Constant Linear Velocity Mode)	**Scan Range**	m/z 50-550
Linear Velocity	51.8 cm/sec	**Scan Interval**	0.5 sec
Injection Method	Split		
Split Ratio	11:1		

■ Sports Doping Test Report Format

In order to present test results in the most effective manner, the results of each analyte must be arranged in an easy-to-view format. For instance, the report must be as compact as possible, displaying chromatograms of the drug and its metabolites side-by-side for easy viewing.

GCMSsolution allows the reporting items to be pasted to the screen and freely positioned to easily generate highly effective doping test reports (Figure 2)

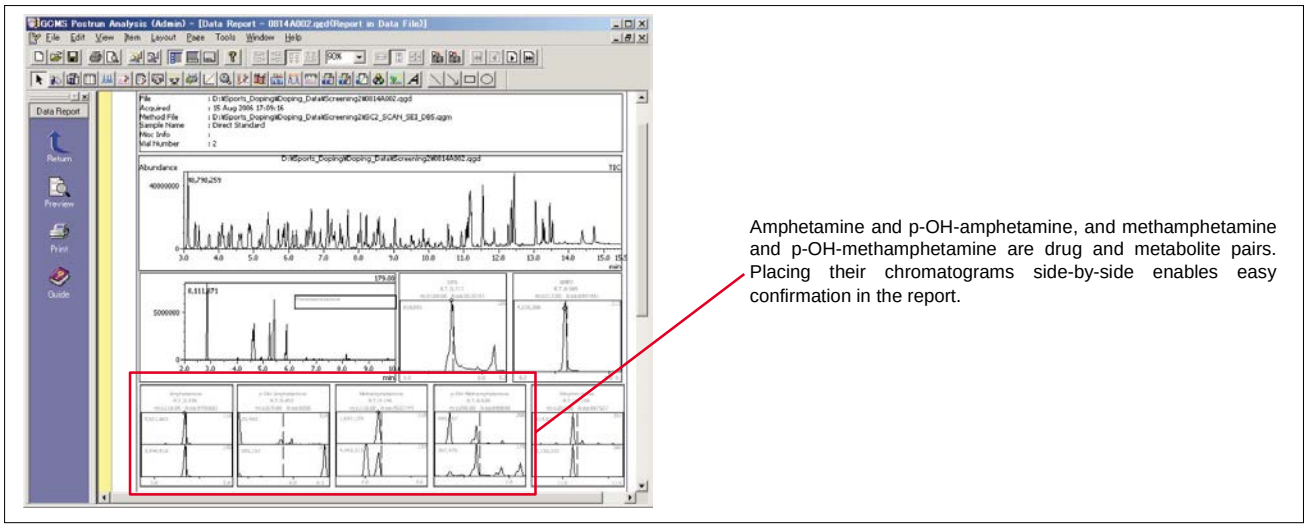

Figure 2: Report Creation Screen

To ensure data reliability, WADA requires various confirmation tests. In the case of Screening Method No. 2, a Minimum Required Performance Limit (MRPL) of 0.5 ug/mL (strychnine only, 0.2 ug/mL) is set to verify the GC/MS sensitivity.[1] In addition, analysis of a control sample, consisting of drug-free urine, and a blank sample is required to ensure the reliability of the pretreatment procedure and system blank.

Figure 3 shows these testing results in a report formatted using GCMSsolution. The chromatograms of the analyte target ions and their identifying ions are positioned one above the other, enabling convenient judgment of the presence or absence of the compound at a glance.

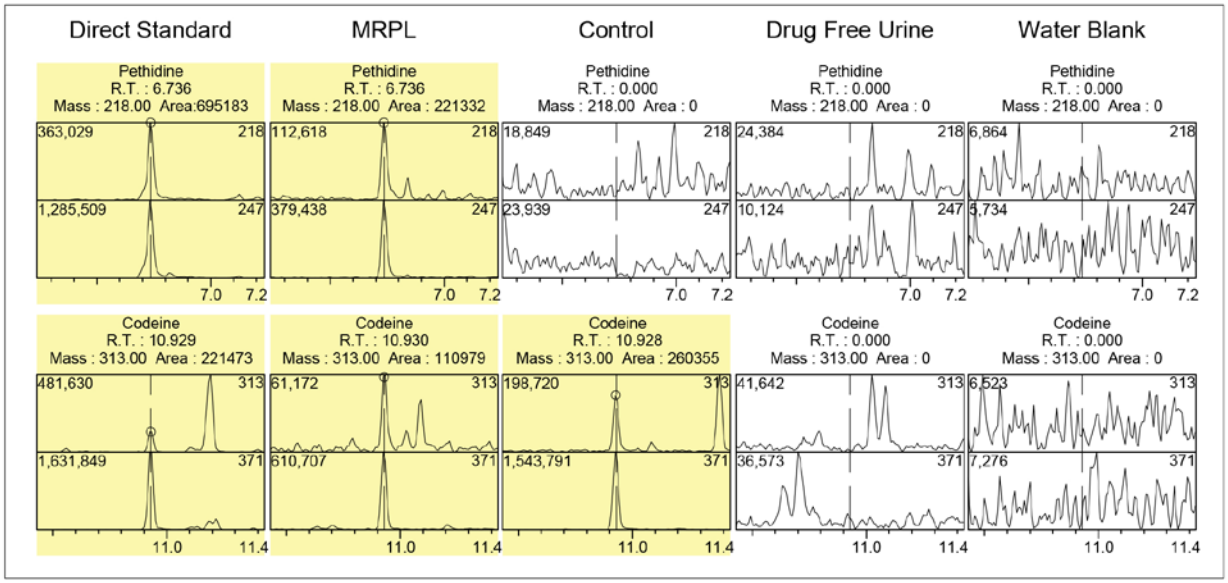

Figure 3: Example of Report Format for Sports Doping Test

Reference

1) MINIMUM REQUIRED PERFORMANCE LIMITS FOR DETECTION OF PROHIBITED SUBSTANCES - WADA Technical Document TD2004MRPL

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Trace Evidence Detection

1. GC

a. An Arson Investigation by using Comprehensive Two-Dimensional Gas Chromatography-Quadrupole Mass Spectrometry

2. Molecular Spectroscopy

a. Analysis of Paint Scrapings Using an Infrared Microscope
b. Contaminant Analysis in Food Manufacturing Process by EDX and FTIR
c. Analysis of Thermally Degraded Plastics Using Thermally Degraded Plastics Library

3. Elemental Spectroscopy

a. Analysis of ICP Atomic Emission Spectrometry in Accordance with the ICH Q3D Guideline for Elemental Impurities Using ICPE-9820
b. Getting the Lead Out – Dangerous Toys

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An Arson Investigation by using Comprehensive Two-dimensional Gas Chromatography-Quadrupole Mass Spectrometry

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Research Article Open Access

An Arson Investigation by using Comprehensive Two-dimensional Gas Chromatography-Quadrupole Mass Spectrometry

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Abstract

The aim of this investigation is to develop a new tool for the investigation of hydrocarbon accelerants. The chemical analysis of fire debris in an arson investigation has gone through several developmental stages. The nature of the analysis has been divided into three essential components due to the complexity of the samples. The first has been the extraction of the accelerants from the debris. The second has been the development of instrumental techniques for the analysis of extracted samples. The interpretation of the results is the final stage. In our investigation, we have used comprehensive two-dimensional gas chromatography-quadrupole mass spectrometry (GCXGC-qMS). GCXGC-qMS is well suited for the analysis of complex hydrocarbon accelerants. We applied the resolution and separation powers of the GCXGC with the high precision mass scanning capabilities of a quadrupole mass spectrometer to investigate these complex samples. Total and selective ion scans were performed on samples obtained from an arson investigation. By using this process, we were able to conclusively determine the presence of hydrocarbon accelerants in fire debris.

Keywords: Comprehensive two-dimensional gas chromatography-quadrupole mass spectrometry; Arson; Accelerants; Gasoline; Kerosene; Diesel

Introduction

During 2010, law enforcement agencies reported 56,825 arsons to the Federal Bureau of Investigation, at an estimated total direct cost of over $1.5 billion in property damage. One out of every four fires is due to arson [1]. The National Fire Protection Association estimates approximately 300 to 350 civilian deaths per year due to arson. The injury rate per fire is 5.6 firefighter injuries per 100 structure fires due to arson [2]. Arson is a felony that costs heavily in human and monetary terms.

An arson investigation usually starts by looking at the four factors that must be present in order to create and sustain a fire. These four factors are known as the fire tetrahedron [3]. The tetrahedron consists of a chemical reaction, flammable substance, starting heat source and oxygen. The starting heat source needs to match the ignition temperature of the flammable substance. In order to classify a fire as arson, the investigator has to prove tampering with at least one of the factors in the fire tetrahedron.

A common arsonist's practice is the use of flammable materials and accelerants; where accelerants are ignitable fluids. Widespread accelerants used in this trade are usually hydrocarbon liquids i.e., kerosene, diesel or gasoline.

Debris from suspected arson fires are routinely analyzed for trace amounts of hydrocarbon accelerants. The samples of debris are usually sealed at the fire scene, in an airtight container, i.e., a new unlined metal paint can. Procedures for labeling the samples are given in ASTM E1459 [4].The samples are brought to the lab for analysis. The procedures for receiving, documenting, storing and retrieving are given in ASTM E1492-11[5]. The analysis is accomplished in three stages. The first stage consists of concentration and extraction of the suspected accelerants from the debris. The second stage involves the instrumental analysis of the concentrated extracted samples. Finally, the last stage involves the interpretation and analysis of results [6].

Much of research has gone into the first stage. Steam distillation [7] and solvent extraction [8] were historically the first methods used in the extraction and concentration of the suspected accelerants from the debris [9]. Distillation of debris samples using steam or high boiling point solvents, i.e., ethylene glycol, produces a floating layer on top of the distillate that can be used in the second stage of the investigation [10]. Distillation is a labor intensive process which can take forty eight hours or more to complete. Furthermore, the distillation rate may influence the extraction [11]. Due to the complexity of this method, the ASTM International subcommittee E30.01 withdrew this standard, ASTM E1385-00, as a procedure. On the other hand, solvent extraction is fast; however, undesirable components may be extracted from the matrixes that interfere with the analysis.

Forensic investigators have searched for better methods for the separation and concentration of the accelerants from fire debris. It involved sampling by direct [12], dynamic [13] or passive headspace with activated charcoal or solid phase microextraction [14,15].

Direct headspace sampling extracts the volatile components in the gas portion above the sample. This method turned out to be less

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sensitive and it was only useful in the detection of highly volatile accelerants, i.e. alcohol and lacquer thinners.

Dynamic headspace involves a flow of an inert gas through the sample vessel. The volatile accelerants are collected into a trap. The trap can be charcoal, Porapak Q, Tenax, Amberlite XAD, etc. Desorption into the analyzing instrument can be done by using a "stripping" solvents or by heating.

Dietz introduced passive headspace with activated charcoal strips in 1985 [16,17]. Charcoal strips traps and concentrate the volatiles in the headspace above the sample. A solvent, i.e. carbon disulfide, n-pentane, desorbs the volatiles absorbed by the strips [18,19]. This is a very sensitive method; which can concentrate and isolate small quantities of accelerants from fire debris.

Solid phase microextraction selectively concentrates fire debris volatiles into an absorbent fiber. This method, as the previous one, is very sensitivity; however, it has the advantage that no solvent is used [20,21]. ASTM International has formalized this method in ASTM E2154 [15].

Instrumentally, forensic investigators have used GC-FID [18,19,22,23], GC/MS [24-26], GC/MS/MS [27], FT-ICR [28], GCXGC [29,30].

ASTM International E30 committee formally withdrew the test method for analyzing fire debris ignitable liquid residues samples by gas chromatography, ASTM E1387 and recommended the use of GC/MS for this type of analysis, ASTM E1618.

Pert et al. [31] commented on the advantages of using GCXGC in the analysis of complex arson samples. It is the purpose of this study is to show that GCXGC-qMS can be used in this type of investigation.

Experimental

GC X GC/MS

The GC/MS system used was a Shimadzu QP 2010 Ultra (Shimadzu Scientific Instruments, Inc., Columbia, MD). This is a gas chromatograph-quadrupole platform. As a general rule, quadrupole mass spectrometers have a relatively slow scan speed and this has become a limiting factor for usage of quadrupoles in comprehensive

Injection	1.0 µL. Injector Temperature 325ºC. Pressure 70.1 kPa. Split: 10:1.
First column	30 m X 0.25 mm X 0.50 µm, 50% Phenyl Polysilphenylene siloxane (BPX50, SGE Analytical Sc. Austin, TX).
Modulator	Period 4.0 s. Con1 330ºC
Second column	2 m X 0.1 mm X 0.1 µm, 100% Polydimethylsiloxane (BPX1, SGE Analytical Sc., Austin, TX).
Oven program	40ºC for 2 min. 40-325ºC @ 5ºC min^{-1} Hold at 325 ºC for 30 min.

Table 1: GC Experimental Parameters.

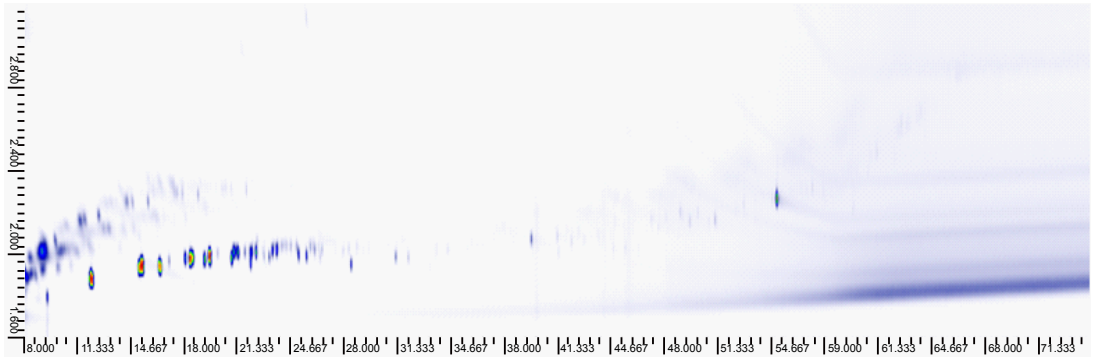

Figure 1: Comprehensive GCXGC-qMS total ion chromatogram of gasoline. X-axis: First dimension, retention time in seconds. Separation based on polarity. Y-axis: Second dimension, volatility-base separation in seconds.

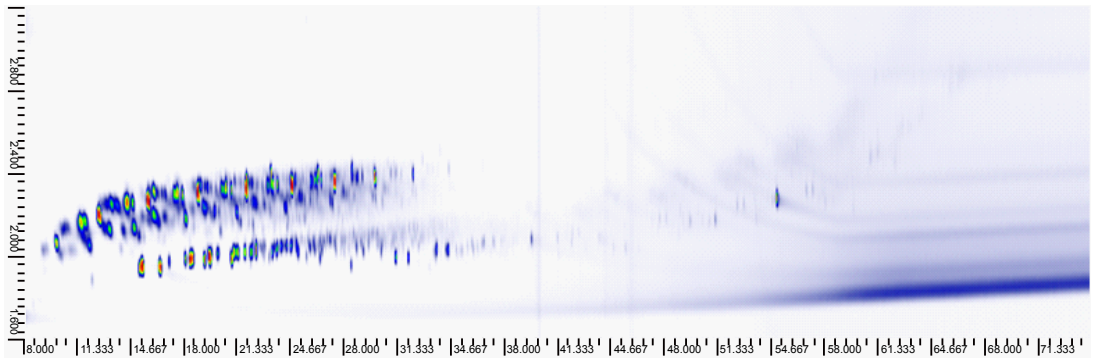

Figure 2: Comprehensive GCXGC-qMS total ion chromatogram of kerosene. X-axis: First dimension, retention time in seconds. Separation based on polarity. Y-axis: Second dimension, volatility-base separation in seconds.

GCXGC-MS. The maximum allowed data acquisition speeds have been 30 Hz. This is too slow to provide enough points for the ultra sharp peaks generated by comprehensive GCXGC. However, this instrument is equipped with a firmware, Advanced Scanning Speed Protocol

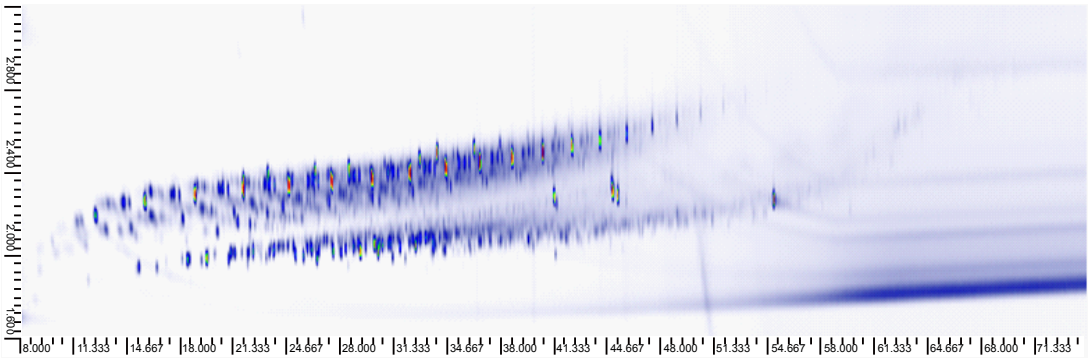

Figure 3: Comprehensive GCXGC-qMS total ion chromatogram of diesel. X-axis: First dimension, retention time in seconds. Separation based on polarity. Y-axis: Second dimension, volatility-base separation in seconds.

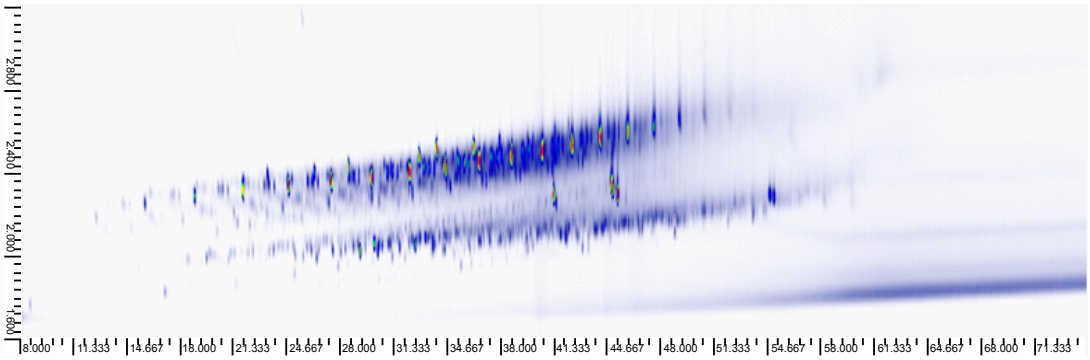

Figure 4: Comprehensive GCXGC-qMS total ion chromatogram of suspected arson sample. X-axis: First dimension, retention time in seconds. Separation based on polarity. Y-axis: Second dimension, volatility-base separation in seconds.

Compounds	m/z[a]
Alkanes	**57, 71, 85, 99**
C1 to C4 alkylbenzenes	**91, 105**, 114
Alicyclics and olefinics hydrocarbon	55, 69, 83, 97
Benzene, C1 to C3 alkylbenzenes	78, 92, 106, 120
C4 to C5 alkylbenzenes	**119, 134**, 148, 162
Alkylnaphthalenes	**128, 142, 156, 170**

[a]Boldfaced ions represent the selected ions

Table 2: Characteristic ions for accelerant pattern identification [31, 40].

Figure 5: Comprehensive GCXGC-qMS profile of selective alkanes ions for gasoline.

(ASSP), and a fast data collection algorithm, which allows for faster collection rates.

A two-stage thermal loop modulator (Zoex Corp. Lincoln, NE) was mounted on top of the GC oven in order to provide comprehensive two-dimensional gas chromatography capabilities. This system employs two gas jets, a cold and a hot jet. The single cold jet cools two different segments of the second column, entrapping the compounds in the two sections of the second column. The hot jet, positioned perpendicular to the cold one, releases the entrapped compounds. A detailed description of the two-stage loop modulator system was provided by E. B. Ledford et al. [32]

Gas chromatograph experimental parameters and columns are listed in table 1. The first column was selected with a high polarity phase and the second was non-polar. The separation occurred via a polar-by-volatile interaction with the samples. Therefore, the samples were separated by polarity in the x-axis and by boiling point y-axis retention times.

The MS data was collected with Shidmazu GC/MS Real Time Analysis. The GCXGC-q MS data was analyzed by using GC Image V 2.1 (Zoex Corp.).

Sample Preparation

Samples of gasoline, kerosene and diesel were prepared by diluting 0.09 g of each sample into 10 milliliters of methylene chloride. Arson samples were extracted with methylene chloride. Purposely, we selected solvent extraction. As previously stated, this sample extraction and concentration technique is rather sensitive, but troublesome. The solvent can extract undesirable compounds from the matrix that can interfere with the analysis.

Results and Discussion

Comprehensive GCXGC works on the principles established by J. B. Phillips [33-35]. It involves the separation by two orthogonal gas chromatographic columns. The sample is separated into fractions and each fraction is transferred and cryogenic focusing to a secondary column with different polarity than the first. The second separation is faster than the first; so, the separation obtained from the first can be maintained. Thus, chemical compounds are separated by their independent chemical properties interacting with two orthogonal column phases. [36-38]. We selected as the first column a polar column. Since the most polar compounds in the sample are aromatic, polyaromatic hydrocarbons and polyaromatic heterocycles, they are regularly spaced along the base of the two-dimensional GCXGC chromatogram. They appear in order of increasing polarity. The first to elute are the mono-aromatic compounds followed by the two, three-ring, and heterocyclic aromatic compounds. The non-polar paraffinic components have the weakest interaction with the first column, and then the second non-polar column separates them. They emerge according to their volatility. They form the top band in the two-dimensional GCXGC chromatogram. Between these two bands, compounds with intermediated polarity and volatility will reside, i.e. alkylated mono-aromatic and polyaromatic compounds.

In order to identify the sample components, we coupled a GCXGC to a quadrupole mass spectrometer. Frysinger et al. [39] coupled a quadrupole mass spectrometer to a GCXGC system. They concluded that due to the high resolution and narrow peaks generated by the GCXGC system, a faster scanning mass spectrometer was required. Our quadrupole mass spectrometer, equipped with ASSP can scan at a faster rate than previous quadrupoles mass spectrometers and therefore, it can handle the high resolution and narrow peaks generated by GCXGC.

Mass spectra data was collected throughout the whole chromatographic runs of gasoline, kerosene, diesel and a suspected arson sample. The software identifies peaks as "blobs". A blob is a collection of the pixels that make up each peak. Each blob contains information, which identifies the two dimensional retention time and the mass spectra of the compound that makes up the collection of pixels. Furthermore, the sum of the pixels value of each blob is proportional to the quantity of the compound that composes that blob. The software allows selection of minimum area, volume and peak area for blob selection. Blob selection can also be performed by computer cursor selection of a specific blob. Total ion chromatograms for the samples (gasoline, kerosene, diesel and suspected arson sample) are shown in figures 1-4.

Total ion chromatograms can generate chromatographic patterns, which can be useful and at the same time deceiving. Pyrolysis of non-accelerant matrices may generate chromatographic patterns that may interfere in recognizing the accelerant distinctive fingerprint patterns. Individual compound identification is insignificant in an arson investigation. The overall fingerprint pattern recognition of the accelerant is the method of confirming their presence.

Fingerprint pattern of selective diagnostic ions that are associated with hydrocarbon accelerants can be used for the screening and identification of a particular accelerant. The diagnostic ions that are used for these types of identifications are given in table 2.

The sets for alkanes (57, 71, 85, 99) m/z[a], alkylbenzenes (91, 105, 119,

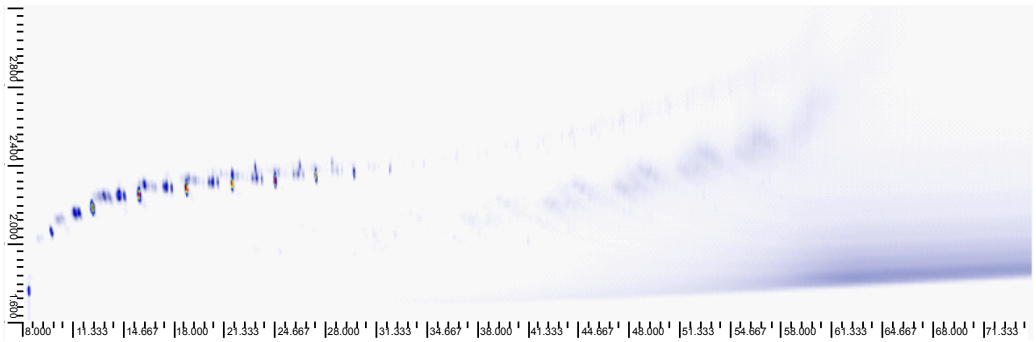

Figure 6: Comprehensive GCXGC-qMS profile of selective alkanes ions for kerosene.

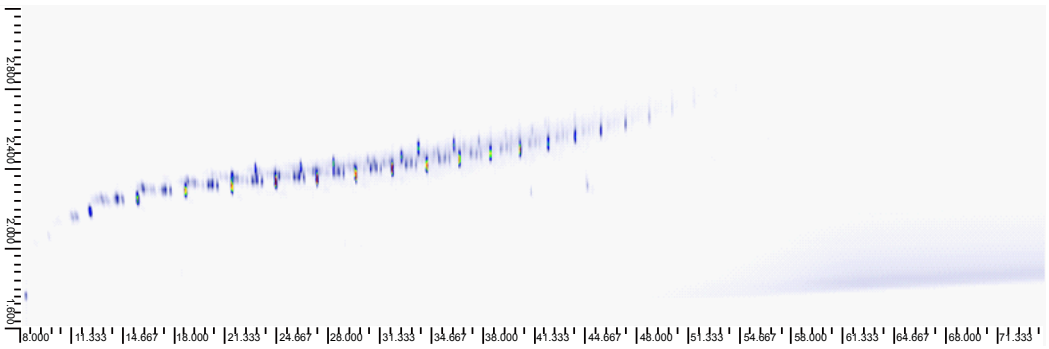

Figure 7: Comprehensive GCXGC-qMs profile of selective alkanes ions for diesel.

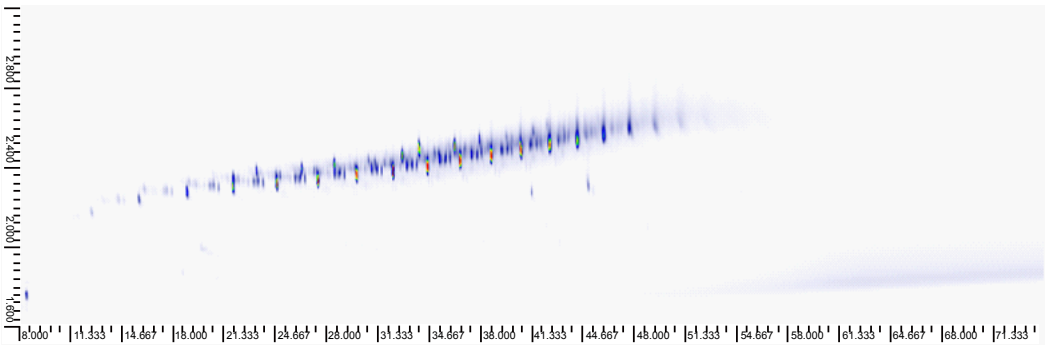

Figure 8: Comprehensive GCXGC-qMS profile of selective alkanes ions for the suspected arson sample.

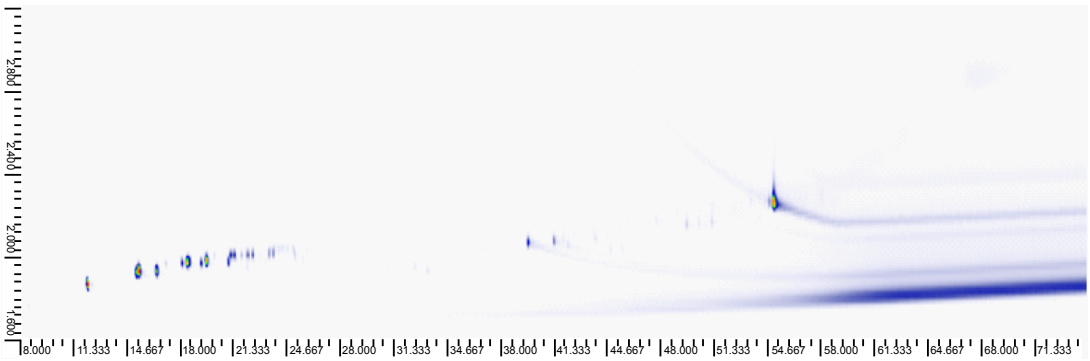

Figure 9: Comprehensive GCXGC-qMS profile of selective alkylbenzenes ions for gasoline.

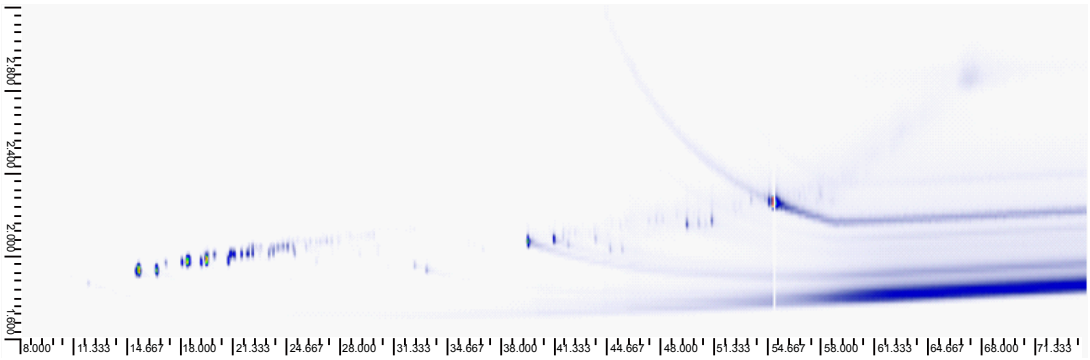

Figure 10: Comprehensive GCXGC-qMS profile of selective alkylbenzenes ions for kerosene.

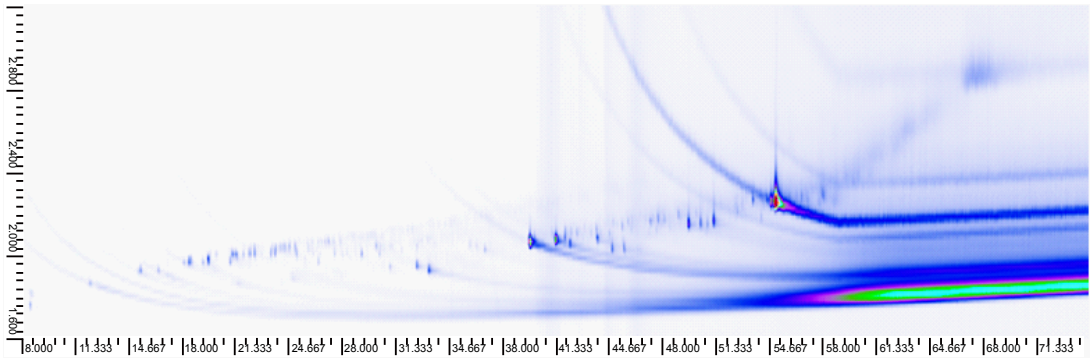

Figure 11: Comprehensive GCXGC-qMS profile of selective alkylbenzenes ions for diesel.

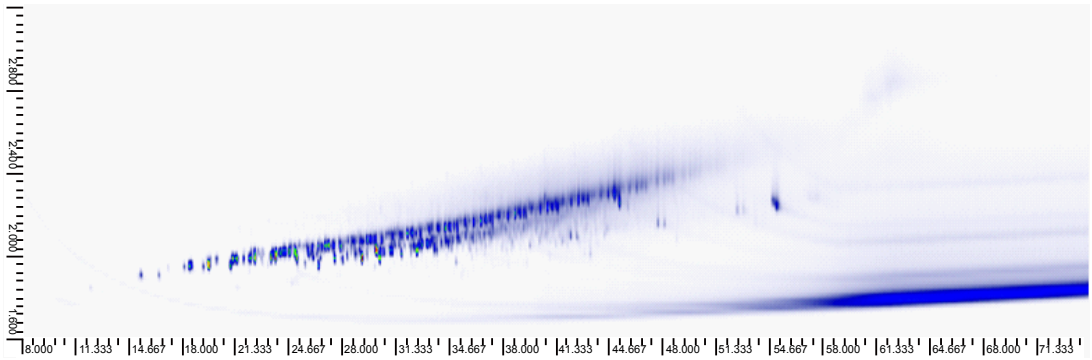

Figure 12: Comprehensive GCXGC-qMS profile of selective alkylbenzenes ions for the suspected arson sample.

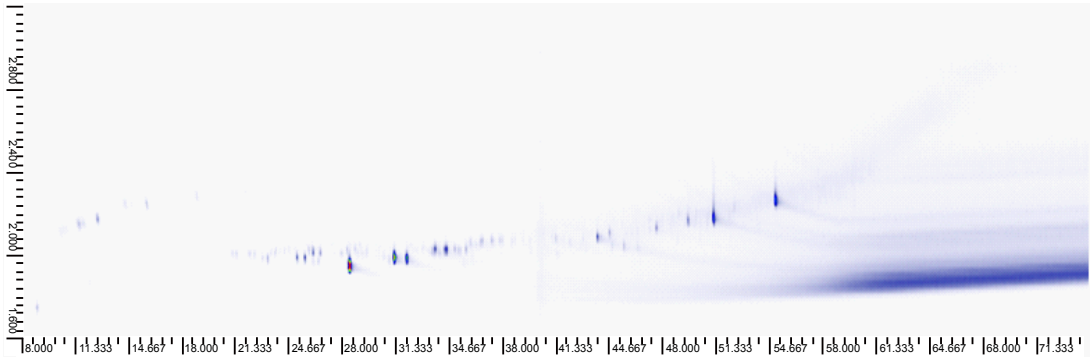

Figure 13: Comprehensive GCXGC-qMS profile of selective alkylnaphthalenes ions for gasoline.

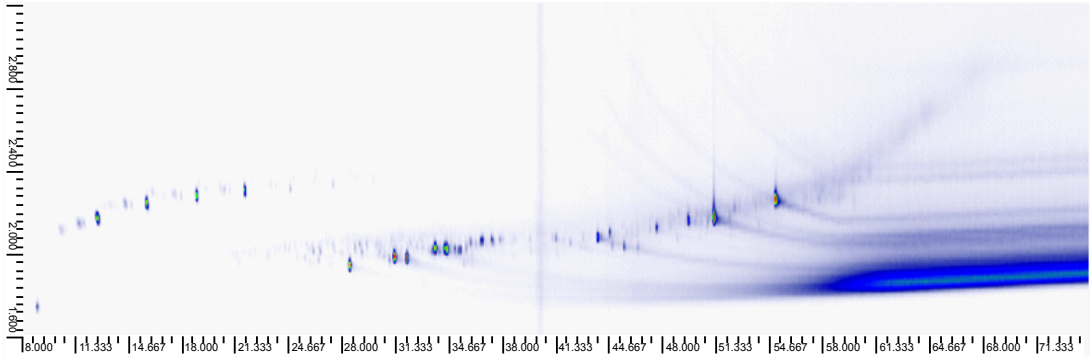

Figure 14: Comprehensive GCXGC-qMS profile of selective alkynapthalene ions for kerosene.

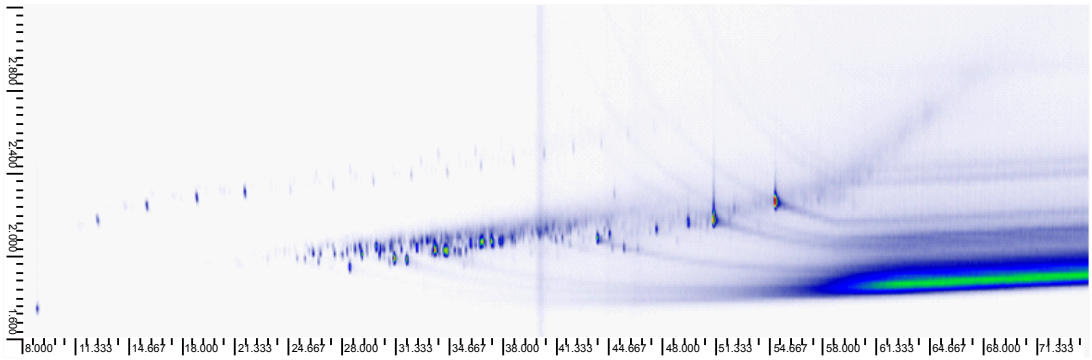

Figure 15: Comprehensive GCXGC-qMS profile of selective alkylnaptthalenes ions for diesel.

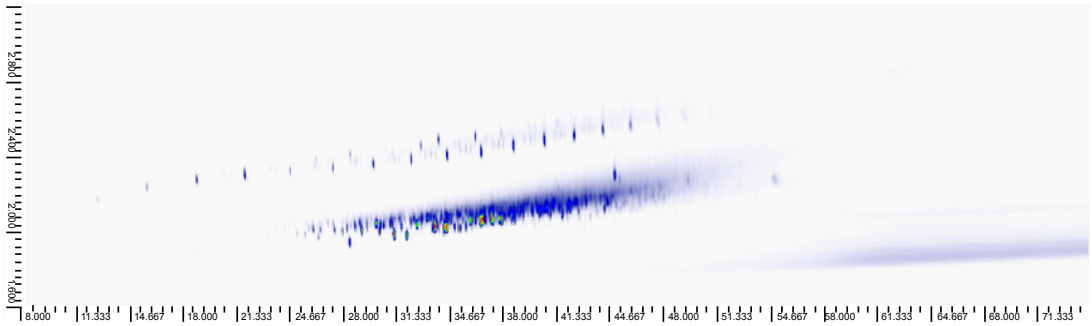

Figure 16: Comprehensive GCXGC-qMS profile of elective alkylnapthalene ions for suspected arson sample.

134) m/z[a] and alkylnaphthalenes (128, 142, 156, 170) m/z[a] were used in this investigation. The selective ion chromatograms for these ions sets for gasoline, kerosene, diesel and the suspected arson sample are given in figures 5-16. From the selective ion chromatograms, it was possible to visualize the fingerprint pattern of an accelerant in the suspected arson sample. A fairly close match was observed between the diesel and the suspected arson sample. The light portions of the chromatographs are slightly different. The low boiling components of the accelerants are usually the first to be consumed by the fire. Therefore, it was possible to positively confirm the presence of accelerant (possibly diesel) in the suspected arson sample.

Conclusions

Hydrocarbon accelerants and a suspected arson sample were analyzed by comprehensive two-dimensional gas chromatography–quadrupole mass spectrometry (GCXGC-qMS). GCXGC-qMS has the advantage of using two orthogonal gas chromatographic columns. The first being a polar column and the second being a non-polar column, the detector was a fast scanning quadrupole mass spectrometer. By using SIM, a two dimensional separation of the samples was achieved. Clearly by using SIM of selective target ions, we can generate fingerprint patterns that can be useful in identifying accelerants in fire debris samples.

Acknowledgements

Alex Mutin of Shimadzu Scientific Instrument provided the GC/MS for conducting the experiment and Edward Ledford of Zoex Corporation provided the modulator and software. Mississippi State Chemical Laboratory provided samples of accelerants. Oktibbeha County Fire Services provided the fire debris samples.

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Application News

No. 279A

Spectrophotometric Analysis

Analysis of Paint Scrapings Using an Infrared Microscope

■ Introduction

There are a number of ways of analyzing paint scrapings employing FTIR spectroscopy. Measurement can be carried out by crushing the collected paint scrapings and employing the KBr pellet method or diffuse reflection method; or by measuring the surface of a coating with the ATR method; or by measuring the minute sample with an infrared microscope.

Introduced here is an example of measurement of paint scrapings from a timber surface.

■ Pretreatment / Results

Measurement was carried out by the transmission method using an infrared microscope after a part of the paint on a chip of wood was scraped off with a needle and crushed using a diamond cell. Figure 1 is a magnified image of the paint scraping after being crushed. Its size is about 70 × 40 µm.

When measuring the paint scraping shown in Figure 1, measurement was carried out with a 30 × 10 µm aperture size, altering the location a few times. The spectra consequently obtained varied slightly with the location (Figure 2). Out of these, a difference spectrum was obtained by subtracting spectrum 3 from spectrum 2. This is shown in Figure 3 (bottom). By searching the paint database, a spectrum (on the top in Figure 3) that matched well with the difference spectrum was found. From this it can be inferred that a pigment thought to be TOLUIDINE RED L (an azo compound) is contained in this paint scraping.

Table 1: Analytical Conditions

Resolution	8 cm^{-1}
Accumulation	100
Apodization	Happ-Genzel
Detector	MCT

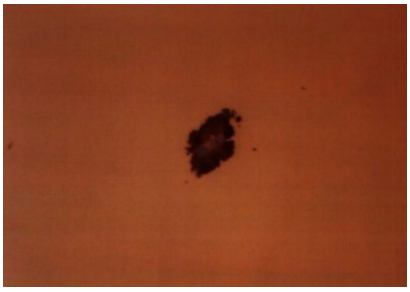

Figure 1: Magnified image of the paint scraping

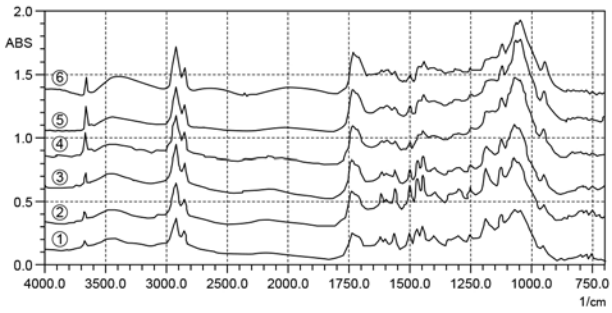

Figure 2: Infrared spectra of the paint scraping

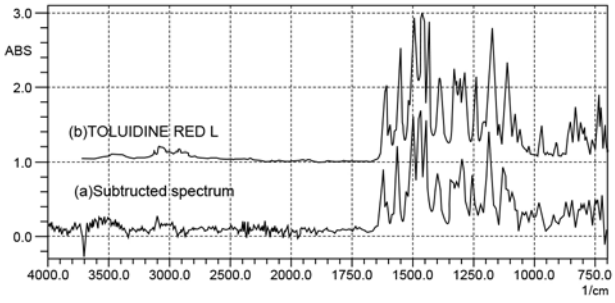

Figure 3: Difference spectrum and the spectrum of TOLUIDINE RED L

■ Explanation

The measurement of automobile coating fragments in criminal investigations by the police are carried out by scraping a part of the coating fragment and employing the transmission method. In actuality, because automobile coatings feature a 3-layer structure consisting of an undercoat, intermediate coat and final coat, measurements are carried out by sampling each layer. This sampling operation requires skill, and depending on the way it is carried out, the quality of the spectrum may vary.

If the size of the sample is over a few mm, the edge of the coating can be scraped off with sandpaper, allowing the use of the microscopic ATR method. Troublesome sampling can thus be omitted.

■ Pretreatment/Results

Figure 4 is a schematic diagram of the automobile coating scraped off with sandpaper. An ATR objective prism (made of Ge) was pressed onto each layer and measured. The spectra for the final coat, intermediate coat, and the undercoat are shown in Figures 5, 6, and 7, respectively. For reference, the undercoat was measured with the transmission method, and overlaid in Figure 8 with the result of the ATR correction of Figure 7.

From this it can be seen that a spectrum similar to the transmission method can be obtained with the microscopic ATR method.

Table 2: Analytical Conditions

Accessory	ATR Objective (IRE: Ge)
Resolution	8 cm^{-1}
Accumulation	40
Apodization	Happ-Genzel
Detector	MCT

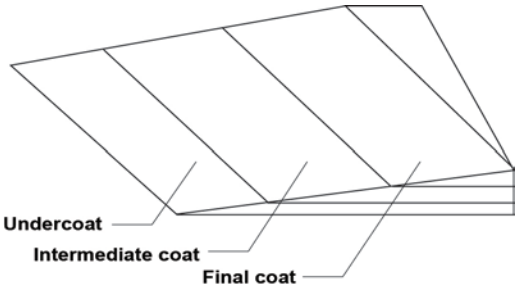

Figure 4: Magnified image of the paint fragment

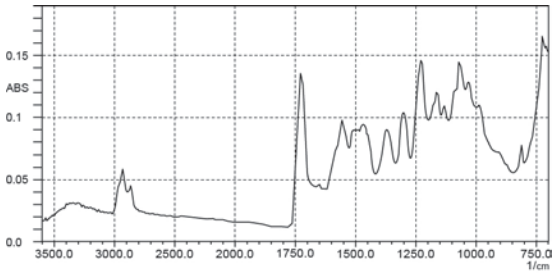

Figure 5: ATR spectrum of the final coat

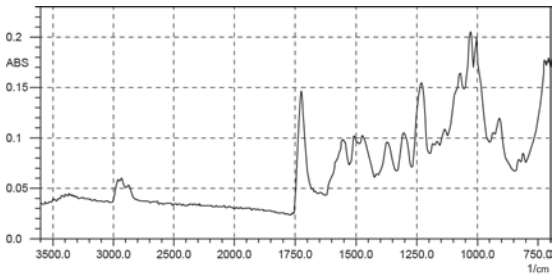

Figure 6: ATR spectrum of the intermediate coat

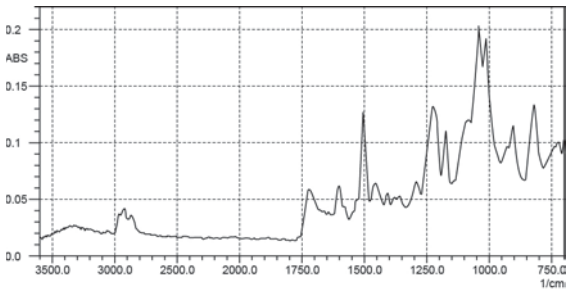

Figure 7: ATR spectrum of the undercoat

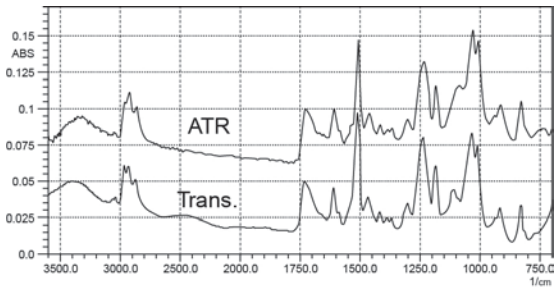

Figure 8: Transmittance spectrum and ATR spectrum of the undercoat

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Application News

No.X261

X-ray Analysis

Contaminant Analysis in Food Manufacturing Process by EDX and FTIR

EDX and FTIR are widely used for analysis of foreign contaminant matter, but recently, these instruments are increasingly being utilized in tandem to conduct contaminant analysis[1]. While identification using any of these instruments and analytical methods independently is limited to some degree, using them in conjunction with one another permits a more detailed elucidation of the contaminant characteristics, thereby enhancing the validity of the respective results.
The analytical method and sample pretreatment method to be used depend on the degree to which a contaminant is to be characterized, whether or not the substance is altered or destroyed due to pretreatment, and the speed that is required to complete the analysis. Introduced here is an example of actual analysis of various types of foreign matter entered during the food manufacturing process.

Samples

Foreign matter that entered during the food manufacturing process Five types of samples: Sample 1, 2, 3, 4, 5

Pretreatment and Analysis Procedures

First, EDX measurement was conducted without conducting any sample pretreatment, and then FTIR measurement was conducted similarly without pretreatment. Next, the foreign matter was removed by rinsing, and then analyzed. This preparation procedure is outlined in the flowchart of Fig. 1.
Depending on the sample, there may be cases in which detailed analysis by ATR measurement using the FTIR main unit will be difficult due to such factors as small sample size relative to the prism, which could result in the sample being crushed, such as in the current situation, or samples consisting of a mixture, etc. It was therefore decided to conduct microscopic ATR measurement with close contact of the prism at the measurement site.

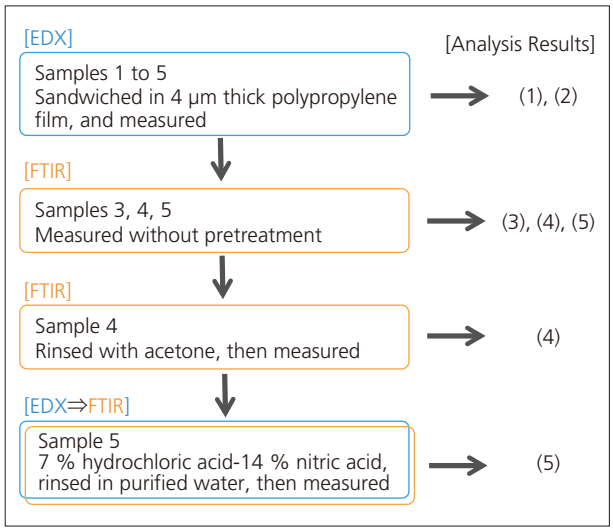
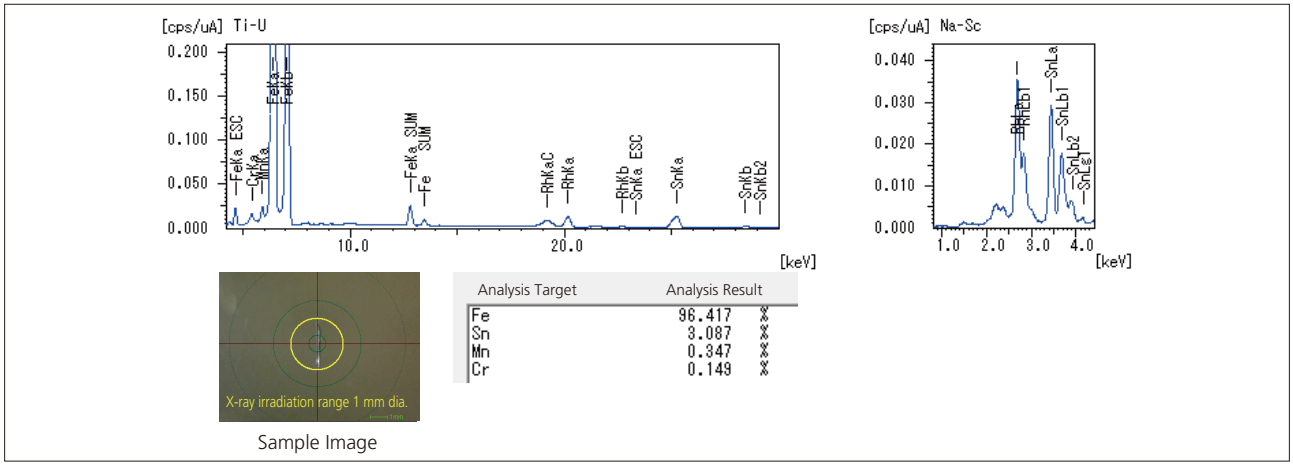


Fig. 1 Pretreatment and Analysis Procedures

Analysis Result

Fig. 2 to Fig. 9 and Table 1 to Table 5 show the analysis results for each sample using EDX and FTIR, in addition to the inferred and specific attributions according to those results.

(1) Sample 1 Characteristics: Metallic luster, hard, silvery white



Sample Image

Fig. 2 Sample 1 Qualitative-Quantitative Results by EDX

Table 1 Analysis Results for Sample 1

	Measurement Result	Possible Source	Total Findings Found by EDX and FTIR
EDX	Principal component is $_{26}$Fe, next prevalent is $_{50}$Sn.	Tin-plated steel sheet, fragment of tin can	Tin-plated steel sheet, fragment of tin can (Clearly metallic according to EDX measurement only)
FTIR	Omitted (Significant peak not detected)	Possibly a metal or inorganic compound	

(2) Sample 2 Characteristics: Metallic luster, hard, silver color

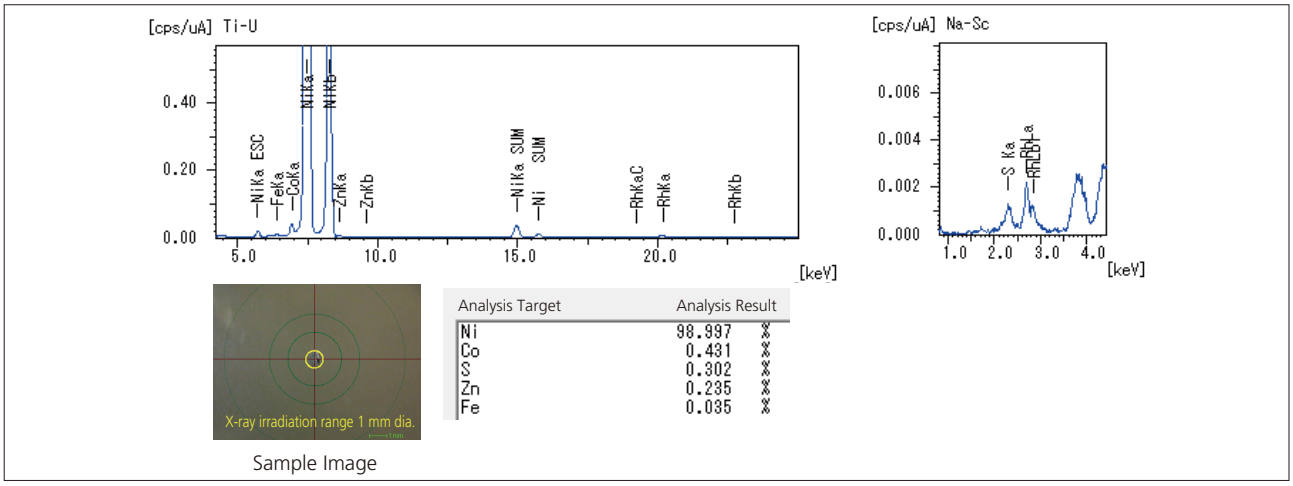


Fig. 3 Sample 2 Qualitative-Quantitative Results by EDX

Table 2 Analysis Results for Sample 2

	Measurement Result	Possible Source	Total Findings Found by EDX and FTIR
EDX	Principal component is $_{28}$Ni, other components are in small quantity.	Nickel, peeling of the nickel plating	Nickel, peeling of the nickel plating (Clearly metallic according to EDX measurement only)
FTIR	Omitted (Significant peak not detected)	Possibly a metal or inorganic compound	

(3) Sample 3 Characteristics: No metallic luster, brittle, brownish red

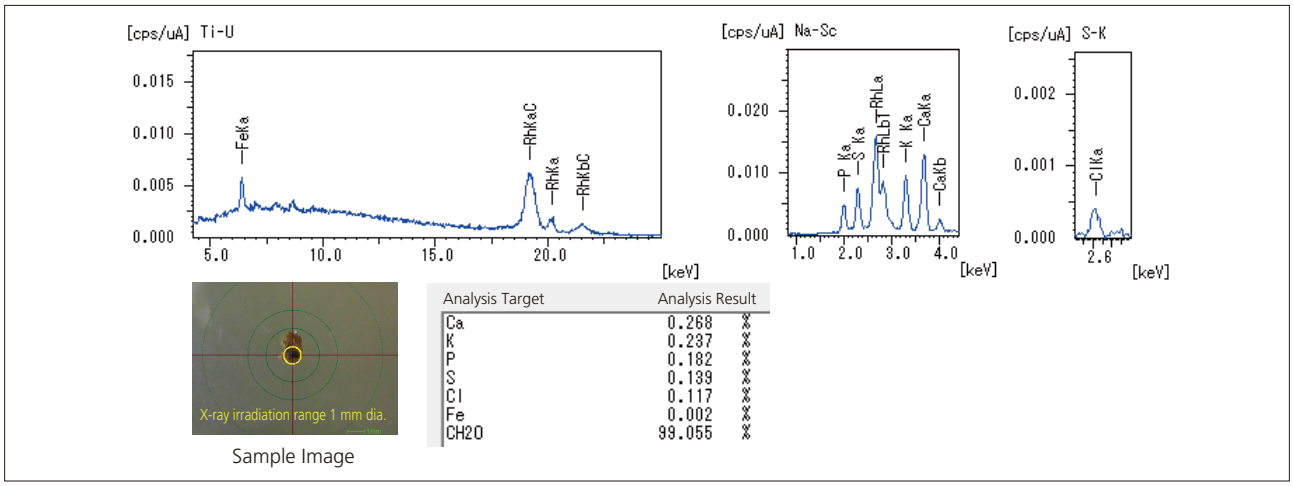


Fig. 4 Sample 3 Qualitative-Quantitative Results by EDX

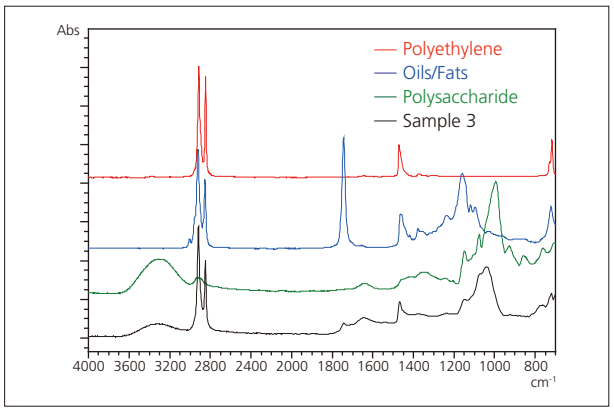


Fig. 5 Infrared Spectrum and Search Results for Sample 3 by FTIR

Table 3 Analysis Results for Sample 3

	Measurement Result	Possible Source	Total Findings Found by EDX and FTIR
EDX	Detected $_{19}$K, $_{20}$Ca, and other food components. Principal component is $_{9}$F and below. (RhKαC is big.[2])	Food clump	Polyethylene with attached food components
FTIR	Polyethylene, oils and fats, polysaccharides	Polyethylene with attached oils/fats and polysaccharides	

(4) Sample 4 Characteristics: Non-metallic luster, hard, black

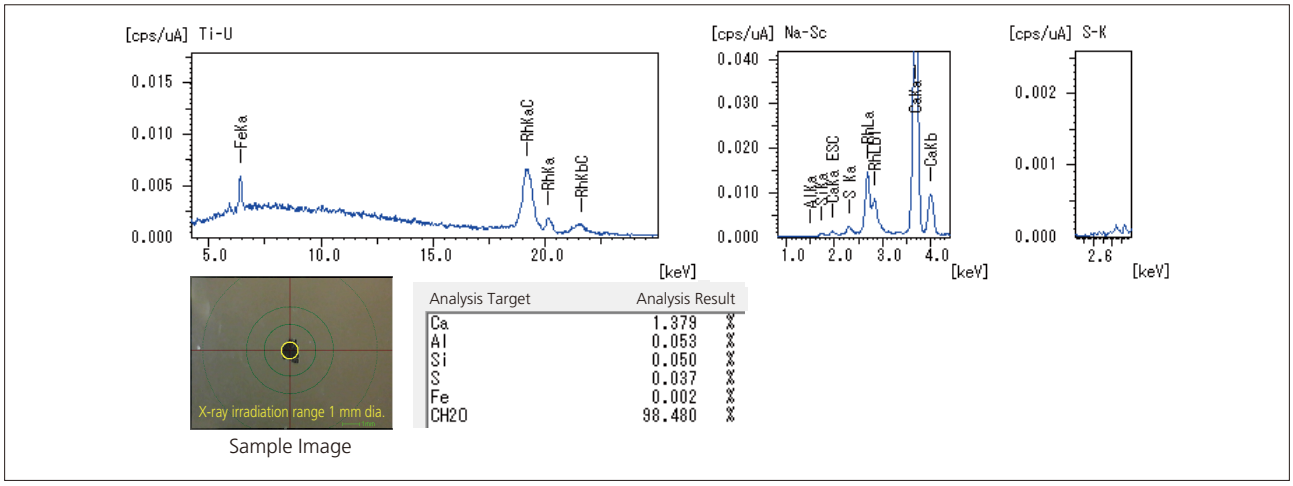


Fig. 6 Sample 4 Qualitative-Quantitative Results by EDX

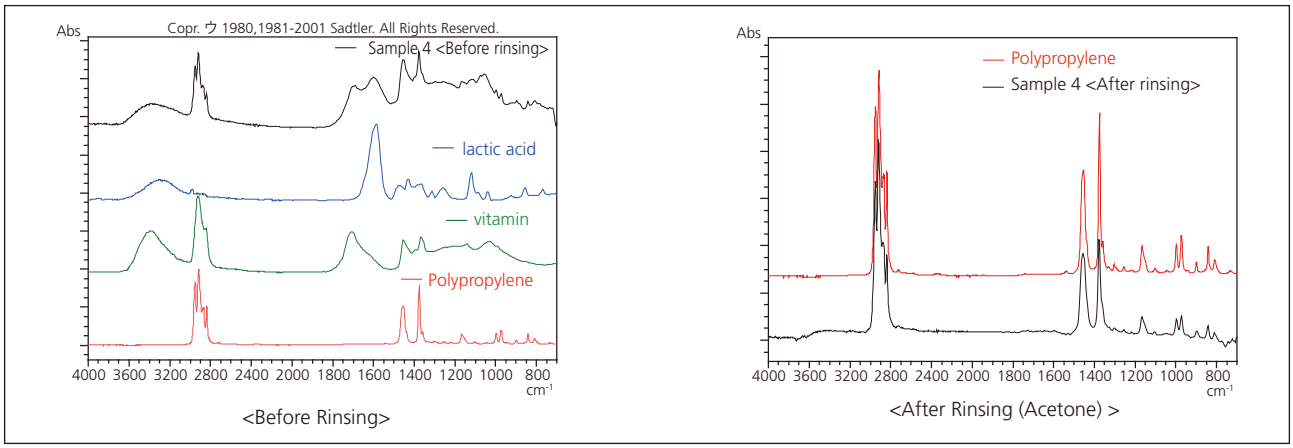


Fig. 7 Sample 4 Infrared Spectra and Search Results by FTIR

Table 4 Analysis Results for Sample 4

	Measurement Result		Possible Source	Total Findings Found by EDX and FTIR
EDX	Detected $_{20}$Ca and other food components. Principal component is $_{9}$F and below.		Food clump, resins, etc.	Polyethylene with attached food components
FTIR	Before rinsing	Polypropylene, lactic acid, vitamins	Food components (lactic acid, vitamins, etc.) adhering to polypropylene	
	After rinsing	Polypropylene		

(5) Sample 5 Characteristics: Some metallic luster, hard, black silver color

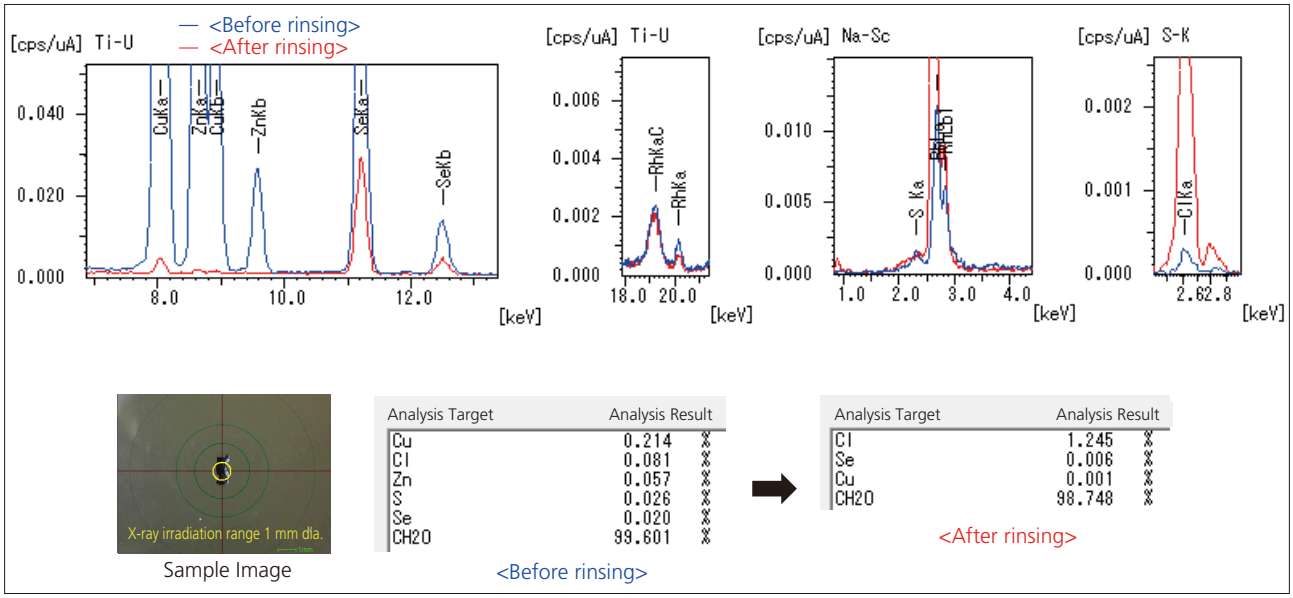
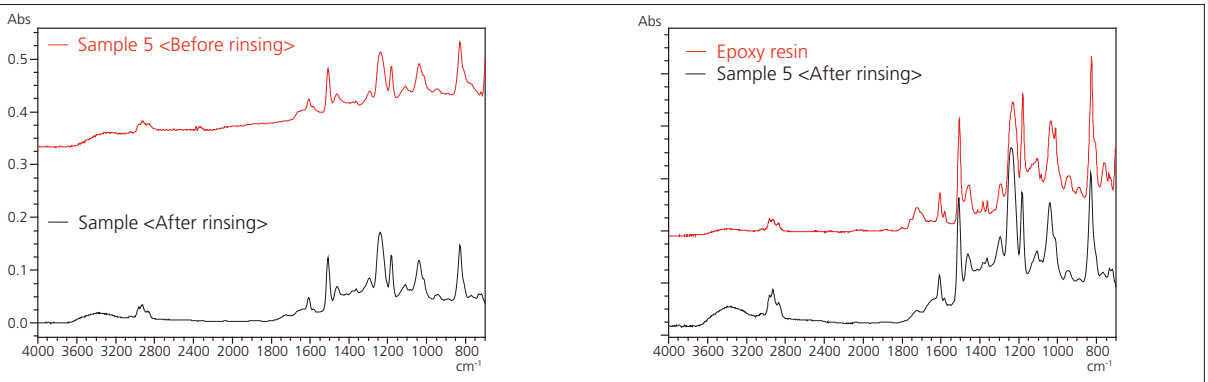


Fig. 8 Sample 5 Qualitative-Quantitative Result by EDX



<Before and After Rinsing (7 % hydrochloric acid-14 % nitric acid) >

<After Rinsing (7 % hydrochloric acid-14 % nitric acid) >

Fig. 9 Sample 5 Infrared Spectra and Search Result by FTIR

Table 5 Analysis Results for Sample 5

	Measurement Result		Possible Source	Total Findings Found by EDX and FTIR
EDX	Before rinsing	Principal components are $_{9}$F and below, large amounts of $_{29}$Cu, $_{30}$Zn, $_{34}$Se.	Copper alloy, resin composite material, zinc, selenium additives	Zinc and selenium food additives adhering to epoxy resin coated on copper thin film
	After rinsing	Principal components are $_{9}$F and below, with $_{29}$Cu, $_{30}$Zn nearly absent due to rinsing, and a small amount of residual $_{34}$Se.	Film	
FTIR	Before rinsing	Epoxy resin (with the presence of metals, etc. suggested due to rising of the infrared baseline)	Composite material consisting of epoxy resin and metal	
	After rinsing	Epoxy resin (no rise in the baseline in infrared spectrum)	Epoxy resin	

- Regarding the EDX quantitative analysis results
  · Organic material is represented by CH_2O, and was balanced.
  · Abundant, small quantity, etc. are relative reference values. (In order to collectively set plating, film and deposits, etc.)

Conclusion

The analysis results by both EDX and FTIR permitted approximate identification of metals, resins, and their compounds or complex materials associated with contaminants introduced during the food product manufacturing process without the need for pretreatment. Further, by conducting relatively simple pretreatment of samples, detailed identification is also possible depending on the sample. In terms of speed and ease, these analytical techniques are quite effective.

[References]

1) Shimadzu Application News No. A452
2) Izumi Nakai (Editor), A Practical Guide for X-ray Fluorescence Analysis, Asakura Publishing, 90 (2006)

Analytical Conditions [EDX]

Instrument	: EDX-7000
Elements	: Na-U
Analytical Group	: Qualitative-quantitative
Detector	: SDD
X-Ray Tube	: Rh target
Tube Voltage [kV]	: 15, 50
Current [μA]	: Auto
Collimator [mm ϕ]	: 1 or 3
Primary Filter	: Non, #2
Atmosphere	: Vacuum
Integration Time [sec]	: 50 /ch
Dead Time [%]	: Max. 30

Analytical Conditions [FTIR]

Instruments	: IRTracer-100, AIM-8800
Resolution	: 8 cm^{-1}
Accumulation	: 40
Apodization	: Sqr-Triangle
Detector	: MCT

First Edition: Jun. 2015

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Application News

No.A501

Spectrophotometric Analysis

Analysis of Thermally Degraded Plastics Using Thermally Degraded Plastics Library

-Applications to Contaminant Analysis-

Introduction

In daily life, we encounter a variety of natural products and manufactured goods. In fields such as food products, pharmaceuticals, and machinery, effort is put into quality assurance, striving to prevent the incorporation of contaminants. However, the incorporation of contaminants does inadvertently occur due to unforeseen factors and problems.

While there are many types of contaminants, the plastic parts used in production line, specific environments, and the vicinity become brittle due to aging and thermal degradation, making their incorporation in part a possibility.

FTIR is optimal for the analysis of such plastic contaminants. However, the infrared spectrum of degraded plastic differs from the spectral pattern before degradation. Accordingly, in searches using commercially available plastics libraries, even if a search result has top ranking, it can inadvertently consist of the spectral pattern for a different substance, making identification and qualification difficult.

In this article, we introduce an example of the changes to the infrared spectrum of a plastic degraded by heat, and a sample search using a library containing data created by changing the heating temperature and time beforehand.

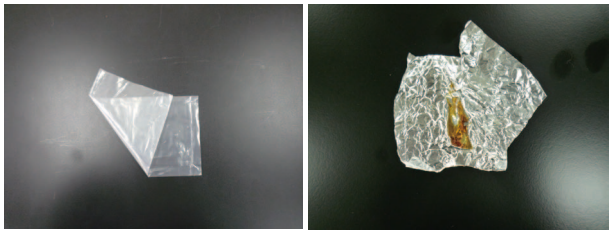
Changes to the Spectrum of a Polyethylene Film Due to Thermal Degradation

A polyethylene film was wrapped in aluminum foil, and then heated on a hot plate. As it was heated in air, the polyethylene film was subject to oxidative degradation.

Fig. 1 is a photograph of the polyethylene film before heating, and then after heating at 200 °C for two hours. Before heating, it was transparent. After heating, however, it evidently turned brown.

Fig. 2 (top) shows the respective infrared spectra. The measurements were performed using the single bounce ATR method. Polyethylene has a repeating $-(CH_2)_n-$ structure, so before heating, peaks due solely to this structure are visible near 3000 cm^{-1}, 1400 cm^{-1}, and 700 cm^{-1}.

As a result of heating, in addition to the original peaks, there are peaks in the 1700 cm^{-1} to 1750 cm^{-1} range due to -C=O, and peaks in the 1100 cm^{-1} to 1200 cm^{-1} range due to -C-O-. These are likely due to oxidative degradation. Fig. 2 (bottom) shows the spectra for polystyrene film before and after an identical heat treatment. Identical changes are visible here as well.



Before Heating　　After Heating

Fig. 1 Polyethylene Film Before Heating, and After Heating at 200 °C for 2 Hours

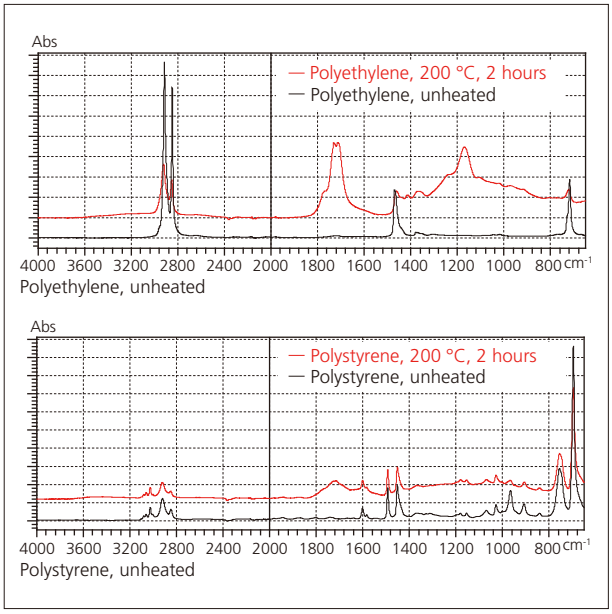


Fig. 2 Top: Infrared Spectra of Polyethylene Film Before Heating (Black) and After Heating at 200 °C for 2 Hours (Red). Bottom: Polystyrene Film Before Heating (Black) and After Heating at 200 °C for 2 Hours (Red)

Analysis of a Contaminant on a Plated Part

A semi-transparent light brown contaminant was visible on a plated product. This area was measured, and the spectrum in Fig. 3 was obtained. A search of the spectrum was performed using a standard library directly, but no equivalent spectra were found.
As shown in Fig. 2, a portion is similar to heated plastic, so it is presumed to be plastic that had been changed by heating.

Searches Using Thermally Degraded Plastics Library

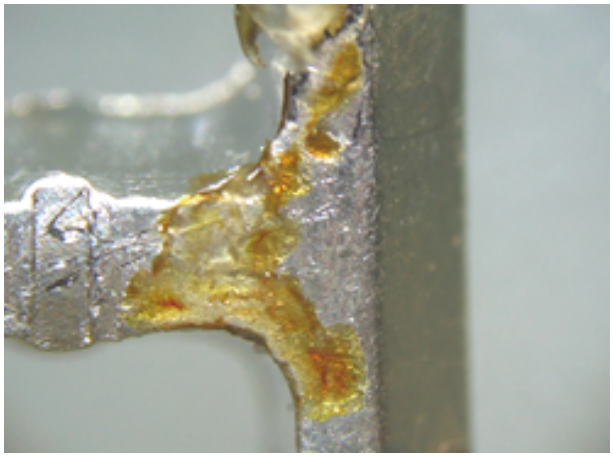
As in Figs. 2 and 3, with heating, the infrared spectral pattern is changed due to oxidation. Typical search libraries contain the infrared spectra for plastic samples measured in an unheated state. As a result, if a search is performed directly, there is a risk that the results obtained will be different than for plastic samples after heating.
This article introduces the Thermally Degraded Plastics Library, a proprietary library compiled by Shimadzu. It consists of spectra obtained by measuring samples at the Industrial Research Institute of Shizuoka Prefecture's Hamamatsu Technical Support Center. The library contains 13 plastics, both unheated and thermally degraded at temperatures from 200 °C to 400 °C.
Fig. 4 shows the results of a search utilizing these. It is evident that the heated plastics have top ranking.
In addition, this library contains infrared spectra changed by heating temperature and heating time, which will be useful for estimating the thermal history of a plastic.
Note that this library is not intended for searches of heating time and heating temperature, but is considered for investigating the thermal history of a sample.

Conclusion

It is evident that qualitative analysis of plastics that have undergone thermal changes can easily be performed by searching the Thermally Degraded Plastics Library.
This library will prove useful in contaminant analysis.

Table 1 FTIR Analysis Conditions

Instrument	: IRTracer-100 MIRacle10
Resolution	: 4.0 cm^{-1}
Accumulation	: 100
Apodization	: Happ-Genzel
Detector	: DLATGS



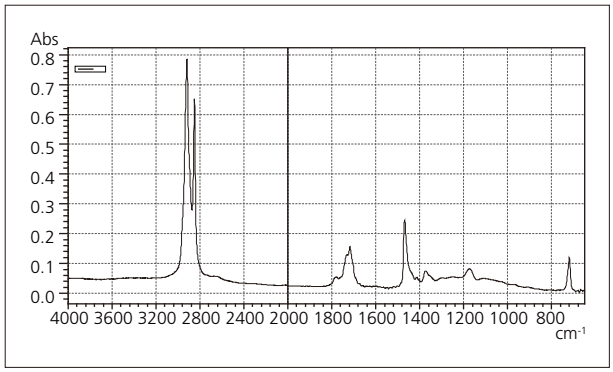
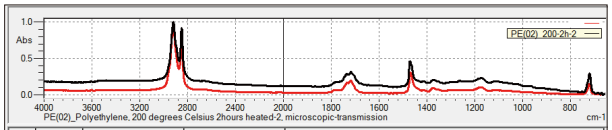


Fig. 3 Photograph of a Contaminant on a Plated Part (Top); Measured Infrared Spectrum (Bottom)



	Score	Library	Name	Comment
1	979	40 - Thermal-Damaged Plastic	PE(02)_200-2h-2	PE(02)_Polyethylene, 200 degrees Celsius 2hours heated-2, microscopic-transmission
2	951	43 - Thermal-Damaged Plastic	PE(05)_250-2h	PE(05)_Polyethylene, 250 degrees Celsius 2hours heated, microscopic-transmission
3	925	44 - Thermal-Damaged Plastic	PE(06)_300-2h	PE(06)_Polyethylene, 300 degrees Celsius 2hours heated, microscopic-transmission
4	908	22 - T_FoodAdditives2	T_Candelilla Wax-4	Candelilla Wax(Sales origin:MIKI CHEMICAL INDUSTRY & CO.,LTD.)@KBr Wafer
5	908	146 - ATR-Polymer2	D_Polyethylene_Oxidized	Polyethylene, Oxidized DuraSamplIR-1U
6	903	41 - Thermal-Damaged Plastic	PE(03)_200-2h-3	PE(03)_Polyethylene, 200 degrees Celsius 2hours heated-3, microscopic-transmission
7	862	21 - A_FoodAdditives2	A_Candelilla Wax-4	Candelilla Wax(Sales origin:MIKI CHEMICAL INDUSTRY & CO.,LTD.)@DuraSamplIR2(diamond)
8	817	60 - Thermal-Damaged Plastic	PMP(05)_300-4h	PMP(05)_Polymethylpentene, 300 degrees Celsius 4hours heated, microscopic-transmission
9	813	42 - Thermal-Damaged Plastic	PE(04)_200-2h-4	PE(04)_Polyethylene, 200 degrees Celsius 2hours heated-4, microscopic-transmission
10	812	81 - Thermal-Damaged Plastic	PP(04)_200-4h-3	PP(04)_Polypropylene, 200 degrees Celsius 4hours heated-3, microscopic-transmission

Fig. 4 Search Results Using the Thermally Degraded Plastics Library

First Edition: Apr. 2016

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Application News

No.J99

Inductively Coupled Plasma Atomic Emission Spectrometry

Analysis by ICP Atomic Emission Spectrometry in Accordance with the ICH Q3D Guideline for Elemental Impurities Using ICPE-9820

Introduction

Analysis of elemental impurities is one of the safety assessments required in the field of pharmaceuticals. In Japan, residual metal catalysts are classified as inorganic impurities according to the guidelines for Impurities in New Drug Substances (No. 1216001, issued by the Evaluation and Licensing Division, the Pharmaceutical and Food Safety Bureau, the Japanese Ministry of Health, Labour and Welfare), and are to be detected appropriately according to the method specified in the Japanese Pharmacopoeia, and evaluated at the stage of drug development. At the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use: ICH, various guidelines were established and harmonized between Japan, Europe, and the US, including guidelines for elemental impurities in pharmaceuticals, referred to as the ICH Q3D, Guideline for Elemental Impurities.

For the analysis of elemental impurities, the methods specified for use as general analytical methods in the First Supplement of the Sixteenth Edition of the Japanese Pharmacopoeia include inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), and atomic absorption spectrometry. Of these, ICP-AES is the most convenient, offering quick and easy multi-element analysis, and low running costs.

Here, we conducted analysis of 24 elements according to the ICH Q3D guidelines using the Shimadzu ICPE-9820 multi-type ICP atomic emission spectrometer. The ICPE-9820 offers simultaneous all element analysis with high sensitivity and high precision, while delivering high throughput. Low running costs are achieved by a unique combination of the reduced-flow mini-torch and vacuum optics, thereby reducing the overall consumption of argon.

Outline of the ICH Q3D Guideline for Elemental Impurities

In the ICH Q3D Guideline for Elemental Impurities, 24 elemental impurities were identified as elements of concern due to their toxicity, and permitted daily exposure limits (PDE) were established. The elements include lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As), referred to as the "big four," as well as residual metal catalysts added intentionally in the synthesis of a drug substance. Table 1 shows the ICH Q3D Guideline (STEP4 draft).

As permitted exposure values for the elemental impurities have been set as PDE values, the PDE values must be converted to concentrations to evaluate the elemental impurity components in the formulations or their component substances. As calculation methods, options 1, 2a, 2b, and 3 are available. Therefore, as long as the formulation is appropriate for the PDE value of the elemental impurity, any of the methods may be selected. Calculation examples for the respective options are shown in Table 2 to Table 5.

Table 1 Permitted Daily Exposure for Elemental Impurities of ICH Q3D (STEP4 draft)

Class	Element	Oral µg/day	Parenteral µg/day	Inhalation µg/day	Class	Element	Oral µg/day	Parenteral µg/day	Inhalation µg/day
1	As	15	15	2	2B	Pt	1000	10	1
	Cd	5	2	2		Se	150	80	130
	Hg	30	3	1		Rh	100	10	1
	Pb	5	5	5		Ru	100	10	1
2A	Co	50	5	3		Tl	8	8	8
	Ni	200	20	5	3	Ba	1400	700	300
	V	100	10	1		Cr	11000	1100	3
2B	Ag	150	10	7		Cu	3000	300	30
	Au	100	100	1		Li	550	250	25
	Ir	100	10	1		Mo	3000	1500	10
	Os	100	10	1		Sb	1200	90	20
	Pd	100	10	1		Sn	6000	600	60

Table 2 Calculation by Option 1: Maximum Permitted Common Concentration Limits of Elemental Impurities Across Drug Product Components for Products with Daily Intake of Not More Than 10 Grams

Component Substance	Max. Daily Intake of Each Substance (g)	PDE (µg)		Max. Permitted Concentration Assuming a 10 g Max. Daily Intake of Formulation (µg/g)		Max. Intake from Each Component (µg)	
				PDE/10 g		Max. Daily Intake (g) of Each Component × Max. Permitted Concentration (µg/g) of Each Component	
		Pb	As	Pb	As	Pb	As
Drug substance	0.2	5	15	0.5	1.5	0.1	0.3
MCC	1.1	5	15	0.5	1.5	0.55	1.65
Lactose	0.45	5	15	0.5	1.5	0.225	0.68
Calcium phosphate	0.35	5	15	0.5	1.5	0.175	0.53
Crospovidone	0.265	5	15	0.5	1.5	0.133	0.4
Magnesium stearate	0.035	5	15	0.5	1.5	0.018	0.05
HPMC	0.06	5	15	0.5	1.5	0.03	0.09
Titanium oxide	0.025	5	15	0.5	1.5	0.013	0.04
Iron oxide	0.015	5	15	0.5	1.5	0.008	0.02
Max. Daily Intake (Total)	2.5					1.25	3.75
PDE (µg/day)						5.0	15

Table 3 Calculation by Option 2a: Maximum Permitted Common Concentration Limits Across Drug Product Component Materials for a Product with a Specified Daily Intake (Assuming That Concentration Remains Constant)

Component Substance	Max. Daily Intake of Each Substance (g)	PDE (µg)		Max. Permitted Concentration (µg/g)		Max. Intake from Each Component (µg)	
				PDE/Max. Daily Intake of Actual Drug (e.g. 2.5 g)		Max. Daily Intake (g) of Each Component × Max. Permitted Concentration (µg/g) of Each Component	
		Pb	As	Pb	As	Pb	As
Drug substance	0.2	5	15	2	6	0.4	1.2
MCC	1.1	5	15	2	6	2.20	6.6
Lactose	0.45	5	15	2	6	0.9	2.7
Calcium phosphate	0.35	5	15	2	6	0.7	2.1
Crospovidone	0.265	5	15	2	6	0.53	1.59
Magnesium stearate	0.035	5	15	2	6	0.07	0.21
HPMC	0.06	5	15	2	6	0.12	0.36
Titanium oxide	0.025	5	15	2	6	0.05	0.15
Iron oxide	0.015	5	15	2	6	0.03	0.09
Max. Daily Intake (Total)	2.5					5.0	15
PDE (µg/day)						5.0	15

Table 4 Calculation by Option 2b: Maximum Permitted Common Concentration Limits Across Drug Product Component Materials for a Product with a Specified Daily Intake (Arbitrary Setting of Maximum Concentration Possible from Actual Value)

Component Substance	Max. Daily Intake of Each Substance (g)	PDE (µg)				Measured Concentration Value (µg)				Arbitrary Setting of Max. Concentration Possible from Actual Value (µg/g)				Max. Daily Intake of Each Componen (µg)			
		Pb	As	Pd	Ni	Pb	As	Pd	Ni	Pb	As	Pd	Ni	Pb	As	Pd	Ni
Drug substance	0.2	5	15	100	200	**	0.5	20	50	**	5	500	200	**	1	100	40
MCC	1.1	5	15	100	200	0.1	0.1	*	**	0.5	5	*	**	0.55	5.5	*	**
Lactose	0.45	5	15	100	200	0.1	0.1	*	**	0.5	5	*	**	0.225	2.3	*	**
Calcium phosphate	0.35	5	15	100	200	1	1	*	5	5	5	*	200	1.75	1.8	*	70
Crospovidone	0.265	5	15	100	200	0.1	0.1	*	**	0.5	5	*	**	0.132	1.3	*	**
Magnesium stearate	0.035	5	15	100	200	0.5	0.5	*	0.5	5	10	*	50	0.175	0.4	*	1.75
HPMC	0.06	5	15	100	200	0.1	0.1	*	**	2.5	5	*	**	0.15	0.3	*	**
Titanium oxide	0.025	5	15	100	200	20	1	*	**	40	20	*	**	1	0.5	*	**
Iron oxide	0.015	5	15	100	200	10	10	*	50	20	100	*	200	0.3	1.5	*	3
Max. Daily Intake (Total)	2.5													4.3	14.5	100	115
PDE (µg/day)														5	15	100	200

*: Since it has been determined that there is no possibility of Pd being present, a quantitative result is not obtained.
**: Below the detection limit

Table 5 Calculation by Option 3: Finished Product
Concentration (µg/g) = PDE (µg/day)/Daily intake of drug product (g/day)

		PDE (µg)				Maximum Permitted Concentration (µg/g)			
	Daily Intake (g)	Pb	As	Pd	Ni	Pb	As	Pd	Ni
Drug Product	2.5	5	15	100	200	2	6	40	80

Sample

· Ophthalmic solution
· Tablet (Daily intake: 1 tablet (0.2 g))

Sample Preparation

1. Pretreatment of sample (ophthalmic solution)

To 2 mL of sample (approximately 2 g), add 0.5 mL hydrochloric acid, 0.5 mL nitric acid and internal standard element Y (0.5 mg/L based on measurement solution concentration). Adjust the volume to 10 mL using distilled water to use as the measurement solution (5-fold dilution). A spike-and-recovery test solution was prepared using a similarly prepared solution spiked with a standard solution of the measurement element.

2. Pretreatment of tablet sample

Two tablets (daily dosage of 1 tablet per day (0.20 g)) were dissolved with 3 mL hydrochloric acid and 2 mL nitric acid using a microwave sample preparation system and a sample pretreatment quartz vessel.
After conducting microwave digestion, the solution volume was adjusted to 20 mL with distilled water to use as the measurement solution (50-fold dilution). At this time, the internal standard elements Y and In (Y at 0.5 mg/L and In at 1.0 mg/L) were added to the solution. Also, prior to digestion, the measurement element was added to prepare a spike-and-recovery test solution.

Instrument and Analytical Conditions

Measurement was conducted using the Shimadzu ICPE-9820 multi-type ICP atomic emission spectrometer. The measurement conditions are shown in Table 6.
The ICPE-9820 is a spectrometer that uses the latest CCD, permitting simultaneous measurement of all elements and all wavelengths, while its high-sensitivity axial observation permits high-throughput measurement. Further, the high-temperature plasma generated by the mini torch assures high sensitivity with low ionization interference to provide acquisition of accurate values. In addition, the mini-torch plasma produced by low-flowrate argon gas, the Eco mode and the vacuum spectrometer greatly reduce running costs.

Table 6 Analytical Conditions

Instrument	: ICPE-9820
Radio frequency power	: 1.2 kW
Plasma gas Flowrate	: 10 L/min
Auxiliary gas Flowrate	: 0.6 L/min
Carrier gas Flowrate	: 0.7 L/min
Sample introduction	: Nebulizer 10
Misting chamber	: Cyclone chamber
Plasma torch	: Mini-Torch
Observation	: Axial (AX) / Radial (RD)

Analysis

Quantitative analysis of the 24 elements subject to the ICH Q3D guidelines was conducted using the calibration curve-internal standard method, and spike-and-recovery testing was also conducted.

Analytical Results

Table 7 shows the results of analysis of the ophthalmic solution. The PDE value of the ophthalmic solution was used as the parenteral value. Table 8 shows the results of the tablet analysis. Good results were obtained in the spike-and-recovery testing for each of the samples (Tables 7 and 8[*1]). In addition, the detection limit calculated as the concentration in the sample (Tables 7 and 8[*2]) adequately satisfied the permitted concentrations (Tables 7 and 8[*3]).

Conclusion

Use of the ICPE-9820 permits quick, accurate analysis of the 24 elements specified in the ICH Q3D guideline.

[References]
1) Impurities in New Drug Substances (No. 1216001, issued by the Evaluation and Licensing Division, the Pharmaceutical and Food Safety Bureau, the Japanese Ministry of Health, Labour and Welfare)
2) First Supplement of the Sixteenth Edition of the Japanese Pharmacopoeia
3) ICH Q3D: Guideline for Elemental Impurities (STEP4 draft)

Table 7 Analytical Results of Eye Drop

Element	PDE value for parenteral	*3 Permitted concentration	Post-treatment concentration	Spike concentration	Measured concentration (Eye drop)	*1 Spike-and-recovery rate	*2 Converted detection limit (3σ) in ophthalmic solution
	μg	μg/mL	μg/mL	μg/mL	μg/mL	%	μg/mL
As	15	15	3	1	<DL	104	0.04
Cd	2	2	0.4	0.4	<DL	101	0.0006
Hg	3	3	0.6	0.3	<DL	105	0.007
Pb	5	5	1	0.3	<DL	102	0.01
Co	5	5	1	0.3	<DL	95	0.001
Ni	20	20	4	0.5	<DL	104	0.003
V	10	10	2	0.5	<DL	98	0.0008
Ag	10	10	2	0.5	<DL	104	0.0008
Au	100	100	20	0.5	<DL	99	0.006
Ir	10	10	2	0.5	<DL	101	0.01
Os	10	10	2	0.5	<DL	103	0.006
Pd	10	10	2	0.5	<DL	102	0.004
Pt	10	10	2	0.5	<DL	99	0.02
Se	80	80	16	0.5	<DL	103	0.02
Rh	10	10	2	0.5	<DL	95	0.007
Ru	10	10	2	0.5	<DL	103	0.003
Tl	8	8	1.6	0.5	<DL	95	0.02
Ba	700	700	140	0.5	<DL	96	0.0006
Cr	1100	1100	220	0.5	<DL	97	0.002
Cu	300	300	60	0.5	<DL	96	0.002
Li	250	250	50	0.5	<DL	99	0.01
Mo	1500	1500	300	0.5	<DL	100	0.003
Sb	90	90	18	0.5	<DL	103	0.01
Sn	600	600	120	0.5	<DL	100	0.01

PDE value for parenteral
Permitted concentration : When 1 mL of the ophthalmic solution is used per day (Option 3 is used when calculating the conversion to the PDE concentration)
Post-treatment concentration : The permitted concentration in the measurement sample after pretreatment of the sample
Spike concentration : Concentration of spiking solution in spike-and-recovery testing
Converted detection limit (3σ) in ophthalmic solution: Detection limit (3σ) in measurement solution × Dilution factor (5)
<DL: Below the detection limit (3σ)

Table 8 Analytical Results of Tablet

Element	PDE value for oral	*3 Permitted concentration	Post-treatment concentration	Spike concentration	Measured concentration (Tablet)	*1 Spike-and-recovery rate	*2 Tablet converted detection limit (3σ)
	μg	μg/g	μg/mL	μg/mL	μg/g	%	μg/g
As	15	75	1.5	0.5	<DL	107	0.5
Cd	5	25	0.5	0.1	<DL	100	0.007
Hg	30	150	3	1	<DL	101	0.1
Pb	5	25	0.5	0.1	<DL	98	0.07
Co	50	250	5	1	<DL	101	0.01
Ni	200	1000	20	1	0.1	100	0.03
V	100	500	10	1	<DL	103	0.01
Ag	150	750	15	1	<DL	104	0.02
Au	100	500	10	1	<DL	105	0.03
Ir	100	500	10	1	<DL	100	0.09
Os	100	500	10	1	<DL	85	0.04
Pd	100	500	10	1	<DL	106	0.05
Pt	1000	5000	100	1	<DL	102	0.3
Se	150	750	15	1	<DL	108	0.3
Rh	100	500	10	1	<DL	101	0.1
Ru	100	500	10	1	<DL	100	0.03
Tl	8	40	0.8	0.1	<DL	103	0.2
Ba	1400	7000	140	1	<DL	102	0.003
Cr	11000	55000	1100	1	<DL	101	0.02
Cu	3000	15000	300	1	<DL	105	0.05
Li	550	2750	55	1	<DL	104	0.1
Mo	3000	15000	300	1	<DL	101	0.03
Sb	1200	6000	120	1	<DL	105	0.1
Sn	6000	30000	600	1	<DL	100	0.03

PDE value for oral
Permitted concentration : Permitted concentration in daily intake (0.2 g) (Option 3 is used for calculation of conversion from PDE to concentration)
Post-treatment concentration : Permitted limit concentration in measurement solution following sample pretreatment
Spike concentration : Concentration of the added spike-and-recovery test solution
Tablet converted detection limit (3σ): Detection limit (3σ) in measurement solution Dilution factor (50)
<DL: Below the detection limit (3σ)

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Application News

X-Ray Analysis

"Getting the Lead Out": Energy Dispersive X-Ray's Role in Uncovering Dangerous Toys

■ Introduction

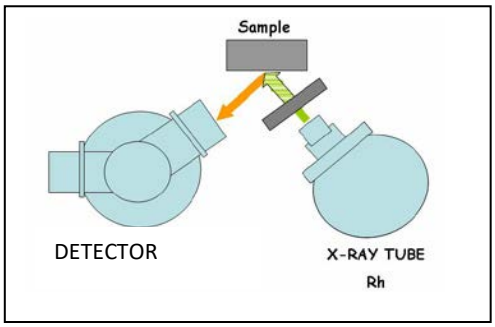
Everyone is becoming more aware of the influx of dangerous substances, specifically lead, being found in our everyday articles. Lead has been found in paints, plastics, and metal commonly used in toys. Compounding the problem even further is the difficulty in identifying which items contain harmful substances and which do not. This article investigates Energy Dispersive X-Ray's ability to identify lead in common constituents of toys, specifically in paints, resins, and metal.

There are two main areas of concern for contamination, the surface followed by the substrate. The surface finish can be a variety of materials, paint, resin, or metal. The surface is the first area of concern because this part has the most direct contact with the user. Contamination in the surface has the highest possibility of exposure or transfer to its user, with oral exposure being of particular concern. The second area of concern is the substrate. Over time the surface will wear off, exposing the user to the substrate and any contamination therein.

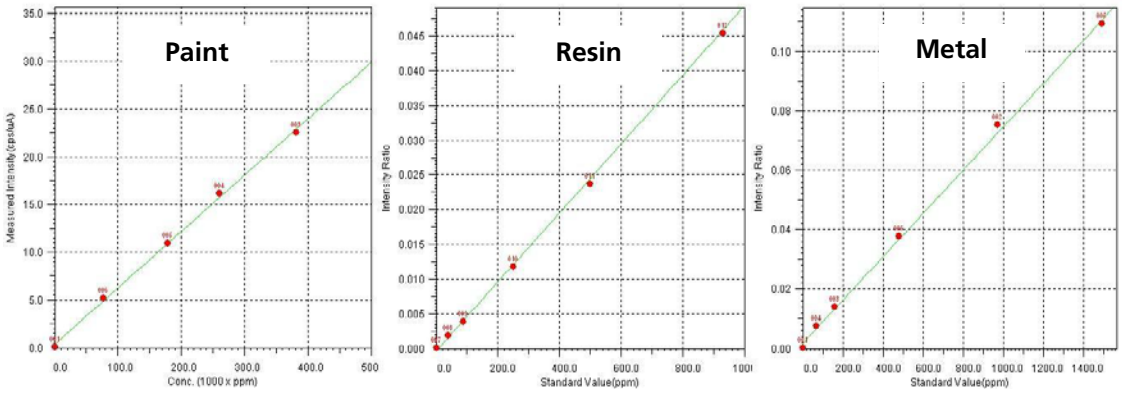
There are many techniques that can be used to identify and quantify harmful substances such as lead. Two very popular and commonly used techniques are Inductively Coupled Plasma (ICP) or Atomic Absorption (AA). Because both of these techniques usually require the sample to be in an aqueous form, intensive sample prep can be required. For samples containing plastics or metal this can be a daunting task requiring a lot of time and dangerous chemicals. Additionally, ICP and AA are both destructive measurements.

Another technique growing in commonality is X-Ray. There are three X-Ray techniques: Energy Dispersive X-Ray (EDX), X-Ray Diffraction, and Wavelength Dispersive X-Ray. Energy Dispersive is the easiest of the techniques and the most cost effective for a screening tool. EDX allows for a non-destructive rapid measurement of elements from Sodium (Na) to Uranium (U) in a variety of sample forms such as metals, resins, powders, and liquids. The functionality of EDX allows it to be easily integrated into any stage in a product's production, from the individual components to the final assembly.

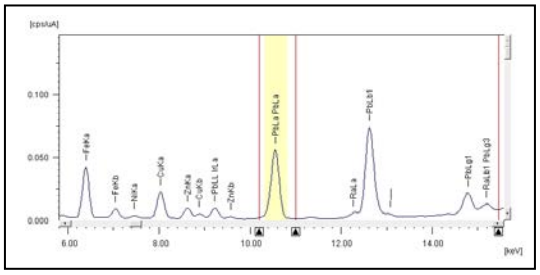
EDX works by a simple process whereby a sample is irradiated by an incidental X-Ray. The sample absorbs the incidental X-Ray and emits characteristic X-Rays for the elements that make up its composition. After analysis the sample is not radioactive or altered in any way.



One important note is the large effect matrix has on the intensity of an emitted X-Ray. The intensity of the characteristic X-Ray decreases with the density of the matrix. Three different matrixes were chosen based on the popularity of use in toy components: paint, resin, and metal. Different amounts of lead contamination in each matrix are used to establish a linear relation between the lead and the matrix.



The data below shows the results of a metallic part of a toy analyzed for lead. The lead peak is highlighted for emphasis. The peak is integrated and compared to a calibration curve to quantify the amount of lead.



■ Conclusion

EDX is a good technique for these types of analyses because it is a rapid, non-destructive analysis involving little to no prep work. The technique can be applied to a variety of materials, elements and matrixes, from levels of a few parts per million to percent levels.

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Forensic Toxicology

1. Elemental Spectroscopy

a. Direct Determination of Pb in Whole Blood by Graphite Furnace Atomic Absorption Spectrophotmetry (GF-AAS)
b. Quantitation of Trace Elements in Blood Using Shimadzu ICPMS-2030 Inductively Coupled Plasma Mass Spectrometer
c. EDXRF Analysis of Cd, Hg, and Pb in Blood
d. EDXRF Analysis of P, Cl, K and Ca in Blood

2. GC

a. Dual Channel Blood Alcohol Content (BAC) Analysis

3. HPLC

a. High Speed Analysis of Delta-Aminolevulinic Acid
b. High Speed with High Resolution Analysis (Part 37): Analysis of Clobazam and Cibenzoline in Serum by the Nexera UHPLC System

4. LC-MS

a. Analysis of Ethylglucuronide and Elthysulfate in Urine, Plasma and Serum by LCMS-8050 using RECIPE ClinMass LC-MS/MS Complete Kit MS8000
b. Determination of delta 9-tetrahydrocannabinol and two of its metabolites in whole blood, plasma and urine by UHPLC-MS/MS Using QuEChERS Sample Preparation
c. Analysis of Carboxy THC Using the LCMS-8030 Triple Quadrupole Mass Spectrometer
d. Analysis of Cannabinoids using the LCMS-2020 Single Quad MS
e. Designer Cannabinoids
f. Tetrahydrocannabinol (THC) Analysis in Oral Fluids Using the LCMS-8050
g. Detecting New Designer Cannabinoids in Herbal Incense Using LC-MS/MS with Fast Precursor Scanning
h. Analysis of Drugs in Puetrefied Human Pleural Fluid Using Triple Quadrupole LC-MS/MS
i. Benzodiazepines and Barbituates
j. Quick Turnaround Analysis of 8 Drugs for Driver Under Influence Using LDTD-MS/MS System
k. Analysis of 90 Multi-Class Drugs with Polarity Switching in Plasma
l. LC-MS/MS Detection of Fentanyl and Related Synthetic Opioids in Biological Matrices Using the Shimadzu LCMS-8060

5. GC-MS

a. Analysis of Blood Alcohol by Headspace with Simultaneous GC-FID and MS Detection
b. Analysis and Quantitation of Cocaine on Currency Using GC-MS/MS
c. GCMS-QP2010 Series Determination of Drugs of Abuse in Oral Fluids
d. Automatic Identifi cation and Semi-Quantitative Analysis of Psychotropic Drugs in Serum Using "GC-MS Forensic Toxicological Database"
e. Analysis of Opioids Using Isotope Dilution with GCMS-TQ8030 GC-MS/MS
f. Analysis of Toxicological Substances in Whole Blood Using Smart Forensic Database
g. Analysis of Volatile Toxic Substances Using Headspace GC/MS Part.1 - Paint Thinner and Alcohol
h. Analysis of Volatile Toxic Substances Using Headspace GC/MS Part.2 - Cyanide and Azide
i. Analysis of a Benzodiazepine-Based Drug Using GC-MS
j. Comprehensive Detection and Structural Elucidation of Synthetic Cathinones Using GC-MS/MS

6. CLAM-2000 (Automated Sample Preparation)

a. A Novel Platform of On-Line Sample Pre-Treatment and LC-MS/MS Analysis for Screening and Quantitation of Illicit Drugs of Abuse
b. Fully Automated Sample Preparation and LCMS Analysis of Drugs in Oral Fluid

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Application News

No. AD-0121Ph

Quantitative Bioanalysis / AA-7000G

Direct Determination of Pb in Whole Blood by Graphite Furnace Atomic Absorption Spectrophotometry (GF-AAS)

Chua, Modesto[1], Parreñas, Thelma[1], Kierulf, Arkaye[1], Ignacio, Austin[1], Argamino, Cris Ryan[2], Castaños, Sara Jane[2] and Torres, Arturo[2]

❑ Introduction

Lead (Pb) is a widely used, cumulative heavy metal which affects numerous body systems, including neurological, hematological, gastrointestinal, cardiovascular and renal [1]. At present, ten (10) µg/dL (micrograms /deciliter) or 100 ppb (parts per billion) was adopted by the Centre for Disease Control & Prevention (CDC) in 1991 as an advisory level for environmental and educational intervention [2]. Detection of Pb in blood can be performed using several methods including atomic absorption spectrophotometry (AAS), anodic stripping voltammetry (ASV) and inductively coupled plasma mass spectrometry (ICP-MS). The method of choice will rely on various aspects including analytical capability (i.e. LOD, LOQ), costs, and technical requirements (i.e. sample preparation) [3]. This application news will report on the analysis of Pb in whole blood using the Shimadzu AA-7000G (Graphite Furnace Atomizer) and the Platform-type Graphite Tube. The method presented is a quick way to determine Pb in whole blood as the sample was mixed with a matrix modifier solution prior to GF-AAS analysis.

❑ Experimental

Preparation of Matrix Modifier, Standards and Samples

In a 100 mL volumetric flask, 5 mL of 10% Triton X-100, 2 mL of NH_4PO_4 and 4 drops of 70% HNO_3 acid were mixed and diluted to volume with deionized water to form the matrix modifier. To prepare a multi-point calibration curve, 0, 50, 100, 300, and 600 ppb Pb working standard solutions were prepared in 1% HNO_3. The final standard solutions were prepared by mixing 100 µL each of the working standard solution and 900 µL of matrix modifier in the autosampler vessels to produce 0, 5, 10, 30, and 60 ppb. These standard solutions were set aside until the bubbles were dissipated.

The samples were prepared by mixing 100 µL of whole blood (with anti-coagulant) with 900 µL matrix modifier. On the other hand, the spiked recovery samples were prepared by mixing 100 µL blood sample, 100 µL working standard solution and 800 µL matrix modifier.

Table 1: Instrument and analytical conditions

AAS	AA-7000 with GFA-7000
Lamp current	10 mA
Wavelength	283.3 nm
Slit width	0.7
Measurement mode	BGC-D2
Graphite tube	Platform type
Autosampler	ASC-7000
Injection volume	10 µL
Workstation	WizAArd version 5.02

Table 2: Temperature Program

Pb

	Temp.	Time (sec)	Heat Mode	Sensitivity	Gas Type	Flow Rate
1	60	3	RAMP	☐	#1	0.10
2	120	20	RAMP	☐	#1	0.10
3	250	10	RAMP	☐	#1	0.10
4	700	10	RAMP	☐	#1	1.00
5	700	10	STEP	☐	#1	1.00
6	700	3	STEP	☑	#1	0.00
7	2000	3	STEP	☑	#1	0.00
8	2500	2	STEP	☐	#1	1.00

Atomization Stage: Step 7

❑ Results and Discussion

Figure 1 shows the Pb calibration curve whereas Figure 2 shows the peak profiles of the standard solutions.

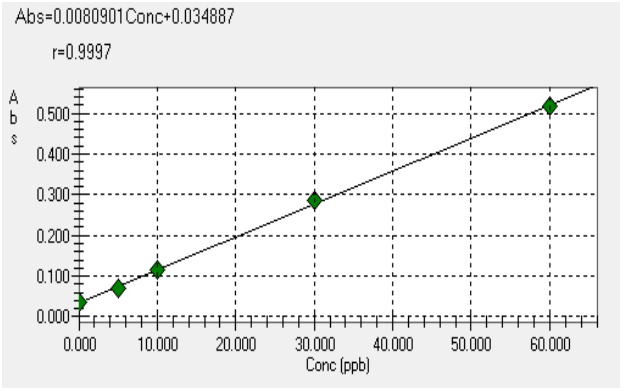

Figure 1: Pb calibration curve

[1]Philippine Institute of Pure & Applied Chemistry, [2]Shimadzu Philippines Corporation

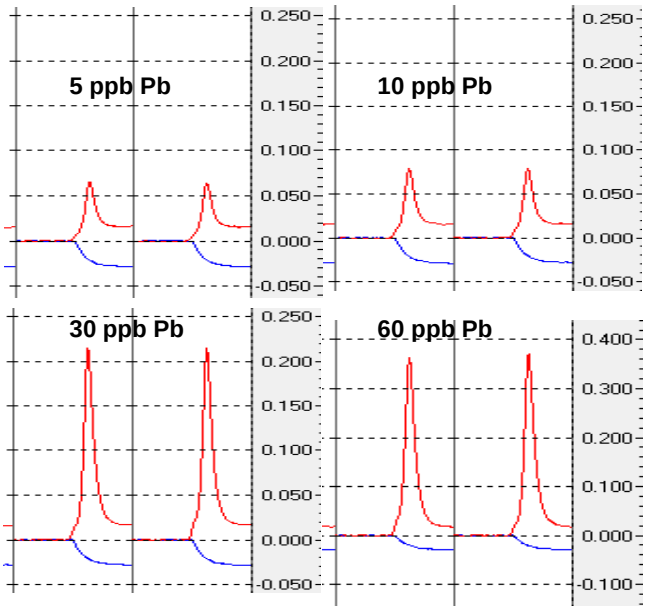

Figure 2: Calibration Standard Peak Profiles

Good percentage recoveries (% R) were obtained for the spiked samples as shown in Table 3.

Table 3: Spiked percentage recovery

Sample + Pb Spike	% R	% RSD
Whole Blood +10 ppb Pb	100.06	5.63
Whole Blood + 30 ppb Pb	109.10	5.71
Whole Blood + 50 ppb Pb	99.65	3.59

The method uses platform-type tube, which allows Pb to be atomized only when the inside of the tube has reached atomization temperature. In addition, the ammonium phosphate-Triton X-100 matrix modifier stabilizes Pb atoms during pyrolysis (>600ºC) and also breaks-up and increase the volatility of the interfering sample matrix.

The platform-type tube, also known as an L'vov platform, is used with integrated peak areas for absorbance measurements [3] through the WizAArd workstation. Figure 3 compares the peak profiles of the sample (whole blood) as well as the same sample spiked with 30 ppb Pb.

The use of the combined approaches above decreases the effect of interference from the sample matrix, which can be verified from the good spiked percentage recovery values as shown in Table 3.

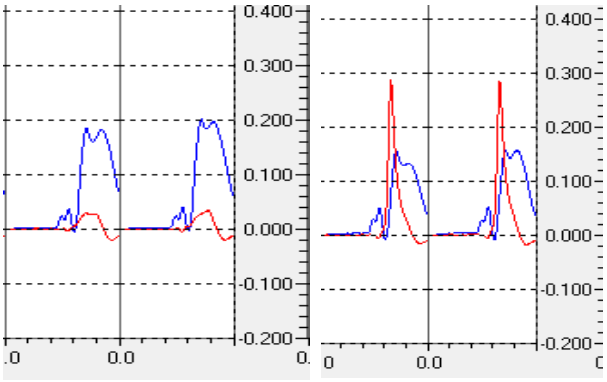

Figure 3: Peak profiles of whole blood (left) and whole blood spiked with 30 ppb Pb (right)

❑ Conclusions

Direct determination of Pb in whole blood using the platform-type graphite tube together with a matrix modifier can be performed accurately on Shimadzu AA-7000 with GFA-7000. This combination provides for an effective way to analyze a volatile element in a complex biological sample matrix. The graphite tube design allows sample atomization to take place under a more uniform temperature condition, whereas the matrix modifier releases and prevents loss of the target element while minimizing matrix interference. The standard solutions used, which were below the CDC Blood Lead Level limit of 10 μg/dL (100 ppb), showed good linearity for the calibration curve (r=0.9997). The good spiked recovery results (99-109%) also provided additional evidence of the instrument and method's suitability for the effective analysis of Pb in whole blood sample.

❑ References

[1] Brief guide to analytical methods for measuring lead in blood. World Health Organization. 2011. (http://www.who.int/ipcs/assessment/public_health/lead_blood.pdf).
[2] Lead Toxicity: What Are the U.S. Standards for Lead Levels? (http://www.atsdr.cdc.gov/csem).
[3] The Lead Laboratory (http://www.cdc.gov/nceh/lead/publications/ 1997/pdf/c1.pdf).

Disclaimer: The Shimadzu AA-7000G system and the data in this Application News are intended for Research Use Only (RUO). Not for use in diagnostic procedures.

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C113-E022

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Accelerating Reliable Performance

Shimadzu ICPMS-2030 Inductively Coupled Plasma Mass Spectrometer

Quantitation of Trace Elements in Blood Using Shimadzu ICPMS-2030 Inductively Coupled Plasma Mass Spectrometer

- A newly developed collision cell provides high sensitivity and low interference.
- A unique system developed by Shimadzu results in the industry's lowest running costs*.

*As of February 2016, based data obtained by Shimadzu

Quantitation of Trace Elements in Blood Using the ICPMS-2030

The quantitation of toxic metals in biological samples such as blood and urine is necessary for assessing the exposure of humans and other animals to such metals through the natural environment, including consumption of metal-laden foods and drinks. An ICP-MS system is able to quickly measure trace quantities of toxic metals with high sensitivity. The quantitation of trace elements in blood performed with the ICPMS-2030 is shown in this report.

Sample

Blood Sample: Seronorm Trace Elements Whole Blood L-1

Sample Pretreatment and Measurement Procedures

About 50 µL of sample was put in a 7 mL TFM insert. 0.5 mL of concentrated nitric acid was added to the TFM insert. Then the sample was decomposed using ETHOS-TC microwave oven digestion system (Milestone). After the decomposition, pure water was added to the TFM insert to make 5 mL of the sample solution. XSTC-622 (SPEX) and a 1000 µg/mL mercury solution (Wako) were diluted with 0.14 M nitric acid solution to make the standard samples for obtaining the calibration curves. The concentrations of As, Cd, Pb, Mn, Hg and Se in the sample solution were determined by the calibration curve method (internal standard method). The measurement results are shown in table 1 and are less than the analytical values. Figure 1 shows the calibration curves of As, Cd and Pb.

System Configuration

▶ ICPMS-2030

▶ AS-10 Autosampler

Measurement Conditions

RF Power	1.2 kW
Plasma Gas Flow Rate	8.0 L/min
Aux. Gas Flow Rate	1.10 L/min
Carrier Gas Flow Rate	0.70 L/min
Sample Introduction	Nebulizer 07
Spray Chamber	Electronically-cooled cyclonic
Torch	Shimadzu mini-torch
Collision Gas	He

Table 1 Measurement Results for Trace Elements in Blood

Measured Element	As	Cd	Pb	Mn	Hg	Se
Mass Number	75	111	208	55	202	78
Analytical Value (µg/L)	2.4	0.36	10.2	20.7	1.50	59
Analytical Uncertainty (µg/L)	0.5	0.02	2.1	4.2	0.30	12
Quantitation Value (µg/L)	2.2	0.35	8.7	20.2	1.5	68

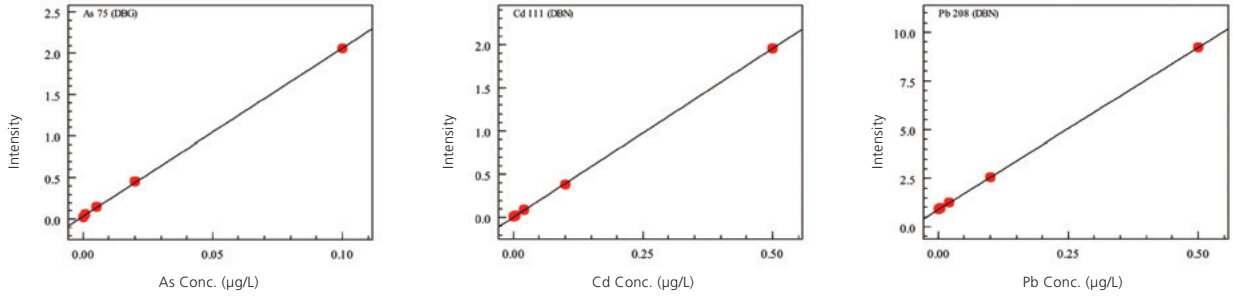

Fig. 1 Calibration Curves for As, Cd and Pb

Note: This analytical system may be used for research applications, and may not be used for clinical diagnosis.

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Application News

No. SCA_125_011

EDX

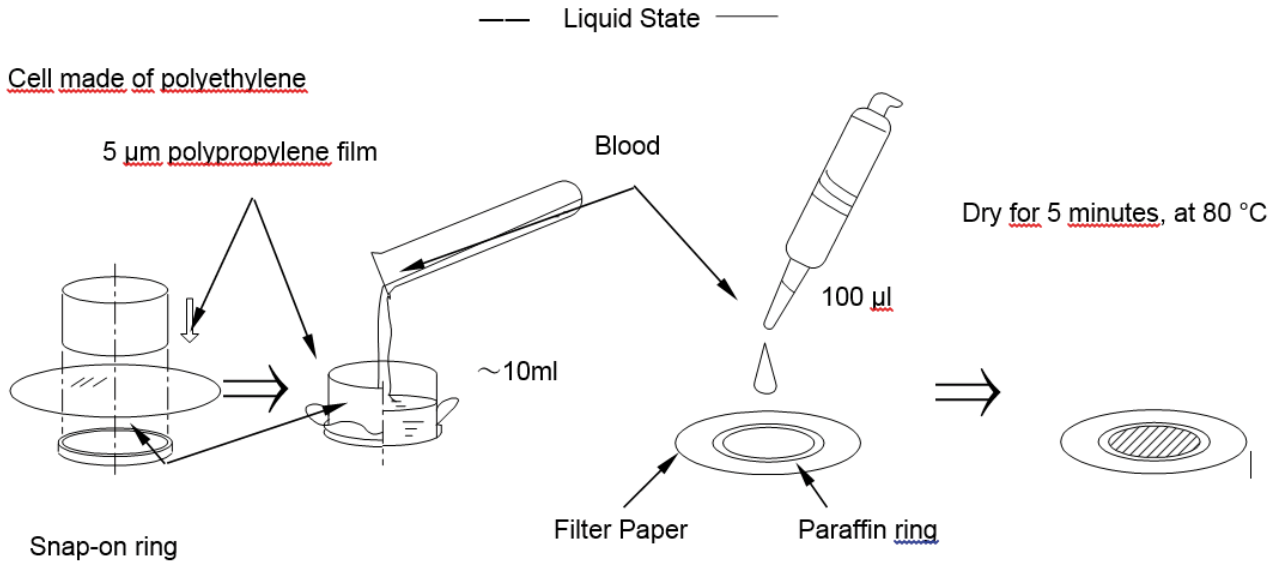
EDXRF Analysis of Cd, Hg, and Pb in Blood

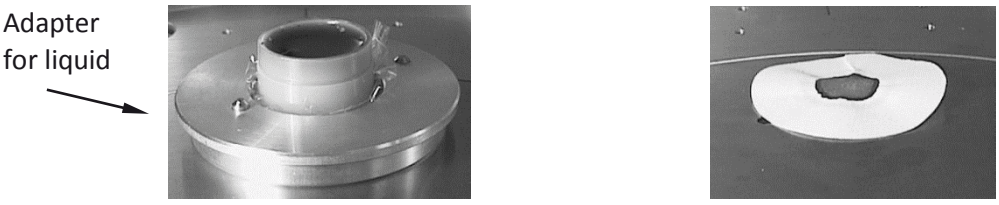
There is a need nowadays for the rapid estimation of the various type of poisons in medicine that have combined with metals in order to be able to give emergency treatment through the administering of an antidote. EDX-700/800 can identify various types of metals after a simple preparation of the sample.
Furthermore, the testing is non-destructive so the sample can be analyzed with a different analyzer after testing. Shown below are examples of qualitative analysis with the sample in liquid form and with the sample dried on filter paper.

Sample Preparation

1) Liquid Cell Method
Approximately 10 ml of the sample was poured into the liquid sample receptacle sealed with 5 µm polypropylene film.

2) Filter Method
After dropping 100 µl of the sample onto filter paper, the paper was dried for 5 minutes at 80 °C



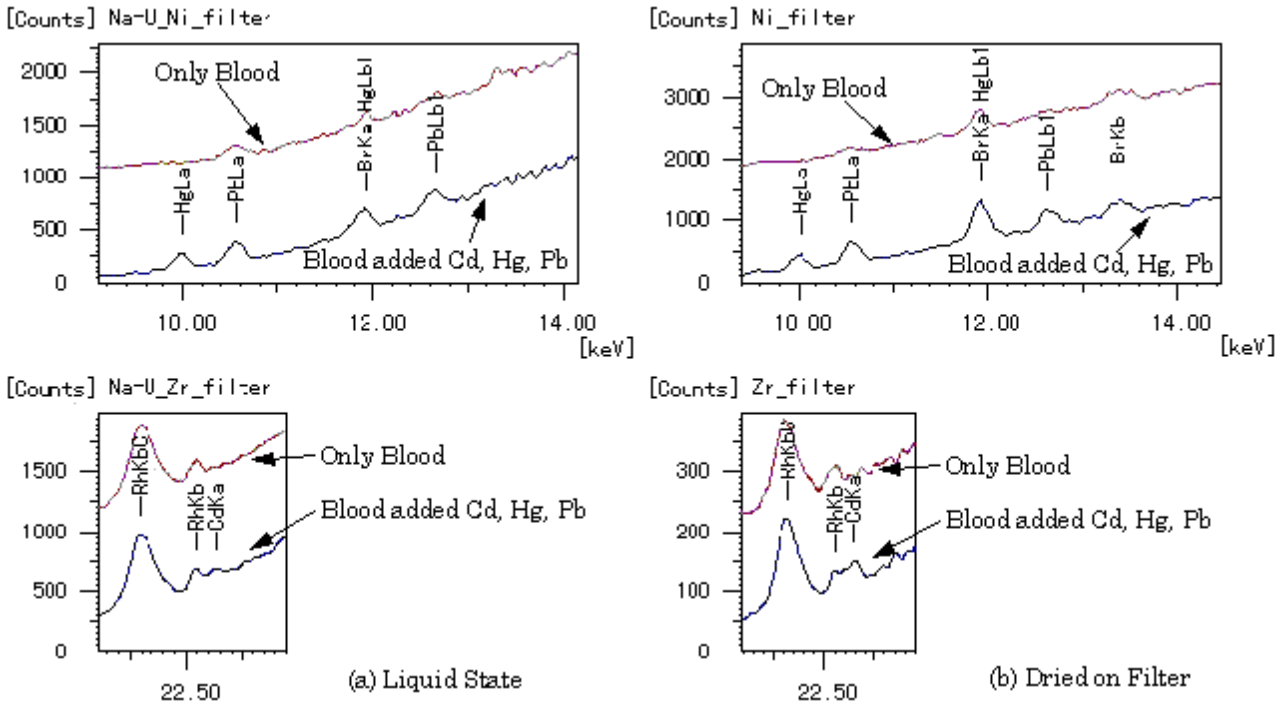


Sample loading (2) Filter method Sample loading

After dropping 100 µl of the sample onto filter paper, the paper was dried for 5 minutes at 80 °C.

Result of the Qualitative Analysis of Blood

The sample of blood and the sample with 10 ppm of Cd, Hg, and Pb were qualitatively analyzed with the liquid cell method and the filter method. The comparative results are shown on Fig. 1 In addition the difference in profile between the blood and that of the sample with the added Cd, Hg, and Pb is compared.



Only Blood" Profile has offset (Backgrounds are same between "Only Blood" and "Blood added Cd, Hg, Pb ")

Fig.1 Qualitative Analysis of Cd, Hg, Pb 10 ppm in Blood

Lower Limit of Detection(L.L.D)

The lower limit of detection of Cd, Hg, and Pb in blood calculated from the results of the qualitative analysis is shown in Table 1.

Element	Liquid State	Dried on Filter
Cd	9.9 ppm	8.0 ppm
Hg	3.4 ppm	1.9 ppm
Pb	2.2 ppm	1.6 ppm

Table 1 L.L.D of Cd, Hg, Pb in Blood

Analytical Conditions

Instrument: EDX-700
X-ray Tube: Rh target
Filter: Ni, Zr
Voltage - Current: 50kV-24-500 μA (Auto)
Atmosphere: Air
Measurement Diameter: 10 mm
Measuring Time: 1000 sec

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Application News

No. SCA_125_012

EDX

EDXRF Analysis of P, Cl, K and Ca in Blood

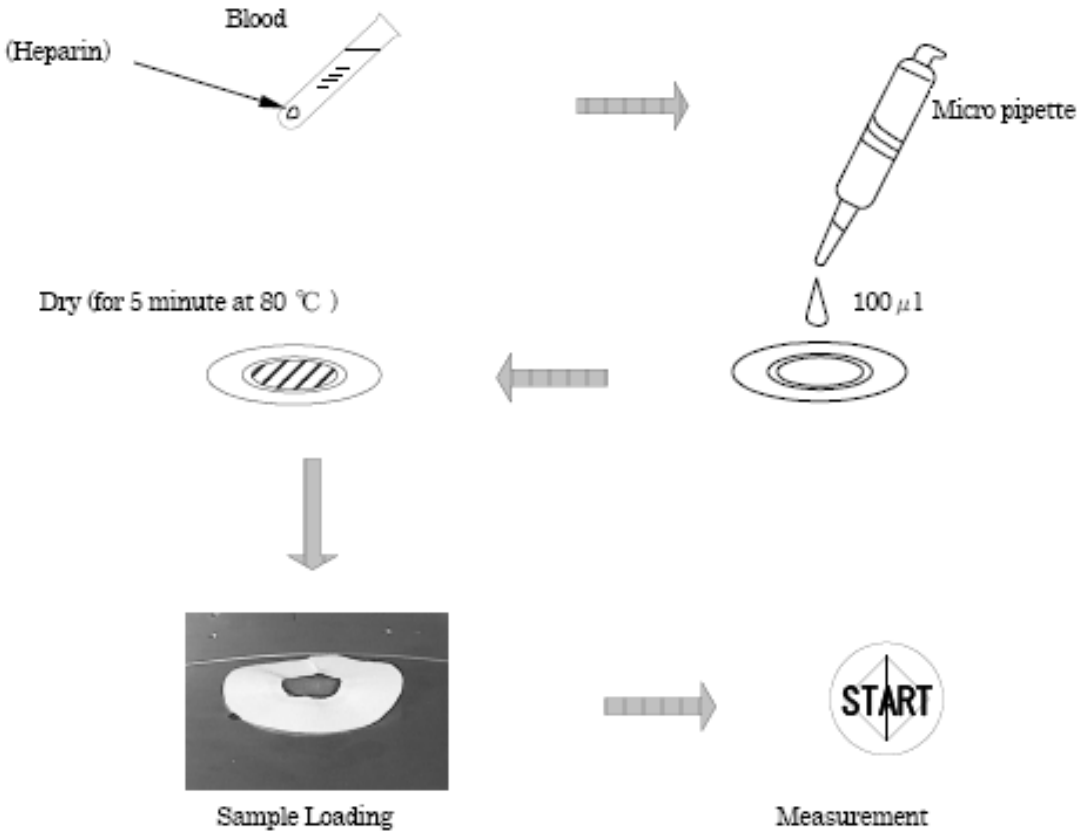
EDX is effective for quick screening analysis of tests for electrolytes (such as P, Cl, K, Ca) in blood. Carrying out the analysis in air with these light elements results in the X-ray fluorescence from the sample being absorbed by the air, consequently lowering the sensitivity. The analysis therefore needs to be carried out either in helium or a vacuum. Though it is impossible to directly analyze a liquid such as blood in a vacuum, the liquid can be dropped onto filter paper, dried, and then analyzed in a vacuum. Shown below is an example of such a qualitative analysis.

Sample

Blood to which the anticoagulant Heparin has been added (Heparin is unnecessary if the sample is prepared as shown below).

Sample Preparation

After 100 µl of the sample has dropped onto the filter paper, the paper is dried for 5 minutes at 80 °C (If heparin is not added the paper is dried at normal temperature). This is described in the diagrams below.



The Result of the Qualitative Analysis of Electrolytes in Blood

The Results of the qualitative analysis of electrolytes such as P, Cl, K, and Ca present in the blood is shown in Fig.1. P-Ca have been detected (S has been detected as well, but it is assumed that this is from the Heparin). The reference values of these constituent elements for an adult male are shown in Table 1.

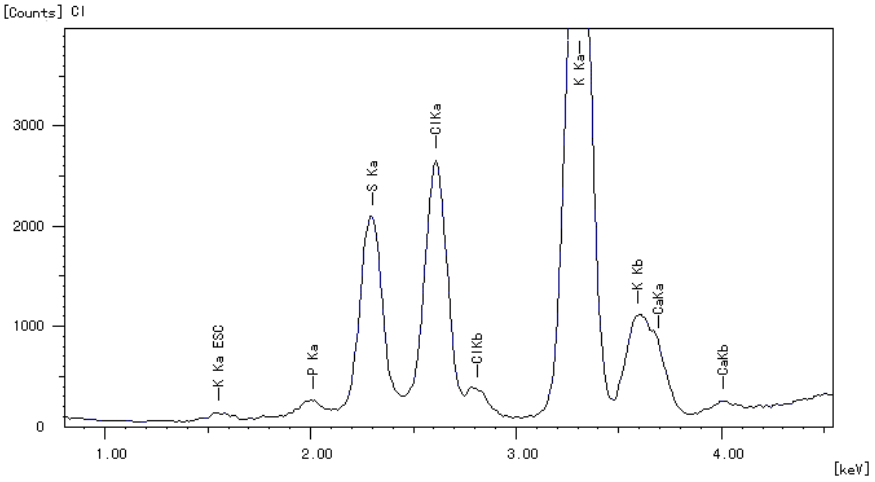


Fig.1 Qualitative Analysis of Electrolytes in Blood

P (Inorganic)	Cl	K	Ca
2.6-4.4 mg/dl (26-44 ppm)	97.8-102.6 mEq/l (0.35-0.36 %)	3.5-4.8 mEq/l (137-187 ppm)	8.6-10.4 mg/dl (86-104 ppm)

Table 1 Reference of Electrolyte in Blood[1)]

Comparison of the Atmosphere in which Analysis was Carried Out

The various sensitivities and methods are compared and summarized in Table 2 for the liquid and filter methods in air, vacuum and helium.

	Air	Vacuum	He Atmosphere
Filter Paper	● Simple, fast, drying not necessary	● Drying necessary	● Drying not necessary
Liquid	● Simple, fast, feasible	× Not possible	● Possible
Sensitivity	● Reduces with elements lighter than Ti	● Good	● Good

Table 1 Reference of Electrolyte in Blood[1)]

Analytical Conditions

Instrument: EDX-700
X-ray Tube: Rh target
Filter: Al
Voltage - Current: 15kV-1000 µA (Auto)
Atmosphere: Vacuum
Measurement Diameter: 10 mm
Measuring Time: 500 sec
Dead Time: 21 %

Reference
Extensive Blood/Urine Chemistry Tests, Immunological Tests – How to Interpret the Values – (First Volume)
Nihon Rin-rin Special Autumn Issue 1985, Nihon Rin-rin sha.

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Application News

No. GC-006

Gas Chromatography

Dual Channel Blood Alcohol Content (BAC) Analysis

■ Introduction

The analysis of ethanol (alcohol) concentration in blood is routinely carried out in forensic labs. The generally accepted method to accurately determine blood alcohol content (BAC) utilizes static headspace sampling and dual column separation by gas chromatography followed by flame ionization detection (GC/FID). Typically, this test is carried out isothermally at 40 °C. High linear velocity of carrier gas coupled with a high split ratio is commonly employed to achieve a short analysis time, but this translates into huge consumption of helium carrier gas. For example, a typical analysis with two 0.32 mm ID columns operating at the linear velocity of 80 cm/sec each and a split ratio of 30:1 consumes over 200 mL of helium carrier gas per minute! Furthermore, the column flow rate (7 mL/min) is well outside of the optimal range for capillary columns (1-3 mL/min) and significant peak tailing is observed under these conditions.

Using the Shimadzu GC-2010 Plus and HS-10 headspace sampler, this application note demonstrates that by moderately increasing column temperature to 50°C and using the optimal flow rate for capillary columns, BAC analysis can be completed in less than 3.5 min without sacrificing peak shapes. More importantly, helium carrier gas consumption was reduced to less than 80 mL/min at the same split ratio (30:1). In addition, good resolution and linearity as well as excellent reproducibility of ethanol concentration measurements have been observed with this set-up.

■ Standards and Sample Preparation

Blood alcohol resolution standard n-P was purchased from Restek, #36010. Ethanol standards (0.010, 0.040, 0.10, 0.40 and 1.00 g/dL ethanol) were prepared by serial dilution from 200 proof ethanol with deionized water to specified concentrations. An internal standard (IS) of 0.020 g/dL *n*-propanol was prepared by diluting *n*-propanol (Sigma, 34817) with deionized water. Aliquots for analyses were prepared by mixing 50 µL of sample with 500 µL of IS solution in 20 mL headspace vials (Shimadzu, 220-94796-01) and sealed with screw caps with PTFE/silicone septa (Shimadzu, 220-94796-02). Deionized water was used as the blank solution.

■ Instrumentation

A Shimadzu GC-2010 Plus equipped with an advanced flow controller (AFC), a split/splitless injector (SPL) and two Flame Ionization Detectors (FID) was used for this study. An HS-10 static headspace sampler with heated transfer line was used for sample preparation and introduction into the GC through the SPL equipped with a 2-way capillary column adaptor (Shimadzu, 221-56222-91). Effluent from HS-10 was split between two columns (SH-Rtx-BAC Plus1, 0.32 mm × 30 m × 1.8 µm, 227-36260-01 and SH-Rtx-BAC Plus2, 0.32 mm × 30 m × 0.6 µm, 227-36260-01). Each column was connected to a separate FID and analyzed simultaneously (Figure 1).

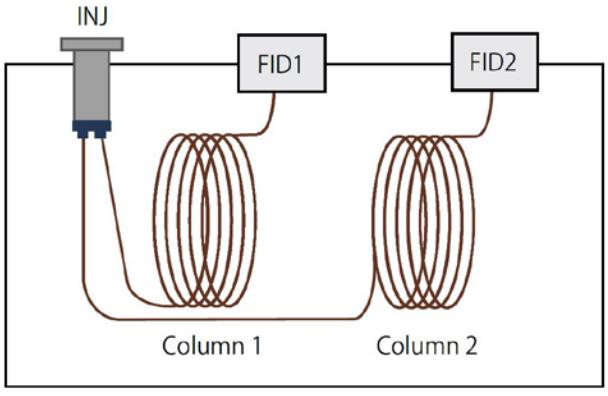


Figure 1: Schematic drawing of the dual column configuration.

■ Analytical Conditions

GC-2010 Plus

- SPL Temp = 150 °C
- Column Temp = 50 °C isothermal (unless specified otherwise in text)
- FID Temp = 250 °C, H_2 flow = 40 mL/min, Air flow = 400 mL/min, Makeup flow = 25 mL/min.
- Carrier gas: Helium
- Flow control mode: constant linear velocity @ 40 cm/sec (unless specified otherwise in text)
- Column flow = 2.5 mL/min
- Split ratio = 30
- Purge flow = 1 mL/min
- Injection volume: 1 mL headspace
- GC run time: 3.5 min

HS-10

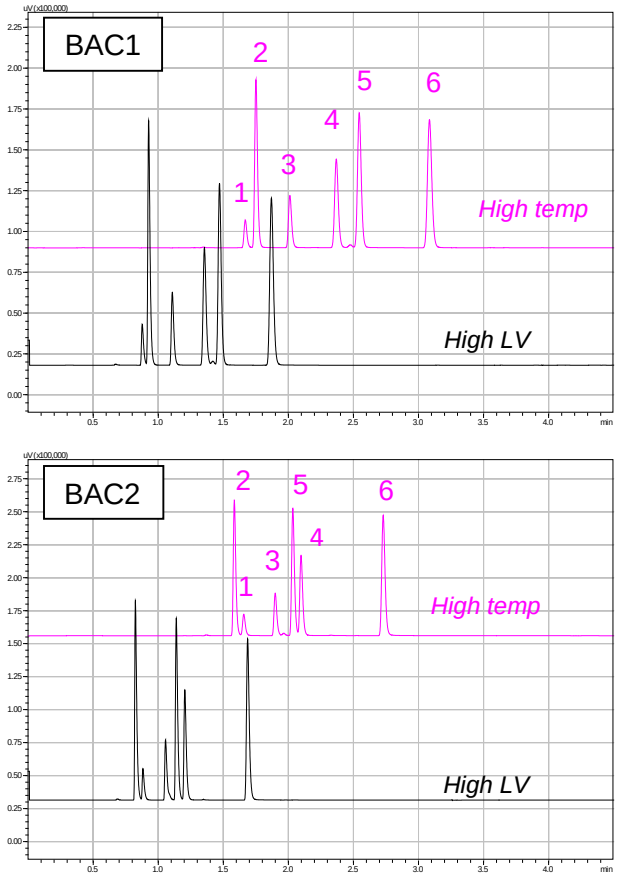
- Vial equilibration: 15 min @ 80 °C
- Sample Pathway Temp = 95 °C
- Transfer Line Temp = 105 °C
- Vial Pressurization: 1.00 min @75 kPa
- Loop Load Time = 0.50 min
- Injection time = 1.00 min

■ Results

Resolution of components in BAC mixture

The headspace sample of a BAC resolution standard was split between SH-Rtx-BAC Plus1 and SH-Rtx-BAC Plus2 column in a nominal 1:1 ratio by using a 2-way capillary column adaptor at the end of the injection port. Compounds eluted from both columns were analyzed simultaneously by two FIDs (Figure 1). The system was operating at the linear velocity of 40 cm/sec so that the column flow rate was within the optimal column flow rates for capillary columns (1-3 mL/min). Column temperature was raised from the standard 40°C to 50°C to shorten the analysis time.

As shown in Figure 2, all six components in the mixture were well resolved and eluted in less than 3.5 minutes using this method. For comparison, the BAC resolution standard was also assayed using the standard high linear velocity (High LV) method. Although shorter analysis was achieved using the high LV method (under 2 min), much better peak shape and overall better resolution were obtained by using the high temp method (Table 1).



1. Methanol
2. Acetaldehyde
3. Ethanol
4. 2-propanol
5. Acetone
6. *n*-Propanol (internal standard)

Figure 2: Analysis of BAC resolution standard on SH-Rtx-BAC Plus1 and SH-Rtx-BAC Plus2 using high temperature method (*High temp,* linear velocity = 40 cm/sec, column temp = 50 °C) or high linear velocity method (*High LV,* linear velocity = 80 cm/sec, column temp = 40 °C). Note that the elution order is the same for either method on the same column, but the elution order is different for SH-Rtx-BAC Plus1 and SH-Rtx-BAC Plus2 columns.

BAC1 analytical line

Peak#	Name	Tailing Factor		Resolution	
		High LV	*High temp*	*High LV*	*High temp*
1	Methanol	1.682	1.277	--	--
2	Acetaldehyde	1.518	1.285	1.625	1.999
3	**Ethanol**	**1.441**	**1.261**	**5.482**	**6.078**
4	2-Propanol	1.294	1.200	6.269	7.273
5	Acetone	1.265	1.178	2.669	3.328
6	*n*-Propanol	1.231	1.172	8.336	9.335

BAC2 analytical line

Peak#	Name	Tailing Factor		Resolution	
		High LV	*High temp*	*High LV*	*High temp*
1	Methanol	1.740	1.243	2.052	1.928
2	Acetaldehyde	1.772	1.372	--	--
3	**Ethanol**	**2.088**	**1.364**	**5.913**	**6.110**
4	2-Propanol	1.563	*n.d.*	2.060	1.560
5	Acetone	1.619	1.337	2.680	3.396
6	*n*-Propanol	1.390	1.265	13.585	14.272

Table 1: Comparison of peak shape and resolution of components in BAC resolution standard assayed under different GC conditions. Tailing factor S is calculated as the following: $S = \frac{W_{0.05}}{2 \times a_{0.05}}$ ($W_{0.05}$ is the peak width at 5% peak height and $a_{0.05}$ is the width of the front half of the peak at 5% peak height). Thus a symmetric peak has a tailing factor of 1.

Calibration

Ethanol standards of concentrations from 0.01 to 1 g/dL (0.01-1%) were assayed to generate the calibration curves. A blank sample was run after the highest calibration standard (1% ethanol) to address potential carryover issue. As shown below, no carryover was detected (Figure 3). Calibration curves with excellent linearity spanning two orders of magnitude in concentration were obtained for both analytical lines (Figure 4).

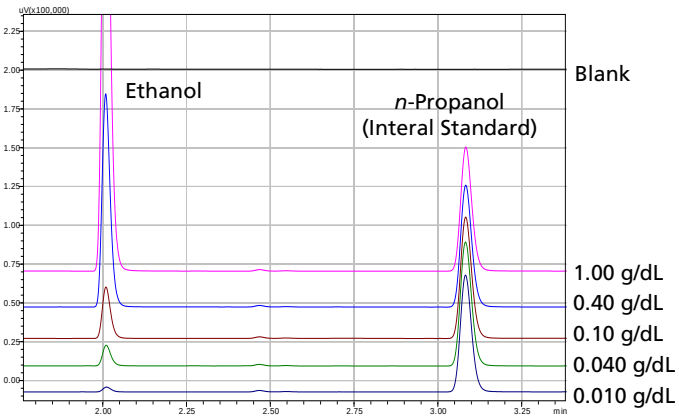


Figure 3: Chromatograms of calibration standards and a blank from BAC1 analytical line. Similar results were obtained from BAC2 analytical line.

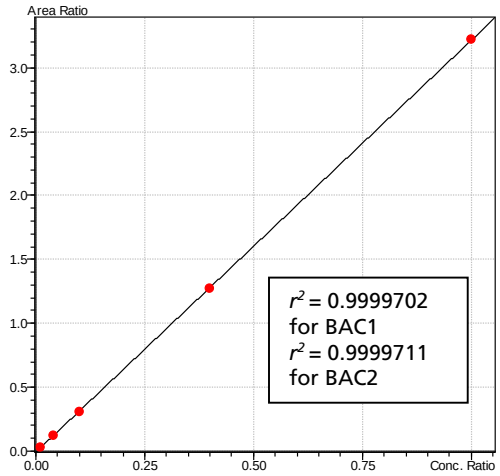


Figure 4: Five-point calibration curve for ethanol from BAC1 analytical line. Each standard (0.010, 0.040, 0.10, 0.40 and 1.00 g/dL) was run in duplicates. Internal standard quantification method was used. Similar results were obtained from BAC2 analytical line. Correlation coefficient (r^2 values) are shown in the inset for both analytical lines.

Reproducibility

30 samples of 0.080 g/dL (0.080%) ethanol control standard were assayed and the internal standard quantification method was used to determine the ethanol concentration. As shown in Table 2, the average (mean) ethanol concentration obtained is 0.0798 g/dL from BAC1 analytical line and 0.0794 g/dL from BAC2 analytical line. And the relative standard deviation (*RSD or coefficient of variation*) is 0.736 % for BAC1 analytical line and 0.983 % for BAC2 analytical line, demonstrating excellent accuracy and repeatability in both cases.

	BAC1	BAC2
AVERAGE RETENTION TIMES (MIN)	2.012	1.899
% RSD (% CV) FOR RET. TIME	0.061	0.072
AVERAGE ETHANOL CONC. (G/DL)	0.0798	0.0794
% RSD (% CV) FOR CONC.	0.736	0.983
STANDARD DEVIATION FOR CONC. (G/DL)	0.000587	0.000780

Table 2: Statistical results for ethanol control standard (*n=30*).

■ Conclusions

In this study, an improved BAC analysis was carried out using the Shimadzu HS-10 static headspace sampler and GC-2010 Plus gas chromatograph. By reducing the linear velocity and increasing column oven temperature to 50 °C, the GC separation was completed in less than 3.5 minutes. In addition, superior peak symmetry and compound resolution as well as excellent linearity and reproducibility of ethanol concentration measurements were obtained for both analytical lines.

Moreover, the amount of carrier gas required was much reduced from the commonly employed high linear velocity method. The gas saver function of the GC-2010 Plus can be turned on to further reduce helium consumption during GC idling time.

First Edition: December 2016

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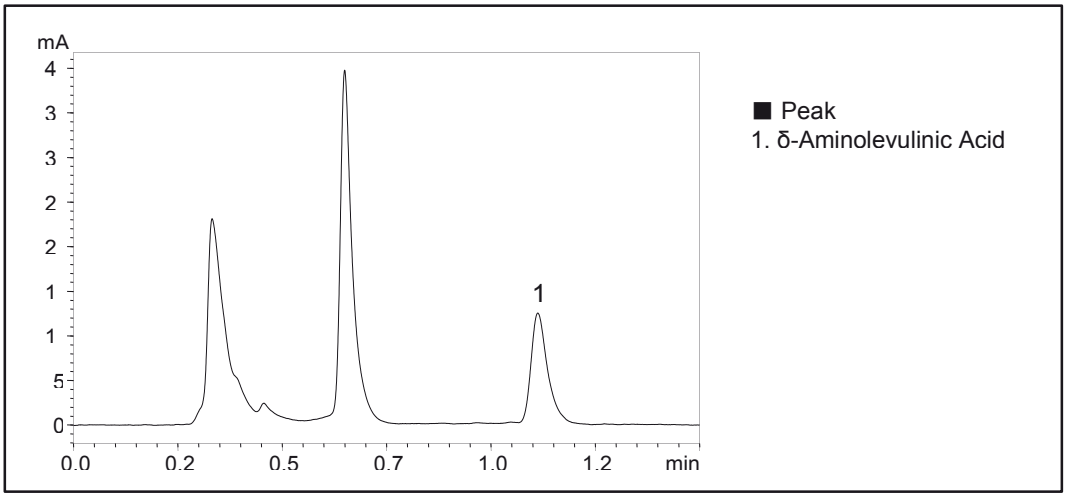
Application News

HPLC

High Speed Analysis of Delta-Aminolevulinic Acid

No. SCA-190-014

Delta-aminolevulinic acid in urine is known as an effective marker of lead (Pb) exposure. This chromatogram shows results of a high speed separation of delta-aminolevulinic acid in urine by Prominence UFLC.



Analysis of Delta-Aminolevulinic Acid in Urine [Note]

[Sample Preparation]

1) 0.45 mL of 8.5% formaldehyde was added to a 20 µL urine sample.
2) 3.5mL of a mixed solution of acetylacetone, ethanol, and distilled water was added to the solution from step 1.
3) After heating the solution for 15 minutes in boiling water then cooling it in a water bath, the solution was injected into the HPLC system.

Note: This data was provided by BML Inc.

Instrument	:	Prominence UFLC system
Column	:	Shim-pack XR-ODS (50 mm × 3.0 mm *i.d.*)
Mobile Phase	:	A) Water, B) Methanol, C) Acetic acidA / B / C = 53 / 45 / 2 (v / v / v)
Flow Rate	:	0.8mL/min
Column Temperature	:	40 °C
Detection	:	Fluorecence (Ex:363nm , Em:473nm) with Semi-micro flow cell
Sample Volume	:	5 µL

Note: The indicated data was not acquired using a system registered by Japanese Pharmaceutical Affairs Act.

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Application News

HPLC

High Speed with High Resolution Analysis (Part 37) Analysis of Clobazam and Cibenzoline in Serum by the Nexera UHPLC System

No. SCA-190-012

HPLC is an important technique used for the analysis of drugs in the blood. However, faster analysis is often required to improve sample throughput and productivity at sites handling many specimens.
Here, we introduce an example of ultra-high-speed analysis of clobazam and cibenzoline in serum using the Nexera UHPLC (Ultra High Performance Liquid Chromatography) system and the Shim-pack XR-ODS III high-speed separation column (particle size: 1.6 µm).

Analysis of Clobazam

Clobazam is a benzodiazepine type of antiepilepsy drug. N-desmethylclobazam, one of the substances formed when clobazam is metabolized in the body, displays activity similar to that of clobazam. Fig. 1 shows the structures of these substances.
A serum sample was analyzed after cleanup by liquid-liquid extraction. Analysis was conducted using 2 different columns, the Shim-pack VP-ODS (particle size: 4.6 µm) for conventional analysis, and the Shim-pack XR-ODS III (particle size: 1.6 µm) for ultra-high-speed analysis. Fig. 2 shows the chromatograms, and Table 1 shows the analytical conditions used.
Conducting ultra-high-speed analysis with the Shim-pack XR-ODS III allowed the analysis time to be shortened to about 1/12 that by conventional analysis. The system back pressure in this analysis was about 85 MPa (12,300 psi).

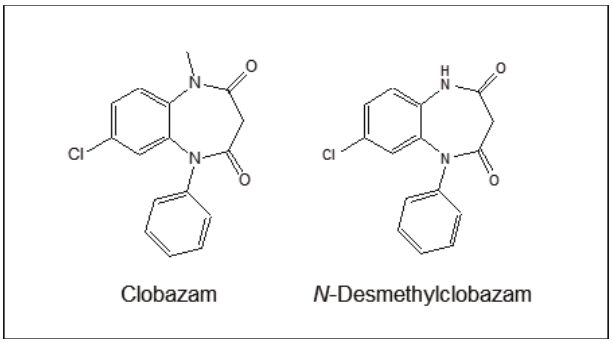

Fig. 1 Structures of Clobazam and N- Desmethyl-clobazam

Table 1: Analytical Conditions

Column	:	Shim-pack VP-ODS (150 mm L. × 4.6 mm I.D., 4.6 µm)
		Shim-pack XR-ODSⅢ (50 mm L. × 2.0 mm I.D., 1.6 µm)
Mobile Phase	:	10 mmol/L NaH_2PO_4 aq./ Acetonitrile = 2 / 1 (v / v)
Flow Rate	:	1.0 mL/min (VP-ODS)
		0.9 mL/min (XR-ODSⅢ)
Column Temp.	:	40 ℃
Injection Volume	:	50 µL (VP-ODS)
		10 µL (XR-ODSⅢ)
Detection	:	SPD-20AV at 230 nm
Flow Cell	:	Conventional Cell (VP-ODS)
		Semi-micro Cell (XR-ODSⅢ)

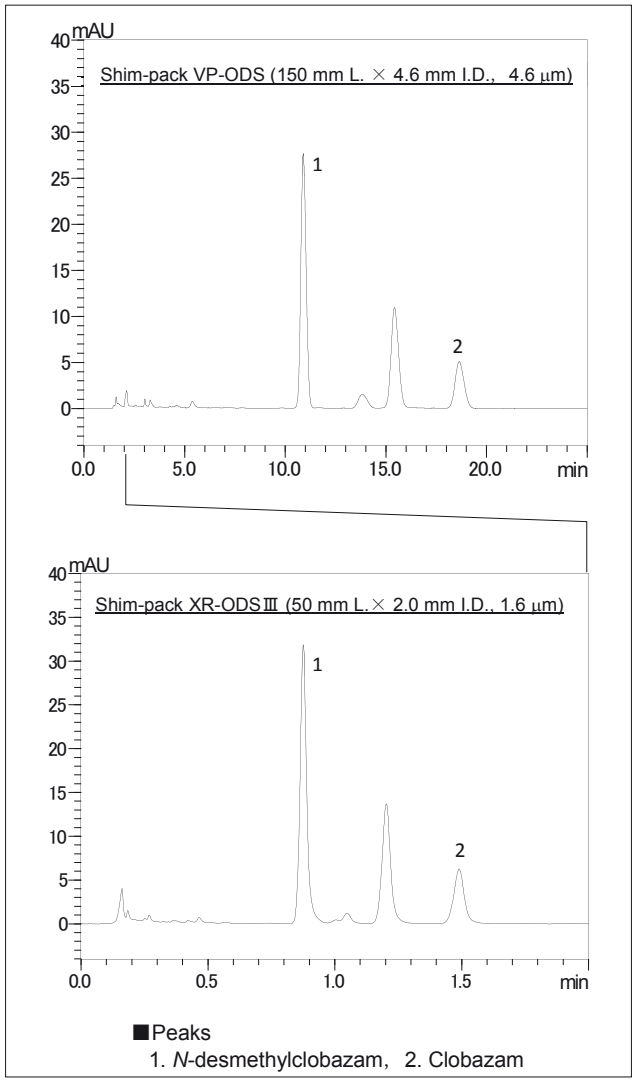

Fig. 2 Chromatograms of Clobazam and N-Desmethylclobazam in Serum Sample
(Upper: Shim-pack VP-ODS, Lower: Shim-pack XR-ODS III)

Analysis of Cibenzoline

Cibenzoline (Fig. 3) is a type of antiarrhythmic drug. A serum sample was analyzed using the Shim-pack XR-ODS III (particle size: 1.6 μm) after cleanup by liquid-liquid extraction. Fig. 4 shows the chromatogram, and Table 2 shows the analytical conditions used. Conducting analysis using these conditions allowed the analysis time to be shortened to about 1/10 that by conventional analysis, which took about 15 minutes. The system back pressure during the high-speed analysis was about 77 MPa (11,100 psi).

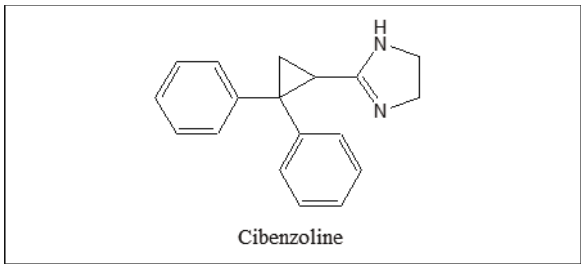

Fig. 3 Structures of Cibenzoline

Tab. 2 Analytical Conditions

Column	:	Shim-pack XR-ODSⅢ (50 mm L. × 2.0 mm I.D., 1.6 μm)
Mobile Phase	:	Phosphate buffer / Acetonitrile / Methanol = 20 / 5 / 4 (v / v / v
Flow Rate	:	0.7 mL/min
Column Temp.	:	40 ℃
Injection Volume	:	10 μL
Detection	:	SPD-20AV at 225 nm
Flow Cell	:	Semi-micro Cell

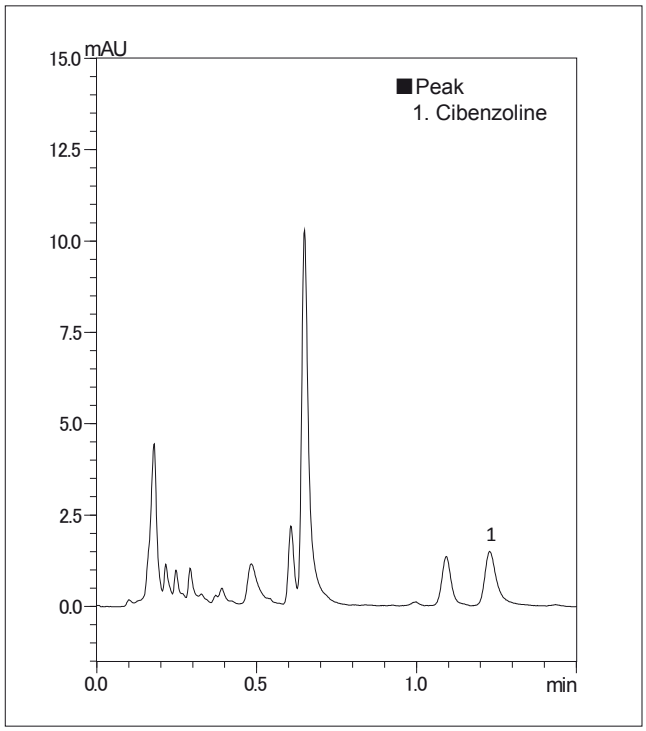

Fig. 4 Chromatogram of Cibenzoline in Serum Sample

Reducing the Total Analysis Time with Overlapping Injection

HPLC productivity is improved when the overall analysis time is shortened. This includes decreasing the run time through method condition changes like temperature and gradient profile, and also reducing the autosampler injection cycle time between samples. The Nexera SIL-30AC autosampler is equipped with an overlapping injection feature that, when enabled, loads the next sample while the current analysis is in progress.

This feature, combined with the world's fastest and cleanest injection performance, greatly shortens the overall analysis time.
Fig. 5 shows the results of overlapping 10 injections of the Cibenzoline sample from Fig. 4. Ten analyses were completed in 15 minutes.

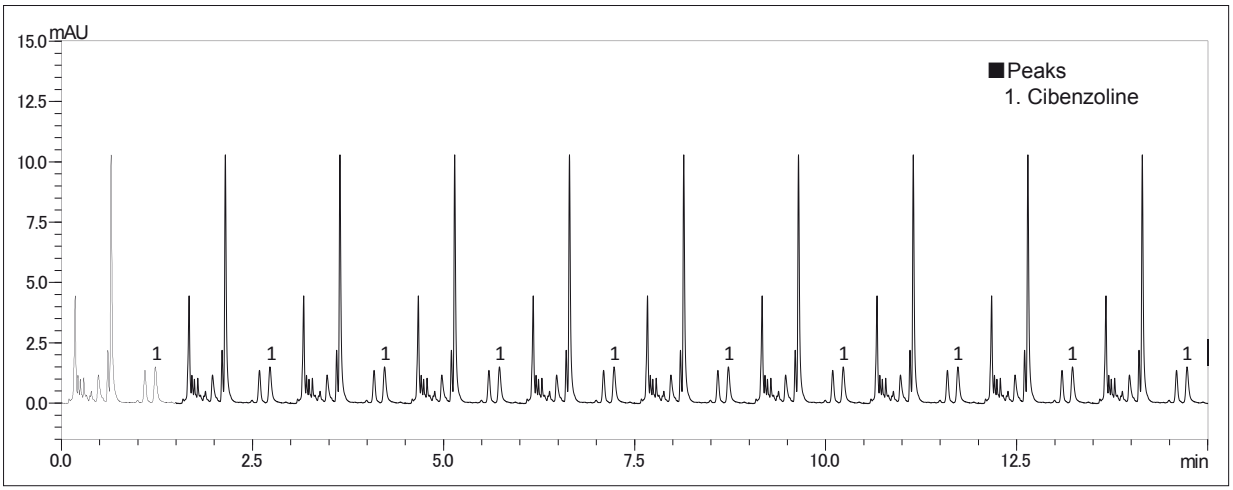

Fig. 5 Chromatograms of Cibenzoline in Serum Sample Using Overlapping Injection (10 Repetitions)

* The published data were acquired with a non-pharmaceutical compliant instrument.

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Application News

No. SCA_210_022

LCMS-8050

Analysis of Ethylglucuronide and Ethylsulfate in Urine, Plasma and Serum by LCMS-8050 using RECIPE ClinMass® LC-MS/MS Complete Kit MS8000

Dr. Johannes Engl (RECIPE Chemicals + Instruments GmbH), Anja Grüning (Shimadzu Europa GmbH)

■ Introduction

In many societies around the world alcohol abuse represents a serious social and economic problem. For alcohol misuse several parameters of laboratory diagnostics are available. They are used for the assessment of acute drinking, chronic abuse (alcoholics), abstinence control and relapse diagnosis.
Acute alcohol abuse, which dates back several hours, is mainly determined by ethanol in the respiratory air (alcohol breath test) and by the blood alcohol level (short-term marker). For the investigation of a long-term, chronic abuse the determination of CDT (Carbohydrate Deficient Transferrin) has been established for routine analysis.
Ethylglucuronide (EtG) and Ethylsulfate (EtS) are formed in the ethanol metabolism and therefore serve in addition to the short-term marker ethanol and the longterm marker CDT for the verification of alcohol abuse. They can be determined in urine in a time range up to 80 h after excessive consume of alcohol. Even in case of low to mid uptake of alcohol, Ethylglucuronide and Ethylsufate can be detected up to 24 h respectively 48 h.

■ Materials and methods

The LCMS-8050 triple quadrupole mass spectrometer was coupled to a Nexera X2 UHPLC system. EtG and EtS were measured using a commercially available test kit ClinMass® Complete Kit for Ethylglucuronide and Ethylsulfate in Urine, Plasma and Serum, MS8000, MS8100 (RECIPE Chemicals + Instruments GmbH, Dessauerstraße 3, 80992 München, Germany). Calibrators, control samples, analytical column and mobile phase solvents were provided by the kit. 50 µL of urine sample was added to 1000 µL of internal standard solution and mixed for 5 sec. 10 µL of the sample was analysed.
For analysis the [M-H]- ion was measured and used as the precursor ion (negative electrospray ionization).

■ Analytical conditions

UHPLC:	Nexera X2 UHPLC
	0.2 mL/min (0.0 - 2.5 min)
	0.5 mL/min (2.6 - 4.8 min)
	0.2 mL/min (4.9 - 5.0 min)
Column temperature:	40 °C
Injection volume:	10 µL
Mass spectrometer:	LCMS-8050
Source conditions:	
Nebulizer Gas:	3 L/min
Heating Gas:	10 L/min
Drying Gas:	5 L/min
Interface temperature:	200 °C
Desolvation Line:	200 °C
Heat Block temperature:	200 °C
Interface voltage:	-2.5 kV
Dwell time:	50 msec
Pause time:	3 msec
Ionization:	Electrospray ionization (ESI) negative mode
Scan Type:	MRM

Table 1 EtG/EtS MRM transitions, retention time (RT). T/I = target or internal standard

Compound		Formula	MRM1	MRM2	RT
EtG	T	$C_8H_{14}O_7$	221>75	221>85	2.08
D5-EtG	I	$C_8H_9D_5O_7$	226>75	226>85	2.07
EtS	T	$C_2H_6O_4S$	125>97	125>80	3.95
D5-EtS	I	$C_2HD_5O_7S$	130>98	130>80	3.92

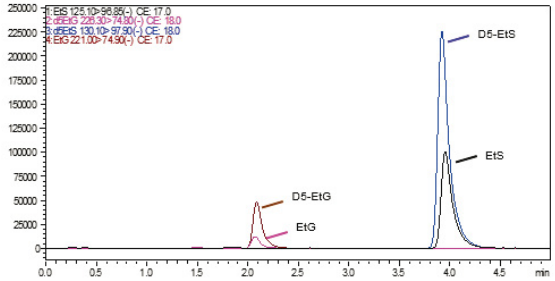
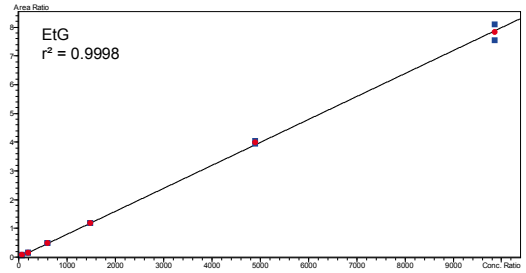


Figure 1 LC-MS separation of EtG / EtS and deuterated standard in five minutes by isocratic chromatography.



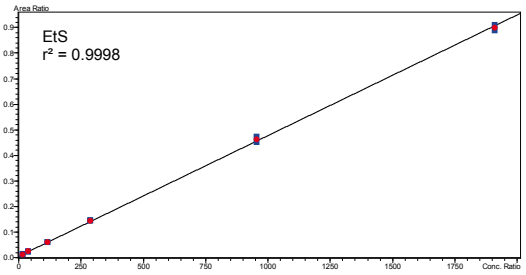


Figure 2 Calibration curves for EtG and EtS.

EtG	Control Level I (111 µg/L)	Control Level II (518 µg/L)	Control Level III (2052 µg/L)
	Conc.	Conc.	Conc.
Control 1	108.055	523.773	2124.918
Control 2	94.895	529.889	2005.968
Control 3	118.378	509.452	2137.082
Control 4	104.611	503.697	2071.541
Mean	106.485	516.703	2084.877
SD	9.691	12.187	59.814
%RSD	9.10	2.36	2.87

Table 2 Reproducibility for EtG

EtS	Control Level I (48 µg/L)	Control Level II (201 µg/L)	Control Level III (799 µg/L)
	Conc.	Conc.	Conc.
Control 1	49.174	209.118	781.053
Control 2	50.413	199.111	772.997
Control 3	46.561	212.100	790.709
Control 4	44.721	200.284	775.448
Mean	47.717	205.153	780.052
SD	2.563	6.434	7.864
%RSD	5.37	3.14	1.01

Table 3 Reproducibility for EtS

■ Results

The rapid elution of EtG and EtS by isocratic chromatography produced excellent peak shape and accuracy with elution in five minutes (Fig. 3).

The calibration curve determined in duplicate showed good linearity over a clinically relevant range of 78.6-9860 µg/L for EtG and 15.3-1910 µg/L for EtS (Fig. 2)

Three control samples at high, mid and low concentration were analyzed in fourfold to measure analytical reproducibility. The percentage relative standard deviation was typically lower than 10% from these measurements.

■ Conclusion

The application of the clinical ClinMass® Complete Kit, for Ethylglucuronide and Ethylsulfate in Urine, Plasma and Serum proved easy to implement and showed good sensitivity and linearity in a clinically relevant concentration range.

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Determination of Δ^9-tetrahydrocannabinol and two of its metabolites in whole blood, plasma and urine by UHPLC-MS/MS using QuEChERS sample preparation

ASMS 2014 ThP600

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CHU Centre hospitalier universitaire Limoges

Determination of Δ⁹-tetrahydrocannabinol and two of its metabolites in whole blood, plasma and urine by UHPLC-MS/MS using QuEChERS sample preparation

Introduction

In France, as in other countries, cannabis is the most widely used illicit drug. In forensic as well as in clinical contexts, Δ⁹-tetrahydrocannabinol (THC), the main active compound of cannabis, and two of its metabolites [11-hydroxy-Δ⁹-tetrahydrocannabinol (11-OH-THC) and 11-nor-Δ⁹-tetrahydrocannabinol-9-carboxylic acid (THC-COOH)] are regularly investigated in biological fluids for example in Driving Under the Influence of Drug context (DUID) (figure 1).
Historically, the concentrations of these compounds were determined using a time-consuming extraction procedure and GC-MS. The use of LC-MS/MS for this application is relatively recent, due to the low response of these compounds in LC-MS/MS while low limits of quantification need to be reached. Recently, on-line Solid-Phase-Extraction coupled with UHPLC-MS/MS was described, but in our hands it gave rise to significant carry-over after highly concentrated samples. We propose here a highly sensitive UHPLC-MS/MS method with straightforward QuEChERS sample preparation (acronym for Quick, Easy, Cheap, Effective, Rugged and Safe).

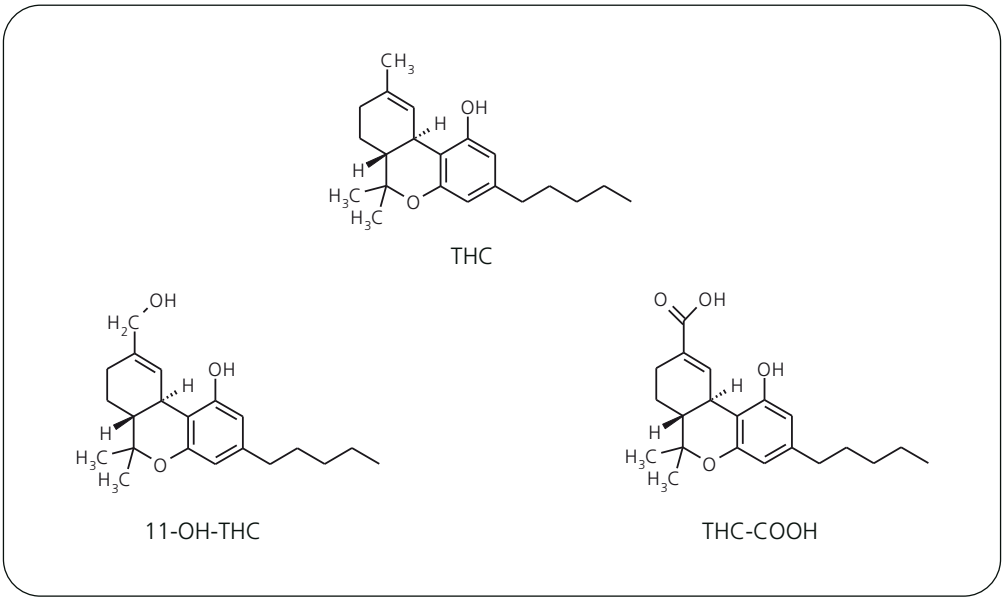


Figure 1: Structures of THC and two of its metabolites

Methods and Materials

Isotopically labeled internal standards (one for each target compound in order to improve method precision and accuracy) at 10 ng/mL in acetonitrile, were added to 100 µL of sample (urine, whole blood or plasma) together with 50 mg of QuEChERS salts ($MgSO_4$/NaCl/Sodium citrate dehydrate/Sodium citrate sesquihydrate) and 200 µL of acetonitrile. Then the mixture was shaken and centrifuged for 10 min at 12,300 g. Finally, 15 µL of the upper layer were injected in the UHPLC-MS-MS system. The whole acquisition method lasted 3.4 min.

Determination of Δ⁹-tetrahydrocannabinol and two of its metabolites in whole blood, plasma and urine by UHPLC-MS/MS using QuEChERS sample preparation

UHPLC conditions (Nexera MP system)

Column	: Kinetex C18 50x2.1 mm 2.6 µm (Phenomenex)
Mobile phase A	: 5mM ammonium acetate in water
B	: CH_3CN
Flow rate	: 0.6 mL/min
Time program	: B conc. 20% (0-0.25 min) - 90% (1.75-2.40 min) - 20% (2.40-3.40 min)
Column temperature	: 50 °C

MS conditions (LCMS-8040)

Ionization	: ESI, negative MRM mode
Ion source temperatures	: Desolvation line: 300°C Heater Block: 500°C
Gases	: Nebulization: 2.5 L/min Drying: 10 L/min

MRM Transitions:

Compound	MRM	Dwell time (msec)
THC	313.10>245.25 (Quan)	60
	313.10>191.20 (Qual)	60
	313.10>203.20 (Qual)	60
$THC-D_3$	316.10>248.30 (Quan)	5
	316.10>194.20 (Qual)	5
11-OH-THC	329.20>311.30 (Quan)	45
	329.20>268.25 (Qual)	45
	329.20>173.20 (Qual)	45
$11-OH-THC-D_3$	332.30>314.40 (Quan)	5
	332.30>271.25 (Qual)	5
THC-COOH	343.20>245.30 (Quan)	50
	343.20>325.15 (Qual)	50
	343.20>191.15 (Qual)	50
	343.20>299.20 (Qual)	50
$THC-COOH-D_3$	346.20>302.25 (Quan)	5
	346.20>248.30 (Qual)	5

Pause time	: 3 msec
Loop time	: 0.4 sec (minimum 20 points per peak for each MRM transition)

Results

Chromatographic conditions

A typical chromatogram of the 6 compounds is presented in figure 1.

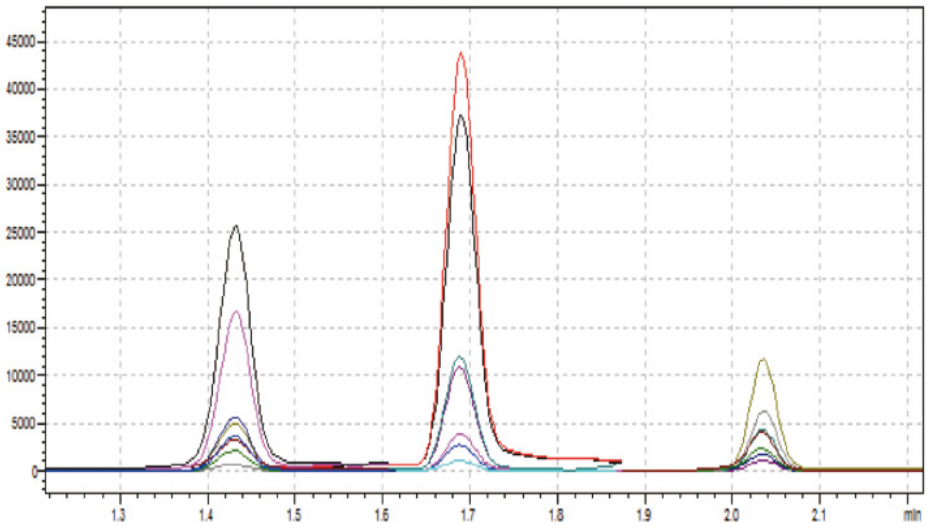


Figure 1: Chromatogram obtained after an injection of a 15 µL whole blood extract spiked at 50 µg/L

Extraction conditions

As described by Anastassiades et al. J. AOAC Int 86 (2003) 412-31, the combination of acetonitrile and QuEChERS salts allowed the extraction/partitioning of compounds of interest from matrix. This extraction/partitioning process is not only obtained with whole blood and plasma-serum where deproteinization occurred and allowed phase separation, but also with urine as presented in figure 2.

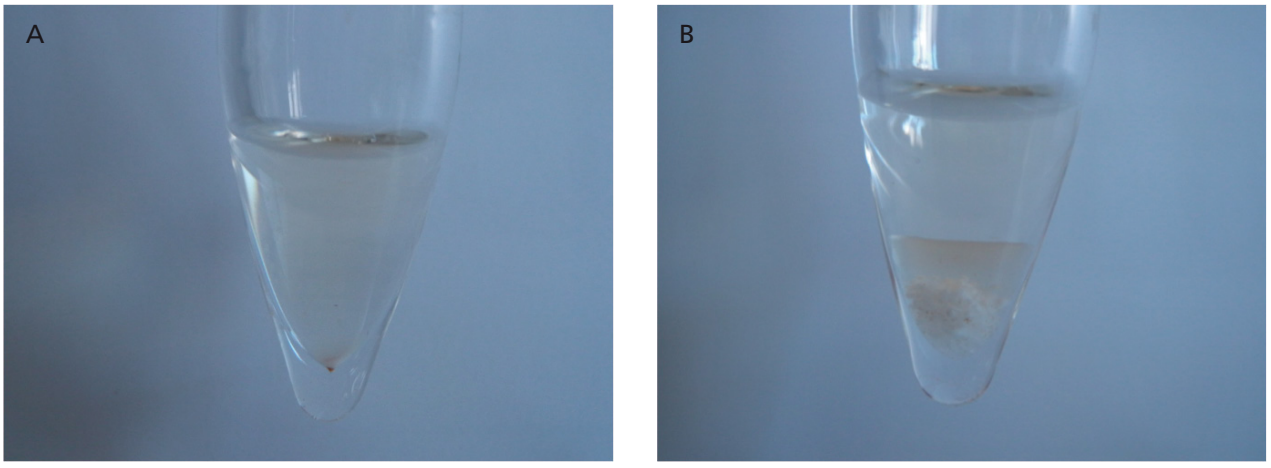
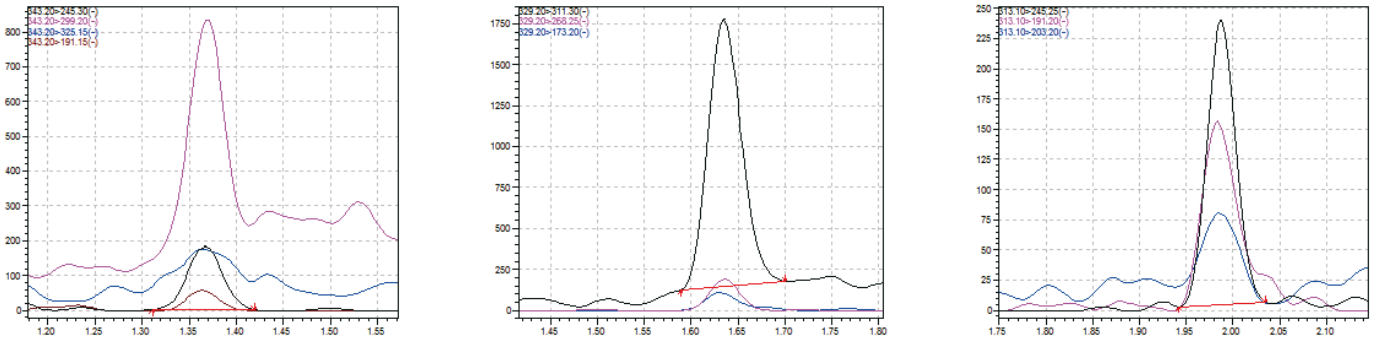


Figure 2: influence of QuEChERS salts on urine extraction/partitioning: A: acetonitrile with urine sample lead to one phase / B: acetonitrile, QuEChERS salts and urine lead to 2 phases.

Determination of Δ⁹-tetrahydrocannabinol and two of its metabolites in whole blood, plasma and urine by UHPLC-MS/MS using QuEChERS sample preparation

Validation data

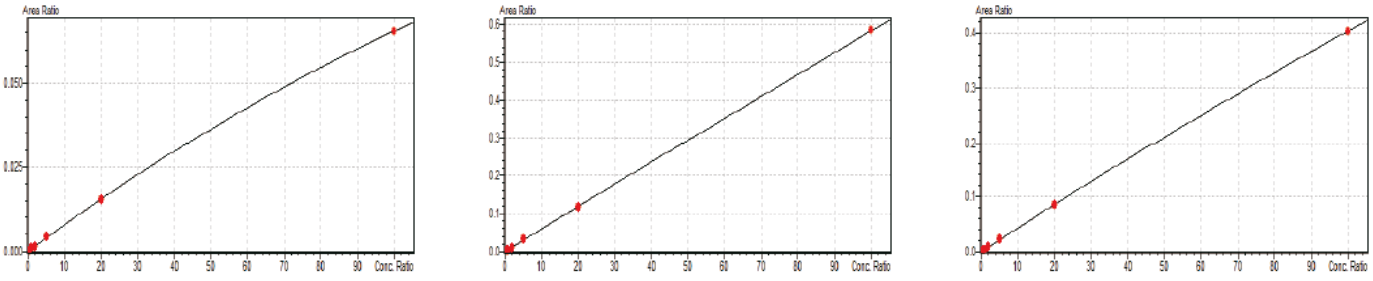
One challenge for the determination of cannabinoids in blood using LC-MS/MS is the low quantification limits that need to be reached. The French Society of Analytical Toxicology proposed 0.5 µg/L for THC et 11-OH-THC and 2.0 µg/L for THC-COOH. With the current application, the lower limit of quantification was fixed at 0.5 µg/L for the three compounds (3.75 pg on column). The corresponding extract ion chromatograms at this concentration are presented in figure 3.



THC-COOH | 11-OH-THC | THC

Figure 3: Chromatogram obtained after an injection of a 15 µL whole blood extract spiked at 0.5 µg/L (lower limit of quantification).

The upper limit of quantification was set at 100 µg/L. Calibration graphs of the cannabinoids-to-internal standard peak-area ratios of the quantification transition versus expected cannabinoids concentration were constructed using a quadratic with 1/x weighting regression analysis (figure 4).



THC-COOH | 11-OH-THC | THC

Figure 4: Calibration curves of the three cannabinoids

Contrary to what was already observed with on-line Solid-Phase-Extraction no carry-over effect was noted using the present method, even when blank samples were injected after patient urine samples with concentrations exceeding 2000 µg/L for THC-COOH.

Determination of Δ^9-tetrahydrocannabinol and two of its metabolites in whole blood, plasma and urine by UHPLC-MS/MS using QuEChERS sample preparation

Conclusions

- Quick sample preparation based on QuEChERS salts extraction/partitioning, almost as short as on-line Solid Phase Extraction.
- Low limit of quantification compatible with determination of DUID.
- No carry over effect noticed.

First Edition: June, 2014

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Application News

SSI-LCMS-043

Liquid Chromatography Mass Spectrometry

Analysis of Carboxy THC Using the LCMS-8030 Triple Quadrupole Mass Spectrometer

LCMS-8030

Summary

Carboxy THC is an important compound found in marijuana. The Substance Abuse and Mental Health Service Administration (SAMHSA) has set 40% of the cutoff value (15 ng/mL) as the target for the lower limit of quantitation (LLOQ) for carboxy THC. The following Application News will describe a Nexera-8030 LC/MS/MS method for measuring carboxy THC for the LLOQ.

Method

(-)-11-nor-9-Carboxy-THC was obtained from Cerriliant Analytical Reference Standards (Round Rock, TX) in an ampoule of 1 mg/mL concentration in methanol (structure of shown in **Figure 1**). The standard was further diluted in methanol for calibration levels for LCMS analysis.

A Shimadzu Nexera-8030 mass spectrometer was used for this application. The flow rate was 0.35 mL/min and the oven temperature was 40 degrees C. A Phenomenex Kinetex pentafluorophenyl (PFP) (100 x 2.1mm x 2.6 µm) column was used with a binary gradient consisting of 5 mM ammonium acetate and methanol. The gradient conditions for the LC method are shown in **Table 1**.

The MS conditions for the method are shown in **Table 2**. Both electrospray ionization (ESI) in positive and negative mode were used to monitor three multiple reaction monitoring (MRM) transitions. The MRM transition 345.15>298.85 was determined to be the optimal MS condition for carboxy THC. MRM optimization (collision energy, Q1 and Q3 prerod-bias) was done automatically by the 8030 and shown in **Table 3**.

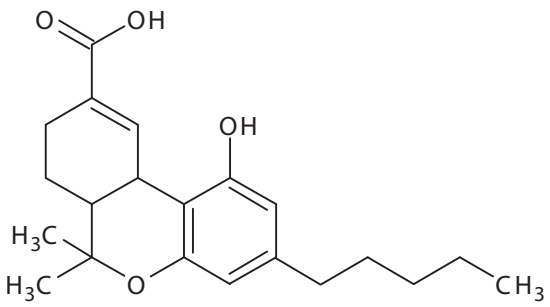


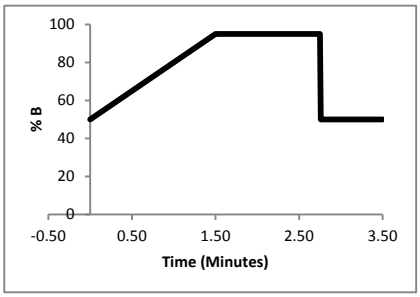
Figure 1: Structure of Carboxy THC.

Six different calibration standards were diluted in a 1:1 solution of Methanol and 5 mM ammonium acetate. **Table 4** lists the amounts injected on column using an injection volume of 10 µL. In addition, the representative concentration of carboxy THC present in urine is also listed in **Table 4**. The initial intensity studies were performed at 0.3 ng on column. This is representative of 20% of the SAMSHA cutoff value assuming 1 mL of urine was prepared and concentrated to 0.1 mL.

Results and Discussion

Carboxy THC was able to be detected and quantified with a single LC/MS/MS method within the SAMSHA cutoff values. The LC/MS/MS chromatograms for the MRM transitions for carboxy THC are shown in **Figure 2**. Calibration curves for the three transitions for carboxy THC are shown in **Figure 3**. Each calibration curve has six points with r^2 values equal to 0.998. In addition, the MS chromatograms for the lowest calibration level, 0.03 ng on column, are shown in **Figure 3**.

Six consecutive injections of carboxy THC were tested for reproducibility of the method and the area counts for each MRM transition are listed in **Table 5**. The percent relative standard deviation (%RSD) for each transition at varying calibration levels is under 6%. The three lowest calibration levels are listed here.



Time (min)	%B
0.00	50
1.50	95
2.75	95
2.76	50
3.50	50

Table 1: Gradient Conditions.

Interface:	ESI
Interface Voltage	4.5 kV
DL Temp:	250 C
Nebulizing Gas Flow:	2 L/min
Heat Block Temp:	400 C
Drying Gas Flow:	15 L/min

Table 2: MS Conditions.

Transition	+/-	Dwell Time (msec)	Q1 Pre Bias (V)	CE	Q3 Pre Bias (V)
345.15>298.85	+	50	-11	-20	-23
345.15>193.00	+	50	-11	-25	-15
343.35>299.35	-	50	13	20	19

Table 3: MRM MS Conditions.

Level	Mass on Column	Representative Concentration in Urine
1	100 ng	1000 ng/mL
2	10 ng	100 ng/mL
3	1.0 ng	10 ng/mL
4	0.3 ng	3 ng/mL
5	0.1 ng	1 ng/mL
6	0.03 ng	0.3 ng/mL

Table 4: Calibration levels of Carboxy THC.

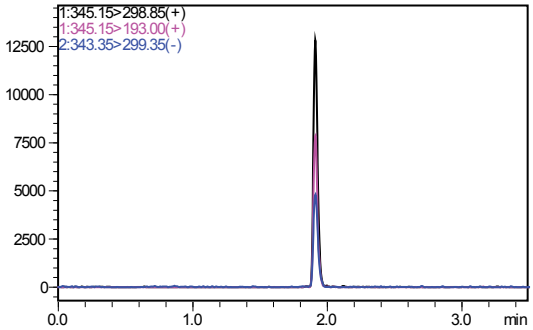


Figure 2: Carboxy THC MRM transitions using 5 mM ammonium acetate and methanol mobile phases.

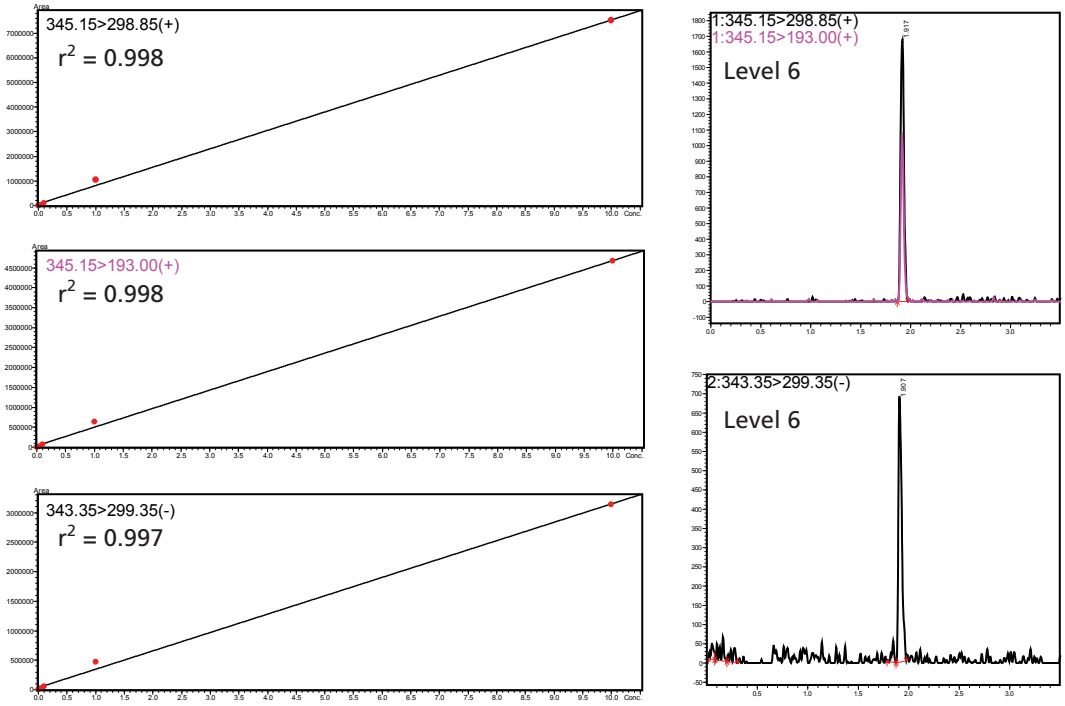


Figure 3: Calibration curves for the three carboxy THC transitions (on left) and respective MS chromatograms for the lowest calibration level 6 (0.03 ng on column).

0.3 ng	+298.85	+193.00	-299.35
1	36729	21479	16133
2	36202	22085	17882
3	35660	21303	17096
4	36573	22043	16648
5	35254	21761	17290
6	35721	22098	16557
Average	36023	21798	16934
%RSD	1.59	1.56	3.65

0.1 ng	+298.85	+193.00	-299.35
1	11663	7224	6223
2	12430	6717	6246
3	12156	7560	6181
4	12843	7568	6157
5	12730	7122	5634
6	12386	7325	5909
Average	12368	7253	6058
%RSD	3.43	4.38	3.97

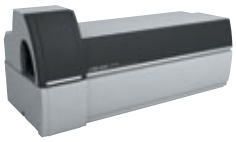
0.03 ng	+298.85	+193.00	-299.35
1	3999	2215	1957
2	3474	2193	1936
3	3630	2144	1792
4	3822	2088	1907
5	3559	2291	1916
6	3558	2334	2002
Average	3674	2211	1918
%RSD	5.38	4.12	3.68

Table 5: Average of six replicate injections for three lowest levels of Carboxy THC. Values are area counts for MRM peaks for the mass on column indicated.

Conclusion

An fast, sensitive and accurate LC/MS/MS method has been developed to identify and quantify carboxy THC within the 20% SAMSHA cutoff value. In summary, Shimadzu 8030 triple quadrupole mass spectrometer coupled with Shimadzu Nexera UHPLC is a powerful system to quantify low levels of carboxy THC.

ULTRA FAST MASS SPECTROMETRY


LCMS-8040


LCMS-8030


LCMS-8080


LCMS-2020


LCMS-IT-TOF

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Application News

SSI-LCMS-045

Liquid Chromatography Mass Spectrometry

Quantitative Analysis of Cannabinoids using the LCMS-2020 Single Quad MS

LCMS-2020

Summary

Evaluation and quantitation of a variety of cannabinoids on an LCMS-2020 single quadrupole mass spectrometer.

Method

Cannabichromene (CBC) and Δ9-THC Acid A (THCA) standards were purchased from Restek (Bellefonte, PA). Cannabinol (CBN), Δ8-THC (d8-THC), Δ9-THC (d9-THC) and Cannabidiol (CBD) were purchased from Cerilliant (Round Rock, TX). A stock concentration of 1 µg/mL for each standard was used for SIM optimization. The standards were combined into one solution and then serially diluted with 70/30 (%) methanol/water using 2-fold dilutions yielding concentrations ranging from 8192 ng/mL to 1 ng/mL. The standards (**Figure 1**) were transferred to autosampler vials and injected into a Nexera-LCMS-2020 system for analysis.

A Thermo Hypersil Gold (1.9 µm x 2.1 mm x 100 mm) was used with a binary gradient of 5 mM ammonium acetate in water and acetonitrile. The gradient conditions are shown in **Figure 2**. The flow rate was 0.5 mL/min with a run time of 6.8 min. The column temperature was 45° C and the injection volume was 5 µL.

LCMS-2020 Analysis

Dual ion electrospray ionization (DUIS) in both positive and negative modes was used for ionization of the analytes on the LCMS-2020. A positive and negative scan and selected ion monitoring (SIM) modes were used simultaneously for analysis. Details of the MS parameters are shown in **Table 1**.

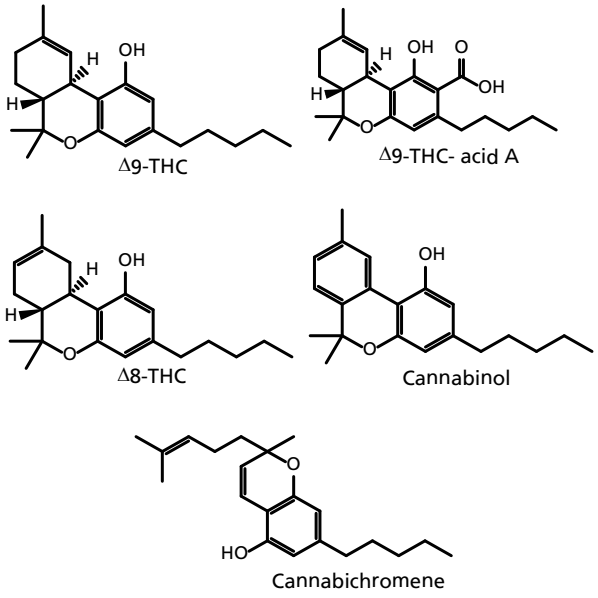


Figure 1. Chemical structures for cannabinoids.

Results and Discussion

Peaks were smoothed using the standard method set to 2 counts and 2 sec for width. The auto integration setting was used with a setting of 1 max peak and 6 sec width. d8-THC was integrated by turning off peak integration from 4.8-5.0 minutes. Linearity was achieved from 0.5-1024ng/mL for CBN and d9-THC, 1-1024ng/mL for d8-THC, 2-128ng/mL for THCA, 2-2048ng/mL for CBD and 4-2048ng/mL for CBC. The calibration curve was weighted using 1/C and not forced through zero. No standards were excluded over the included range and r^2 values ranged from 0.998-0.999. A representative chromatogram and calibration curve for each analyte are shown in **Figures 3-8**.

Time (min)	%B
0.0	70
3.0	75
4.9	85
5.0	95
5.7	95
5.8	70
6.8	70

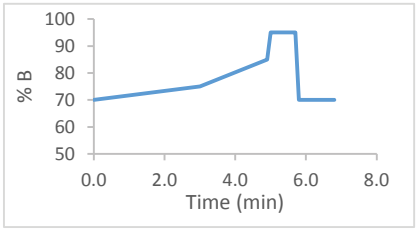
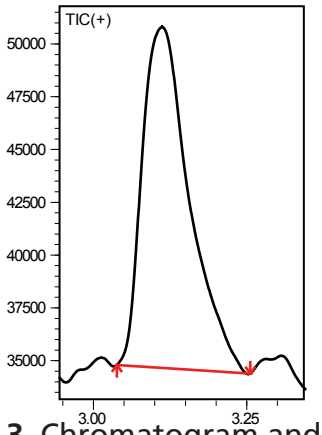


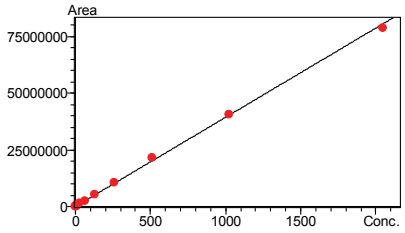
Figure 2. Gradient conditions.

MS Interface Parameters

Interface	DUIS
Nebulizing gas flow	2 L/min
Drying gas flow	15 L/min
DL Temperature	300 °C
Heat Block Temperature	500 °C
Polarity	(+) CBD, CBN, d8-THC, d9-THC, CBC (-) THCA

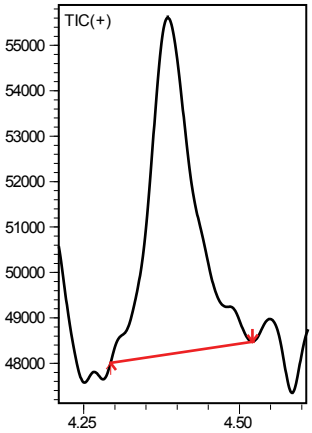
Table 1. MS Interface parameters.

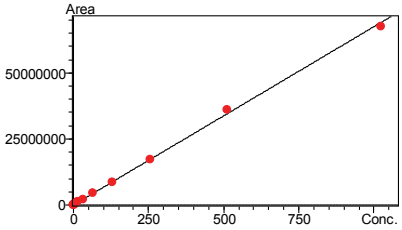




CBD - LLOQ: 2 ng/mL
Linear Range: 2-2048 ng/mL
r^2 = 0.999

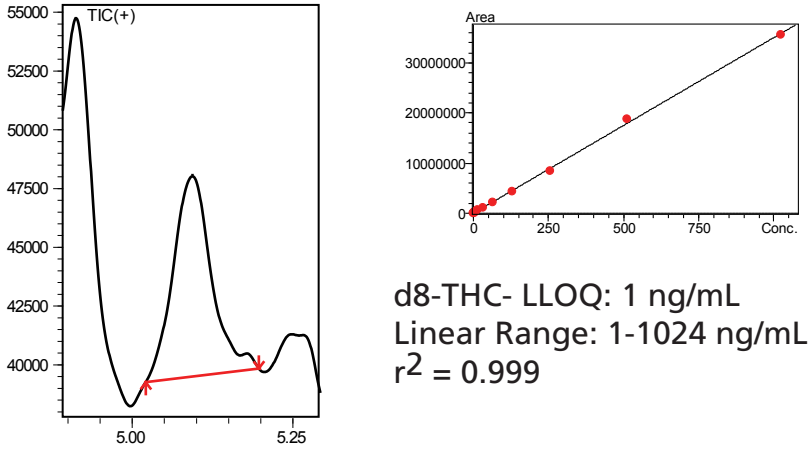
Figure 3. Chromatogram and calibration curve for cannabidiol (CBD).





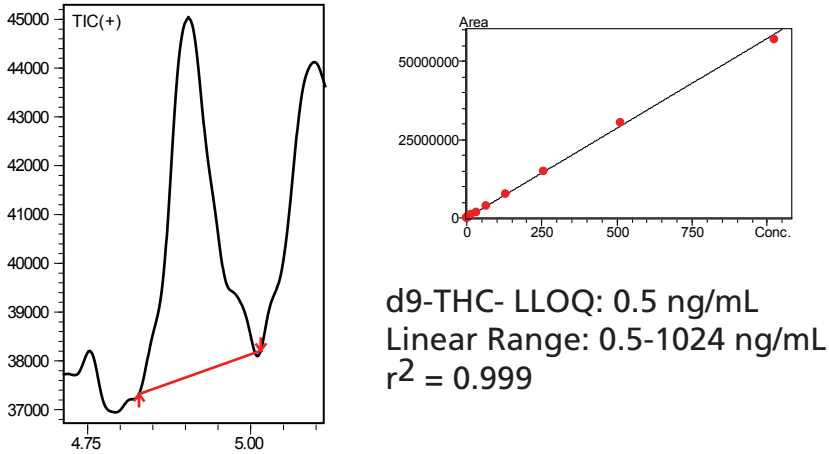
CBN - LLOQ: 0.5 ng/mL
Linear Range: 0.5-1024 ng/mL
r^2 = 0.999

Figure 4. Chromatogram and calibration curve for cannabinol (CBN).



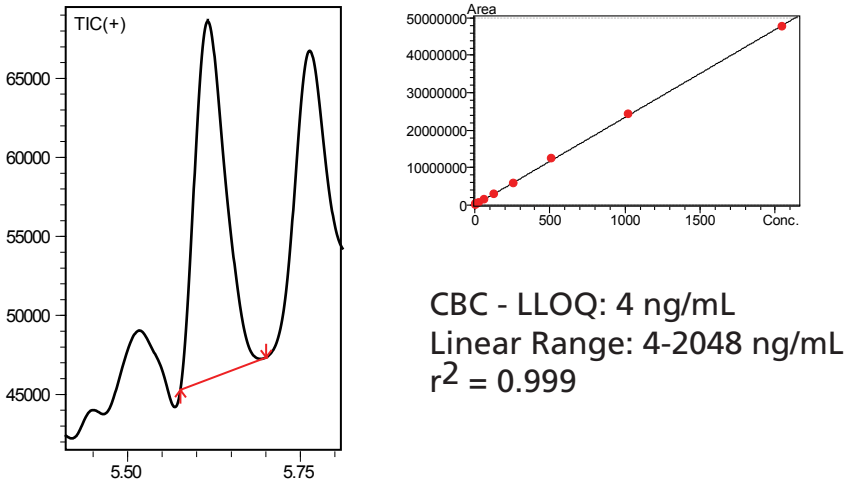
d8-THC- LLOQ: 1 ng/mL
Linear Range: 1-1024 ng/mL
r^2 = 0.999

Figure 5. Chromatogram and calibration curve for d8-THC.



d9-THC- LLOQ: 0.5 ng/mL
Linear Range: 0.5-1024 ng/mL
r^2 = 0.999

Figure 6. Chromatogram and calibration curve for d9-THC.



CBC - LLOQ: 4 ng/mL
Linear Range: 4-2048 ng/mL
r^2 = 0.999

Figure 7. Chromatogram and calibration curve for cannabichromene (CBC).

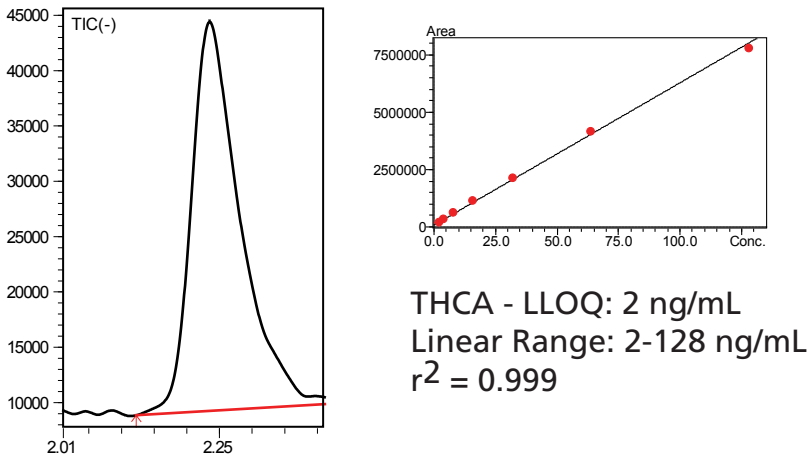


Figure 8. Chromatogram and calibration curve for d9-THC Acid A (THCA).

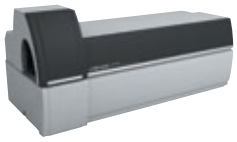
Conclusion

A method for rapid quantitation of various cannabinoids was requested utilizing UHPLC equipment and LCMS to increase the speed and sensitivity of analysis. A 7 minute method was developed, with the main limiting factor being the separation of d8-THC and d9-THC. If d8-THC and d9-THC do not need to be individually quantified then the method could be shortened further.

It is important to note that these results were achieved using standards without matrix, but the ability of the Nexera X2 system and LCMS-2020 mass spectrometer to enable rapid, sensitive quantitative analysis of cannabinoids is highlighted by this method.

ULTRA FAST MASS SPECTROMETRY


LCMS-8030


LCMS-8040


LCMS-8050


LCMS-2020


LCMS-IT-TOF

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Application News

SSI-LCMS-010

Liquid Chromatography Mass Spectrometry

Designer Cannabinoids

LCMS-8030

Summary

A rapid LC-MS-MS method for determination of designer cannabinoids in smokeable herbs was developed.

Background

Designer cannabinoids are synthetic compounds designed to mimic the effects of cannabis. Lawmakers have banned many of these substances, however drug designers can create new analogues as quickly as the old ones are banned. These analogues usually contain only minor modifications that do not affect activity yet render the substance undetectable in routine MRM-based LC-MS-MS assays.

Ultrafast precursor ion or neutral loss scanning on the Shimadzu LCMS-8030 offers a unique way of rapidly detecting and characterizing new designer cannabinoids.

Method

Authentic standards of a variety of synthetic cannabinoids were obtained. Standards were diluted for MRM optimization. A K2 Spice product marketed as "ban-compliant" was purchased from a local gas station.

A Restek Ultra Biphenyl (5 μm, 2.1 x 50 mm) column was used with a binary gradient of 0.1% formic acid (Pump A) and 0.1% formic acid in acetonitrile (Pump B). The linear gradient program started at 5% B and increased to 95% B over 10 min, followed by a 2 minute equilibration. The flow rate was 0.5 mL/min and the column oven was maintained at 40 °C.

Electrospray ionization was used in positive mode. The DL temperature was 250 °C, the Nebulizing gas was 2 L/min, the Heater Block temperature was 400 °C, and the drying gas was 15 L/min.

MS methods were used to search for both known and unknown designer cannabinoids. For known designer cannabinoids, Multiple Reaction Monitoring (MRM) of the transitions for each compound was used. MRM optimization using an automated wizard was performed to determine the highest intensity product ions as well as the optimum ion optics voltages and collision energies.

To search for unknown cannabinoids, precursor ion scanning was used. Analogues of the napthoyl-indole cannabinoids share one or more common fragment ions of *m/z* 155, 127, and 144. Precursor ion scans corresponding to these fragments were added to the MS method at a scan speed of 5,000 u/sec. In addition, data-dependent MS-MS at a scan speed of 15,000 u/sec was used to collect full product ion spectra for each precursor detected in the precursor ion scan. This information was used for library searching and to characterize unknown compounds as designer cannabinoid analogues.

Samples of K2 Spice product were divided into 100 mg portions, then mixed with 1 mL methanol, followed by vortexing and sonication. The sample was filtered and then diluted 100-fold in 50:50 water:methanol. The injection volume was 1 µL.

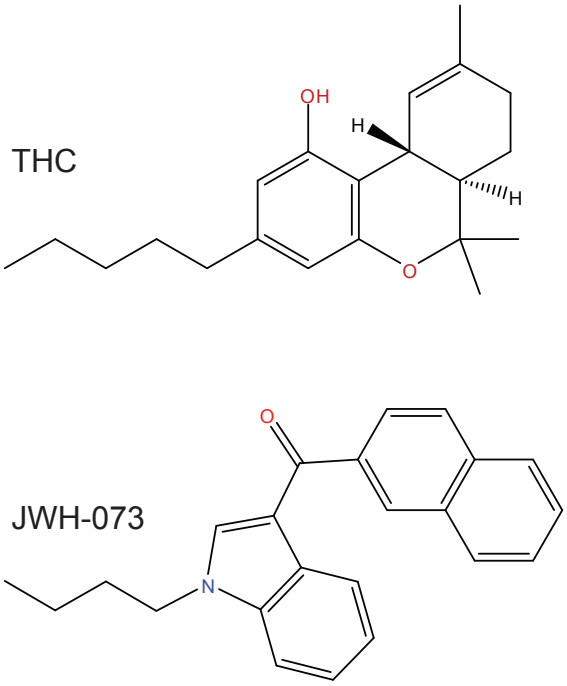


Figure 1: Structures of THC, the active component in marijuana, and JWH-073, a designer cannabinoid

Figure 2: Typical herbal incense product

Results and Discussion

Product ion scans of several representative cannabinoid standards are shown in **Figure 4**. Common product ions were observed in these and other designer cannabinoid analogues. As shown in the figure, the fragments of *m/z* 155 and 127 arise from cleavage on either side of the carbonyl linking the napt halene group from the indole group. The fragment of *m/z* 144 likely results from an intra-molecular cyclization and elimination, involving the indole group and the alkyl side chain.

Designer cannabinoids of the napthoyl-indole type can be synthesized with one or more modifications to the napthalene group, the indole group, or to the N-alkyl chain. These modifications can include

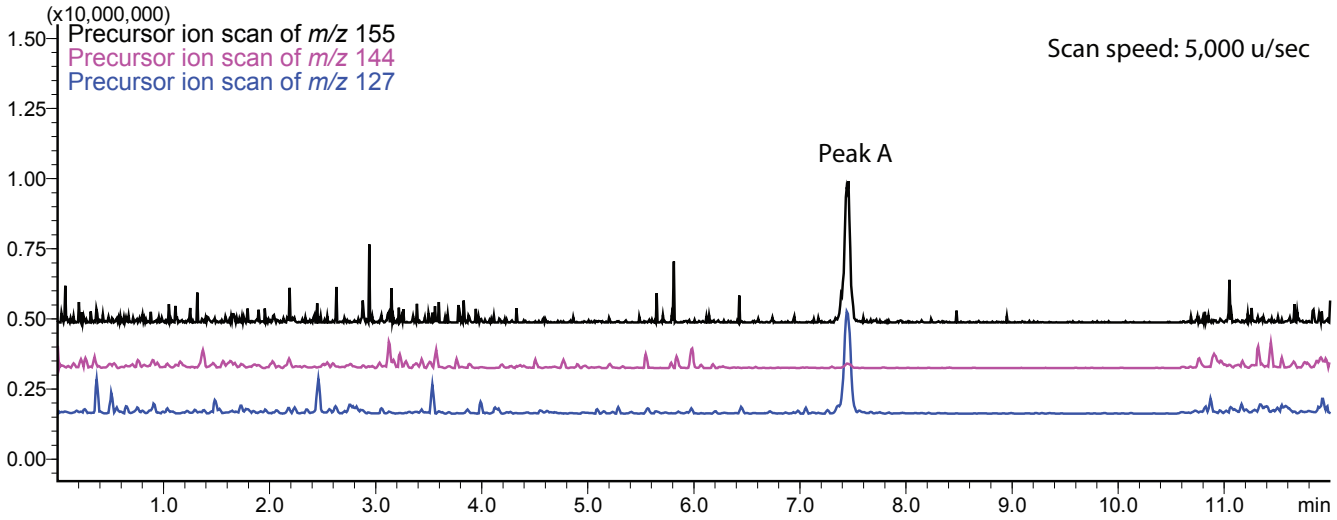


Figure 3: Chromatograms of three precursor ion scans for the K2 Spice cannabinoid product. Peak A, eluting at 7.5 min, was investigated as a designer cannabinoid.

the addition of new functional groups, and they are introduced to alter the potency of the compound or to evade detection. Precursor ion scans of *m/z* 155 and 127 can detect any analogues with modification to the N-alkyl chain or the indole group. Any analogue with modifications to the napthalene group could be detected by the fragments from the indole group using a precursor ion scan of *m/z* 144. Therefore this method has the capability to detect a wide variety of modified napthyolindole designer cannabinoids.

Because the possibility remains that some designer cannabinoids might still not be detected by these precursor ion scans, full scan MS at ultrafast scan speeds combined with ultrafast data-dependent product ion scanning can be used to further complement the semi-targeted screening approach using precursor ion scanning.

The total loop time of all events including MRMs, precursor ion scans, full scans, and data dependent scans was 520 msec. This was sufficient to collect at least 18 points per chromatographic peak.

The LC-MS-MS chromatogram of the precursor ion scans of the K2 Spice product is shown in **Figure 3**. One major peak, **Peak A**, is observed at a retention time of 7.5 min. The precursor ion scans corresponding to this peak are shown in **Figure 5**. The peak at *m/z* 342 is observed as the base peak in each spectrum, indicating the presence of a compound at this mass that fragments to the three products at *m/z* 155, 127, and 144.

The data-dependent MS-MS of Peak A is shown in Figure 7. The spectra were searched in a library containing tandem mass spectra of commercially available designer cannabiniod standards. The top hit from the search was the synthetic cannabinoid JWH-018. Other hits in the search were metabolites of synthetic cannabinoids that, while having some product ion similarities with JWH-018, could be easily rejected because they either contained different product ions or had different precursor masses.

If no hits had been found in the database search, the data dependent tandem mass spectra could be used to further characterize the unknown compound by comparing the product ions to those of known cannabinoids.

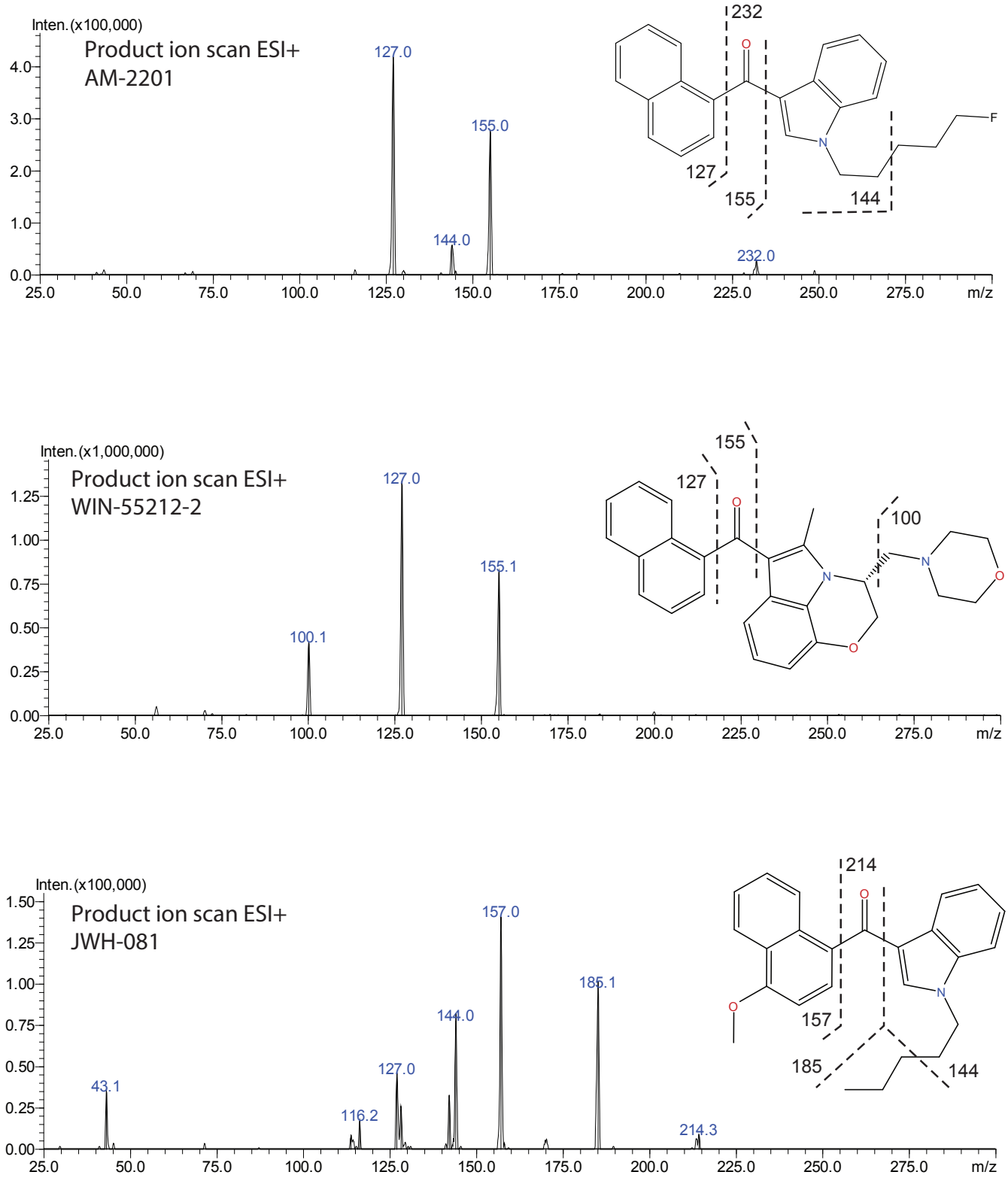


Figure 4: Tandem mass spectra of three representative designer cannabinoids showing common product ions and neutral losses

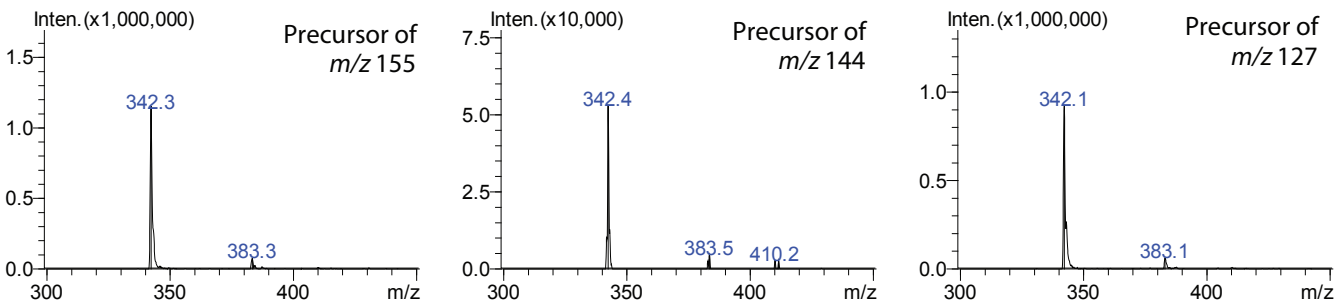


Figure 5: Precursor ion spectra for Peak A

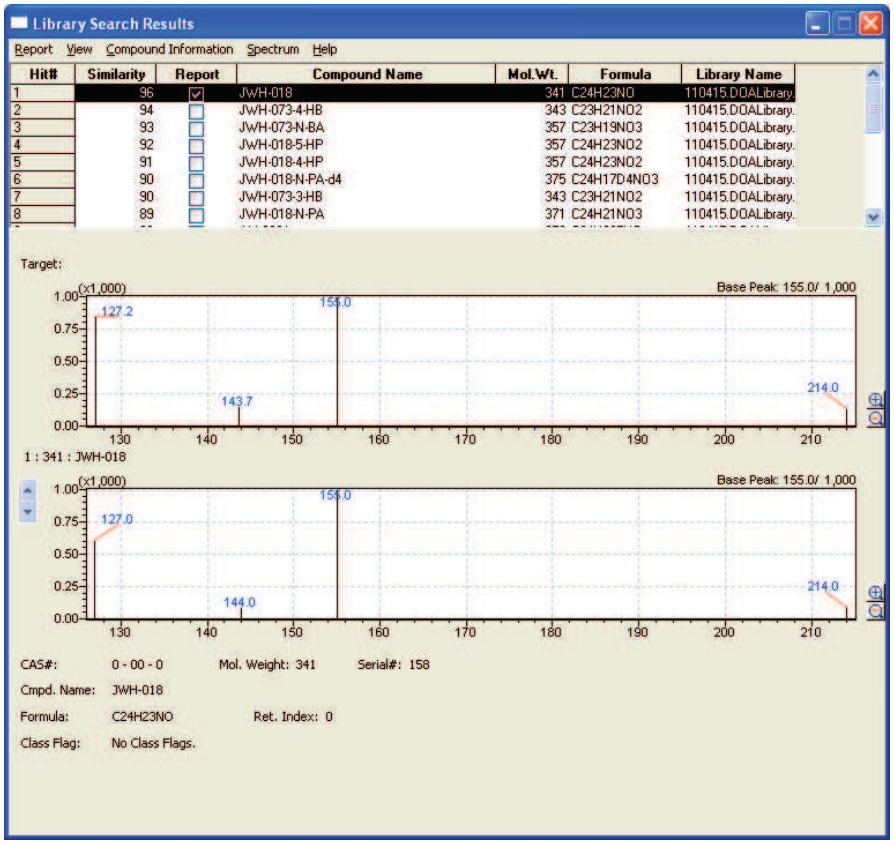


Figure 6: Library search results for the tandem mass spectrum of Peak A. The top hit is the designer cannabinoid**JWH-018**. The other hits found are metabolites of synthetic cannabinoids which have different precursor masses and therefore can be distinguished from JWH-018.

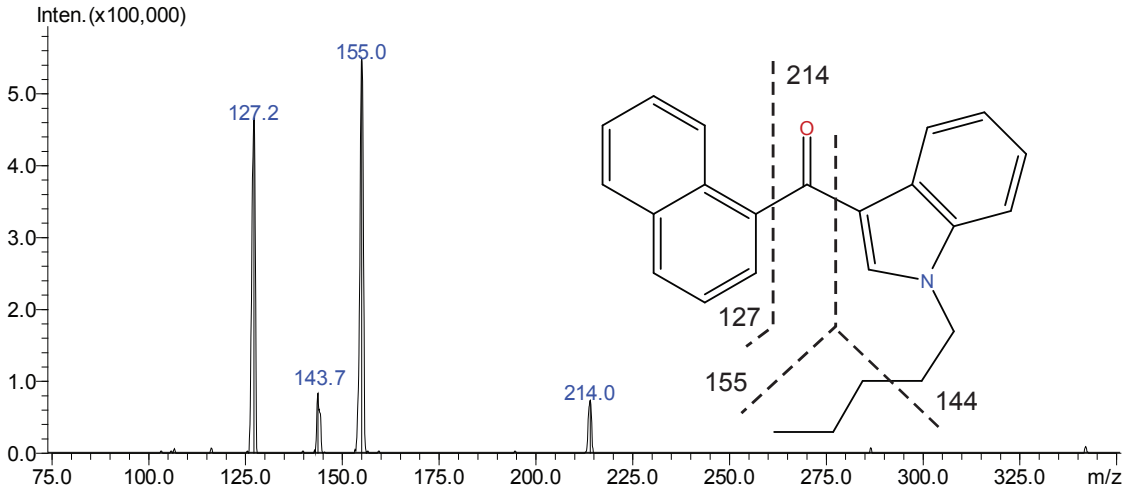


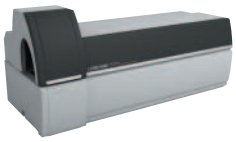
Figure 7: Tandem mass spectrum of Peak A, identified as JWH-018, and fragment assignment

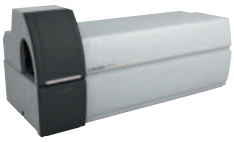
It should be noted that even at the ultrafast precursor ion scan speeds of 5,000 u/sec and data dependent product ion scan speeds of 15,000 u/sec that no significant shift in precursor or product ion masses were observed and no sensitivity was lost.

Conclusion

The fast scan capabilities of the LCMS-8030 enabled MRM, precursor ion scanning and full scanning with data dependent MS-MS for detection of known and unknown designer cannabinoids in commercially available herbal incense products.

ULTRA FAST MASS SPECTROMETRY


LCMS-8040


LCMS-8030


LCMS-8080


LCMS-2020


LCMS-IT-TOF

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Application News

SSI-LCMS-034

Liquid Chromatography Mass Spectrometry

Tetrahydrocannabinol (THC) analysis in oral fluids using the LCMS-8050

Summary

A rapid four minute method for analyzing Tetrahydrocannabinol (THC) in oral fluids with complete separation from matrix and a limit of quantitation of 40pg on column was achieved (Figure 1). A calibration curve in undiluted oral fluid was generated and yielded a quantitation range of 0.4 ng/mL – 60 ng/mL.

Introduction

Rapid collection and screening of THC has quickly become necessary because of the nationwide push for the legalization of Marijuana. Many oral fluid methods struggle to meet the demands of the customer due varying factors, some of which include sensitivity, speed of analysis, or cost. In states where Marijuana is legal, law enforcement agencies have a great need for a fast, sensitivity, and cost effective method in order effectively enforce laws such as D.U.I.D. where evidence preservation is absolutely critical.

Materials and Methods

Samples were obtained by using an oral swab and 0.5mL of oral fluids was then extracted via SPE, evaporated to dryness and reconstituted in matched mobile phase. A 10 µL injection of enriched sample was then made on the LCMS-8050 with a dwell time of 5ms and a runtime of four minutes.

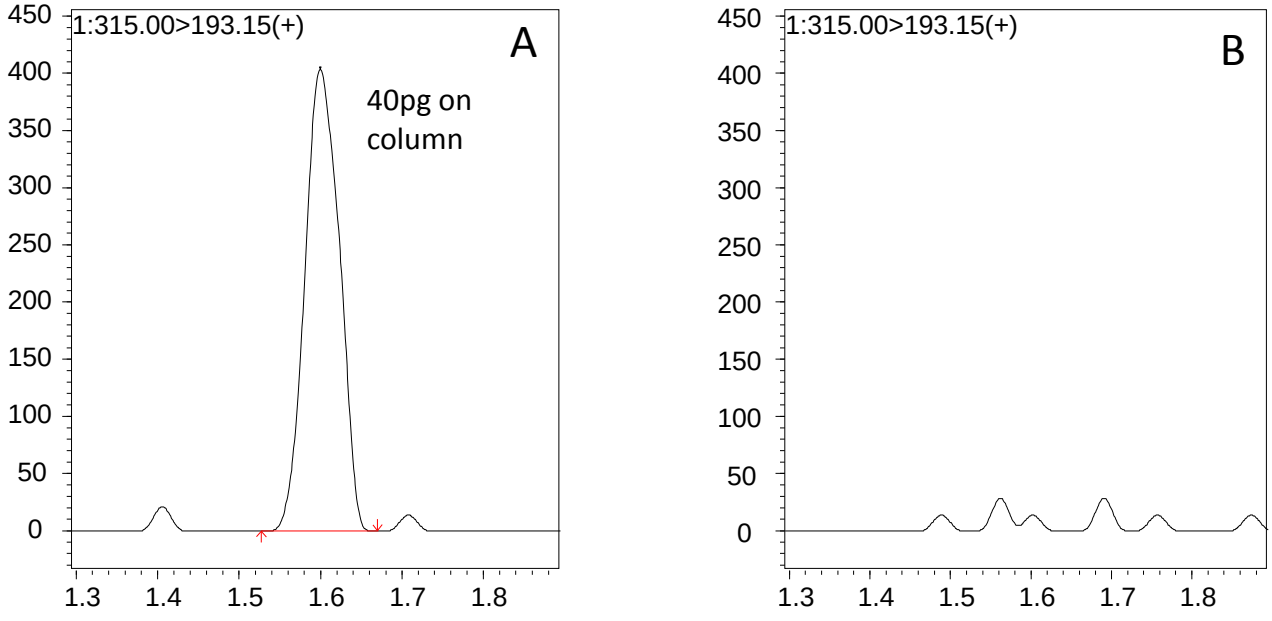

Figure 1. Representative chromatograms showing: (A) the LOQ of THC in oral fluids and (B) a matrix blank of THC in oral fluids.

Results

Detection of THC was optimized using flow injection analysis. Three MRM transitions were selected that were (i) most abundant and (ii) previously reported in the peer-reviewed literature. An LC gradient was established and a standard curve was generated (Figure 2) with a concentration range from 0.4 ng/mL to 60 ng/mL. A limit of quantitation (LOQ) was established at 40 pg on column.

Table 1 shows all of the quantitative results for the generated calibration curve. The THC had a retention time of 1.595 minutes and complete separation from the matrix was observed on column as shown in Figure 3. Matrix blanks showed no quantifiable presence of THC as seen in Figure 1.

Table 1. Quantitative results from the analysis of THC by LCMS-8050. All levels exceeded requirements for signal to noise, accuracy and calculated concentrations.

Level	Std. Conc.	Calculated Conc. (ng/mL)	Accuracy[%]	%Dev	S/N
1	0.4	0.43	106.7	6.73	21.68
2	1	0.88	88.0	-12.04	28.69
3	2	1.74	87.0	-12.97	100.46
4	4	4.16	104.0	3.98	258.12
5	10	10.76	107.6	7.62	479.45
6	20	18.59	92.9	-7.07	835.28
7	40	43.44	108.6	8.60	1,880.96
8	60	63.09	105.2	5.15	837.39

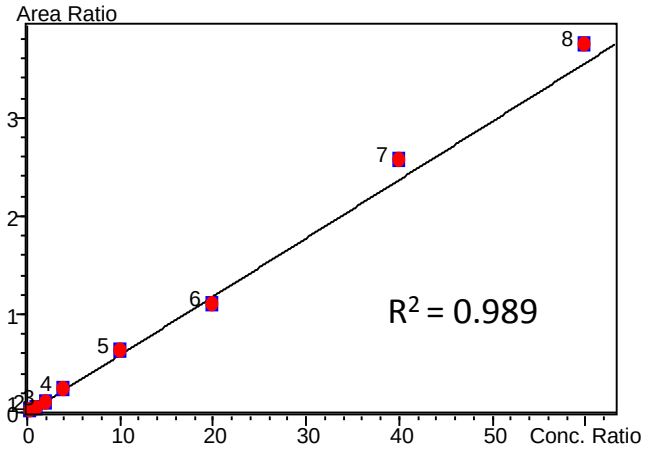

Figure 2. Calibration curve of THC in oral fluids. The standard was injected from 0.4 ng/mL – 60 ng/mL. The LOQ was determined to be 40 pg on column, and the $R^2 = 0.989$.

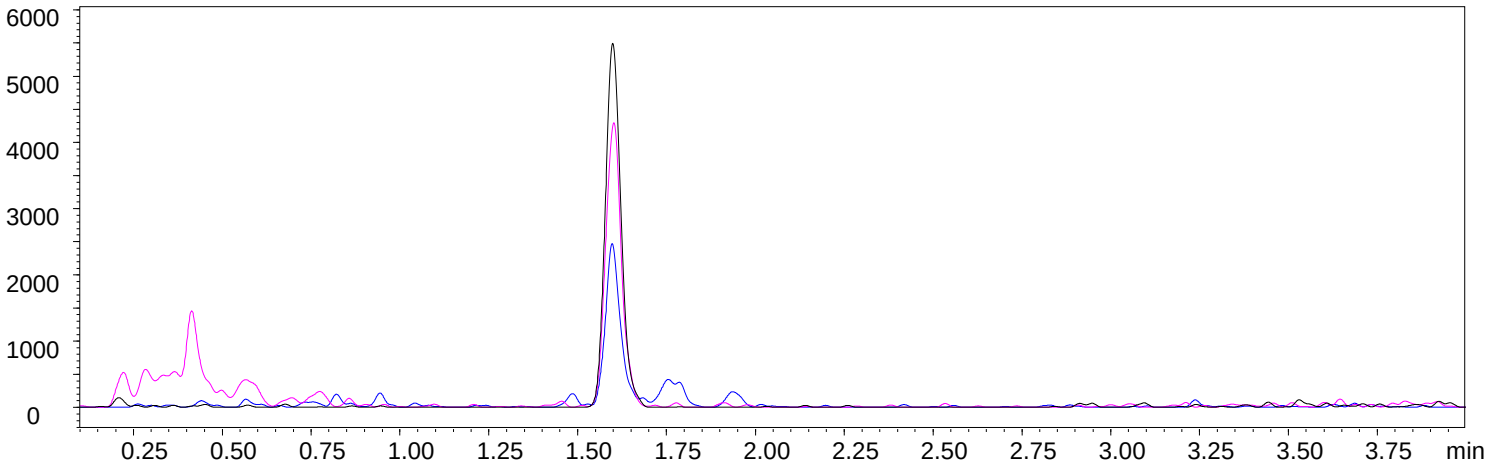

Figure 3. A representative chromatogram of THC extracted from oral fluids with a concentration of 0.4ng/mL.

Conclusions

This work demonstrates a rapid and reproducible method for the detection of THC in oral fluids using the LCMS-8050 allowing for THC analysis while maintaining sufficient sensitivity (LOQ of 40 pg on column).

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Detecting New Designer Cannabinoids in Herbal Incense using LC-MS-MS with Fast Precursor Ion Scanning

TIAFT 2012 P-182

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[1]Shimadzu Scientific Instruments, Columbia, Maryland, and [2]Restek Corporation, Bellefonte, Pennsylvania

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Detecting New Designer Cannabinoids in Herbal Incense using LC-MS-MS with Fast Precursor Ion Scanning

Introduction

Forensics and anti-doping labs rely on LC-MS-MS for detection of controlled and banned substances. LC-MS-MS methods use MRM analysis for the highest sensitivity and selectivity, however these methods only detect analytes whose MRM transitions are known in advance. In order to circumvent drug laws, designer drugs are synthesized which are not detected by traditional MRM-based methods. Because designer drugs often share common product ions and neutral losses, precursor ion or neutral loss scanning could be used to detect them.

We developed LC-MS-MS methods that utilize extremely fast precursor ion scanning for detection of designer cannabinoids in herbal incense products. The urine of human subjects who reported synthetic cannabinoid exposure was also analyzed using a newly developed high sensitivity triple quadrupole mass spectrometer from Shimadzu, the LCMS-8040.

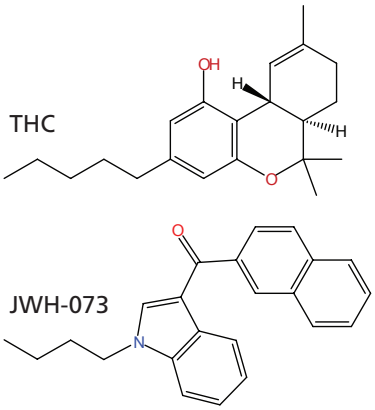


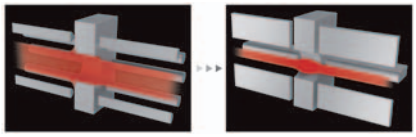
Fig. 1 Structures of THC, the active component in marijuana, and JWH-073, a designer cannabinoid

Fig. 2 Typical herbal incense product

Method

Electrospray ionization with continuous polarity switching was used on a new fast-scanning, high sensitivity triple quadrupole mass spectrometer, the LCMS-8040, and a Nexera ultra high performance liquid chromatograph. A precursor ion scan for each common product ion was carried out at a scan speed of 5,000 u/sec. Data dependent MS-MS were carried out at 15,000 u/sec. A Restek 3 µm Ulta Biphenyl column was used for improved LC separation of isomers and metabolites. The mobile phase was 0.1% formic acid in water (Pump A) and 0.1% formic acid in acetonitrile (Pump B), and the flow rate was 0.5 mL/min.

Improved ion optics of the LCMS-8040 for Ultra fast scan speed with enhanced sensitivity



UF Lens for better ion transmission

UF Sweeper-II® collision cell for better CID efficiency

Detecting New Designer Cannabinoids in Herbal Incense using LC-MS-MS with Fast Precursor Ion Scanning

Results and Discussion

The fragment ions at *m/z* 155 and 127 were observed as a common products among the napthoyl-indole cannabinoids. The fragments result from cleavage on either side of the carbonyl group. Precursor ion scans for these products were used to screen for designer cannabinoids of the napthoyl-indole class.

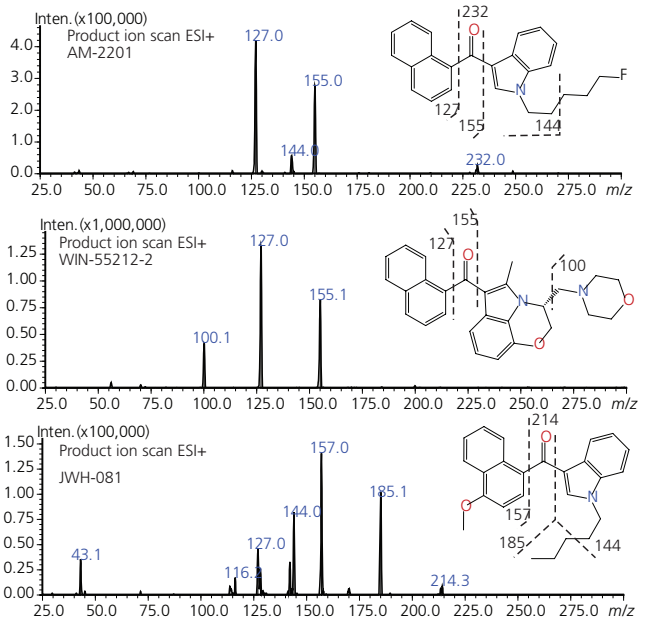


Fig. 3 Tandem mass spectra of three representative designer cannabinoids showing common product ions.

The precursor ion scan chromatograms of an extracted herbal incense product are shown in Fig. 5. The precursor ion spectra of the peak at 7.5 min indicate a precursor ion of *m/z* 342. Data dependent MS-MS of the *m/z* 342 peak is shown in Figure 8. As in this case, the product ion scans should detect any designer cannabinoids with modifications to the N-alkyl chain or indole group. Any designer cannabinoid with modifications to the napthalene group could be detected by the fragment of *m/z* 144 from the indole group. Therefore this method has the capability to detect a wide variety of modified napthoyl-indole designer cannabinoids.

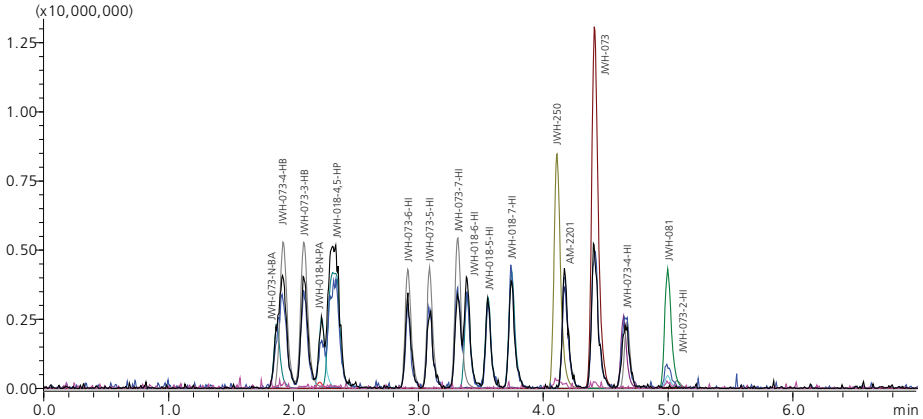


Fig. 4 Improved chromatography of cannabinoids and their metabolites using a Restek Ultra Biphenyl column (2.2 µm, 2.1 × 50 mm)

Detecting New Designer Cannabinoids in Herbal Incense using LC-MS-MS with Fast Precursor Ion Scanning

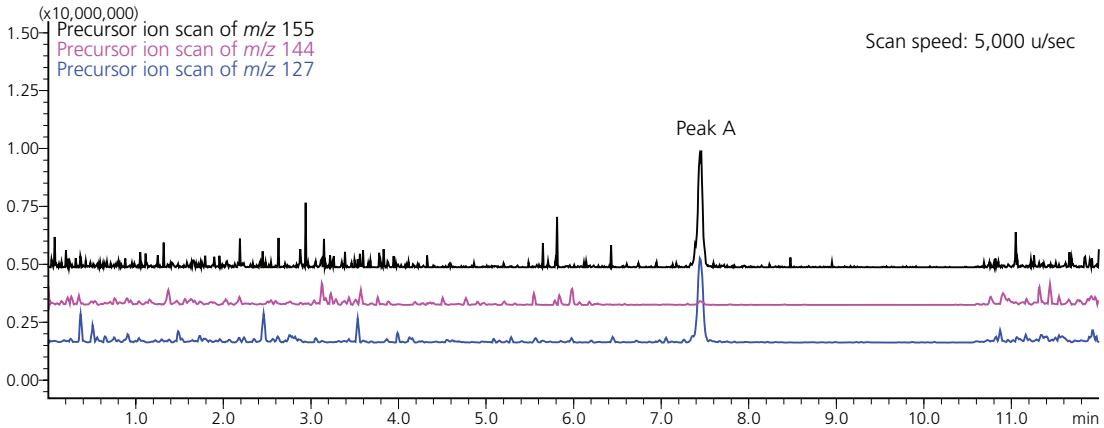


Fig. 5 Chromatograms of three precursor ion scans for the K2 Spice cannabinoid product. Peak A, eluting at 7.5 min, was investigated as a designer cannabinoid.

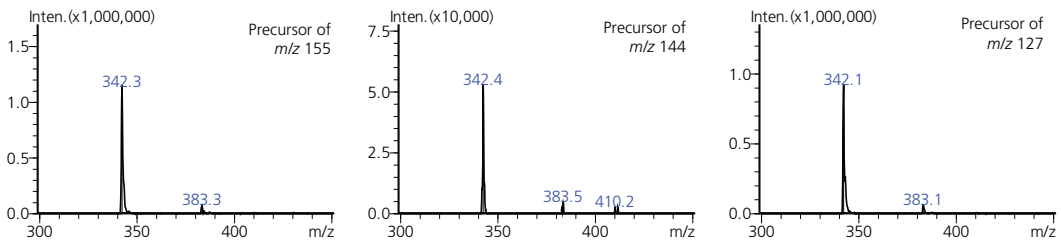


Fig. 6 Precursor ion spectra for Peak A

Because the possibility remains that some designer drugs might still not be detected by these precursor ion scans, full scan MS at a fast scan speed combined with fast data-dependent product ion scanning was used. A similar approach, using tailored fast precursor ion and neutral loss scanning with data dependent tandem MS, could be used to screen for designer barbiturates, amphetamines, and other classes of drugs as well.

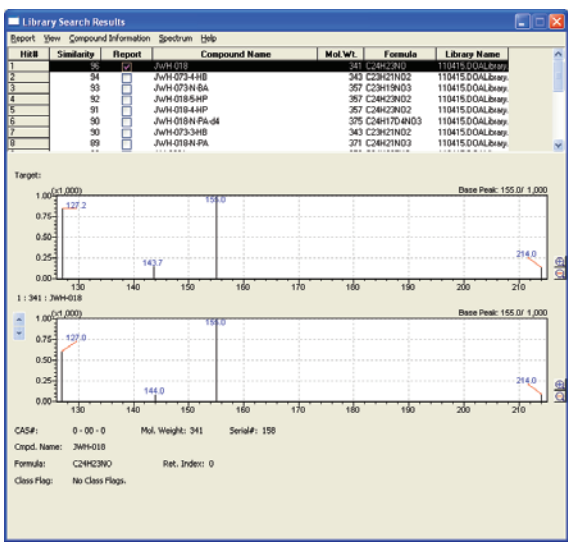


Fig. 7 Library search results for the tandem mass spectrum of Peak A. The top hit is the designer cannabinoid JWH-018. The other hits found are metabolites of synthetic cannabinoids which have different precursor masses and therefore can be distinguished from JWH-018.

Detecting New Designer Cannabinoids in Herbal Incense using LC-MS-MS with Fast Precursor Ion Scanning

A spectrum search in a private library matched several cannabinoids, including JWH-018. The retention time anrd mass spectra of the unknown matched an authentic standard of JWH-018. Urine of a human subject testing positive for synthetic cannabinoid exposure also revealed the presence of synthetic cannabinoid metabolites of JWH-018 as shown in Fig. 9.

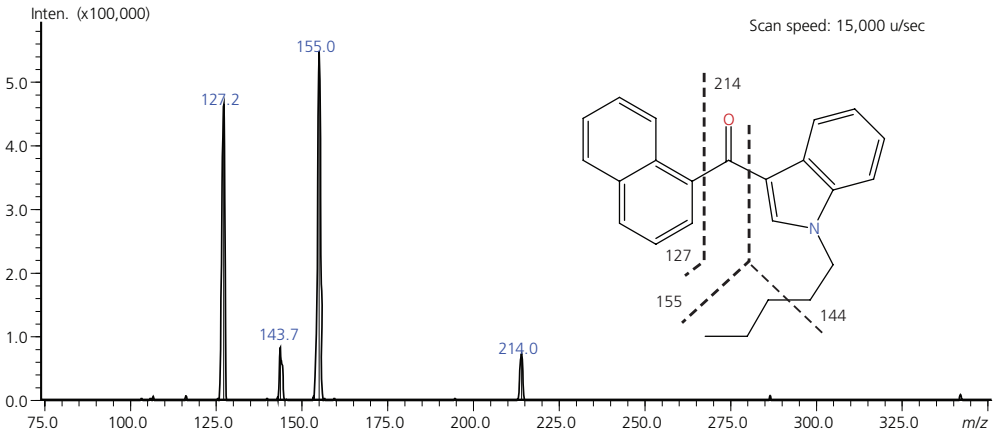


Fig. 8 Tandem mass spectrum of Peak A, identified as JWH-018, and fragment assignment

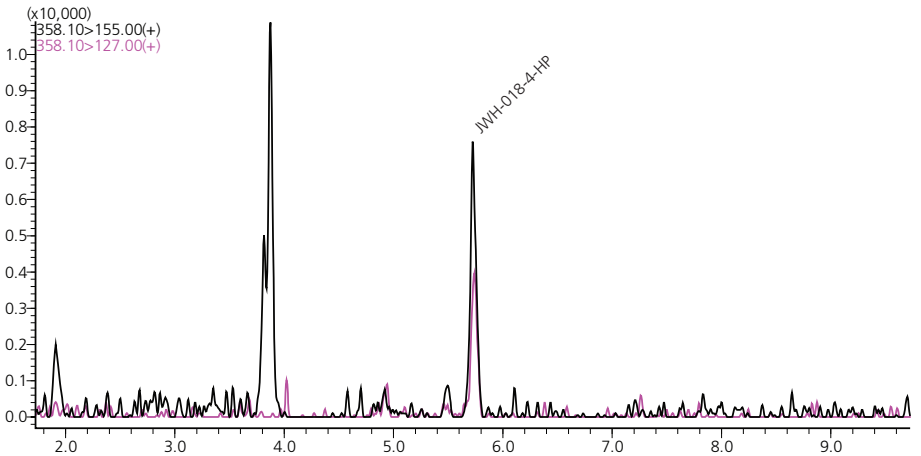


Fig. 9 Chromatograms for the metabolite JWH-018-4-HP from urine of a human subject with reported synthetic cannabinoid exposure.

Conclusion

Fast precursor ion scanning with data dependent MS-MS was used to detect designer drugs in herbal incense products. Metabolites of the drugs could also be detected using the higher sensitivity of the LCMS-8040 in the urine of human subjects with reported synthetic cannabinoid exposure. This fast precursor ion scanning method will enable screening for the latest designer drugs even before they are discovered by law enforcement.

First Edition: June, 2012

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Application Data Sheet

No.46

LC-MS
Liquid Chromatograph Mass Spectrometer

Analysis of Drugs in Putrefied Human Pleural Fluid using Triple Quadrupole LC/MS/MS

This application illustrates a drug screening method in putrefied human pleural fluid using the Shimadzu UFMS triple quadrupole mass spectrometer, LCMS-8050.

In forensic and toxicology fields, it is important to develop a highly sensitive and exhaustive methodology for screening and identifying drug substances. A wide range of these compounds in various biological matrices, such as whole blood, urine and tissue, need to undergo a simple and uniform sample pretreatment protocol prior to Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC-MS/MS) analysis. This data sheet presents a drug screening method using the LCMS-8050 with newly developed sample preparation protocol in putrefied human pleural fluid.

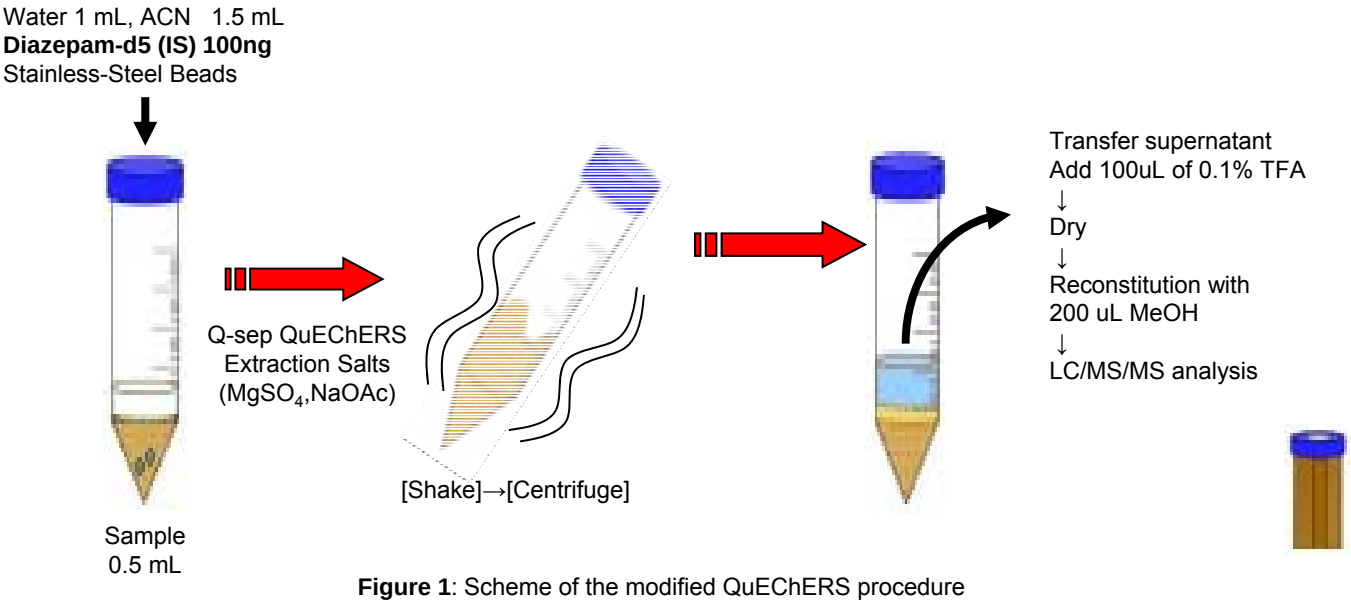

Figure 1: Scheme of the modified QuEChERS procedure

Table 1: Analytical Conditions

Liquid Chromatography

• Column	:Shim-Pack FC ODS (150x2mm, 3µm)
• Temperature	:40 °C
• Injected volume	:5 µL
• Mobile phases	:A: Water + 10 mM Ammonium Formate B: Methanol
• Flow rate	:0.3 mL/min
• Gradient	:5%B (0 min) – 95%B (15 min – 20min) – 5%B (20.1 min – 30 min)

Mass Spectrometry

• Configuration	:LCMS-8050
• Ionization mode	:Heated ESI positive and negative
• Nebulizing gas flow	:2 L/min
• Drying gas flow	:10 L/min
• Heating gas flow	:10 L/min
• DL temperature	:250 °C
• HB temperature	:400 °C
• Analysis mode	:MRM

Traditional sample preparation strategies for biological fluids, such as protein precipitation and solid phase extraction, require multiple time-consuming steps. In addition, commercially available sample preparation techniques lack the ability to extract all compounds of interest. A modified QuEChERS protocol for drug screening in biological fluids described here illustrates stable recoveries for drug substances regardless of sample or chemical properties.

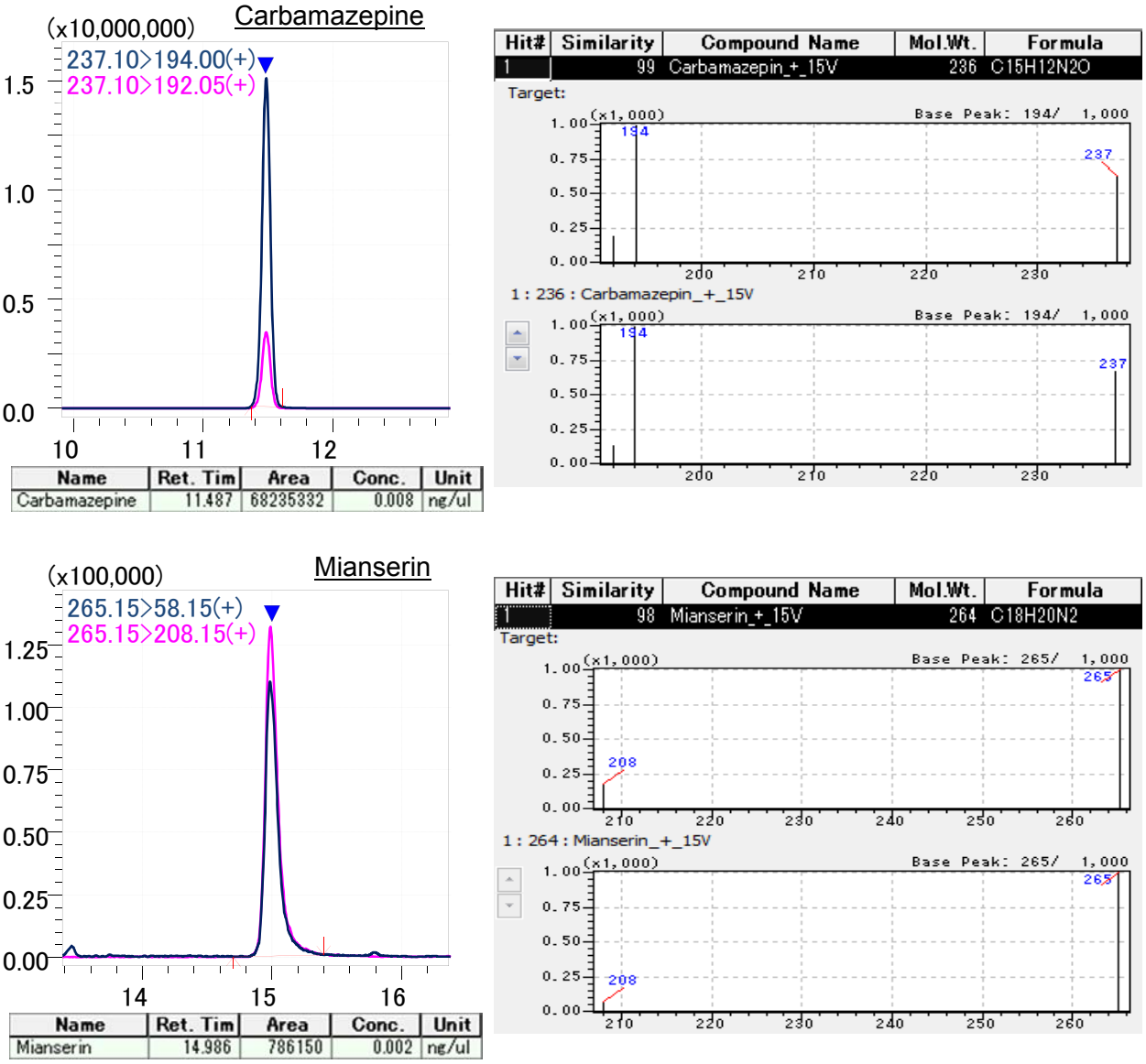

Figure 3: MRM Chromatograms, semi-quantitative value and Library Search results of Carbamazepine and Mianserin in putrefied human pleural fluid

Shimadzu's LCMS-8050 UFMS can perform Synchronized Survey Scan® (SSS), which automatically conducts a product ion scan triggered by preset MRM intensity thresholds. SSS provides both quantitative (MRM chromatograms) and qualitative data (Product ion spectrum) in a single run.

Furthermore, utilizing diazepam-d5 as an internal standard, semi-quantitative results can be determined using the method's built-in calibration curves (slope and intersection).

This software functionality is a very effective way for one to understand the quantitative values obtained during a simultaneous analysis.

First Edition: June, 2014

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Application News

SSI-LCMS-004

Liquid Chromatography Mass Spectrometry

Benzodiazepines and Barbiturates

LCMS-8030

Summary

A rapid, accurate, and reliable LC-MS-MS method was developed for the determination of 21 controlled substances in urine.

Background

Controlled substances play an important role in the practice of medicine but have the potential to be abused. Urinary testing for these drugs is an important tool in preventing diversion and abuse of controlled substances while ensuring patients with legitimate illness receive the medicines they need.

Method

Authentic standards for 21 benzodiazepines and barbituates were obtained from a licensed chemical supplier. Dilutions were made for optimization of mass spectrometry parameters. Positive and negative ion electrospray with polarity switching was used in Multiple Reaction Monitoring (MRM) mode for analysis. Optimized parameters are shown in Table 1. Quantifier and qualifier ions (not shown) were used for each compound.

A Shimadzu Shimpack XR-ODS III column (1.6 um, 2 x 50 mm) was used with a binary gradient of 25% ACN and water increasing to 85% ACN over 36 seconds. The column was equilibrated for 30 seconds. The flow rate was 0.6 mL/min and the column temperature was 50 °C. The injection volume was 10 mL. The standard autosampler needle rinse was used after each injection to eliminate the possibility of carry-over.

Calibration curves for standards in solution were prepared between 4.12 ng/mL and 1 mcg/mL. Four selected standards (alprazolam, diazepam, amobarbital, and talbutal) were prepared in a matching matrix (synthetic urine)in the concentration range of 37 ng/mL up to 2 mcg/mL. A weighting factor of 1/X was used, and all calibration points were repeated in triplicate. Low and High concentration QC samples containing each standard at 100 ng/mL and 1 mcg/mL respectively were prepared. Four test samples were prepared containing various amounts of the selected drugs along with an unspiked fifth test sample containing only synthetic urine (see Table 2). The urine spiked samples and the matrix matched calibration curve were diluted five-fold with mobile phase prior to analysis (dilute and shoot). Each test sample and QC sample was measured five times to determine intra-day repeatability.

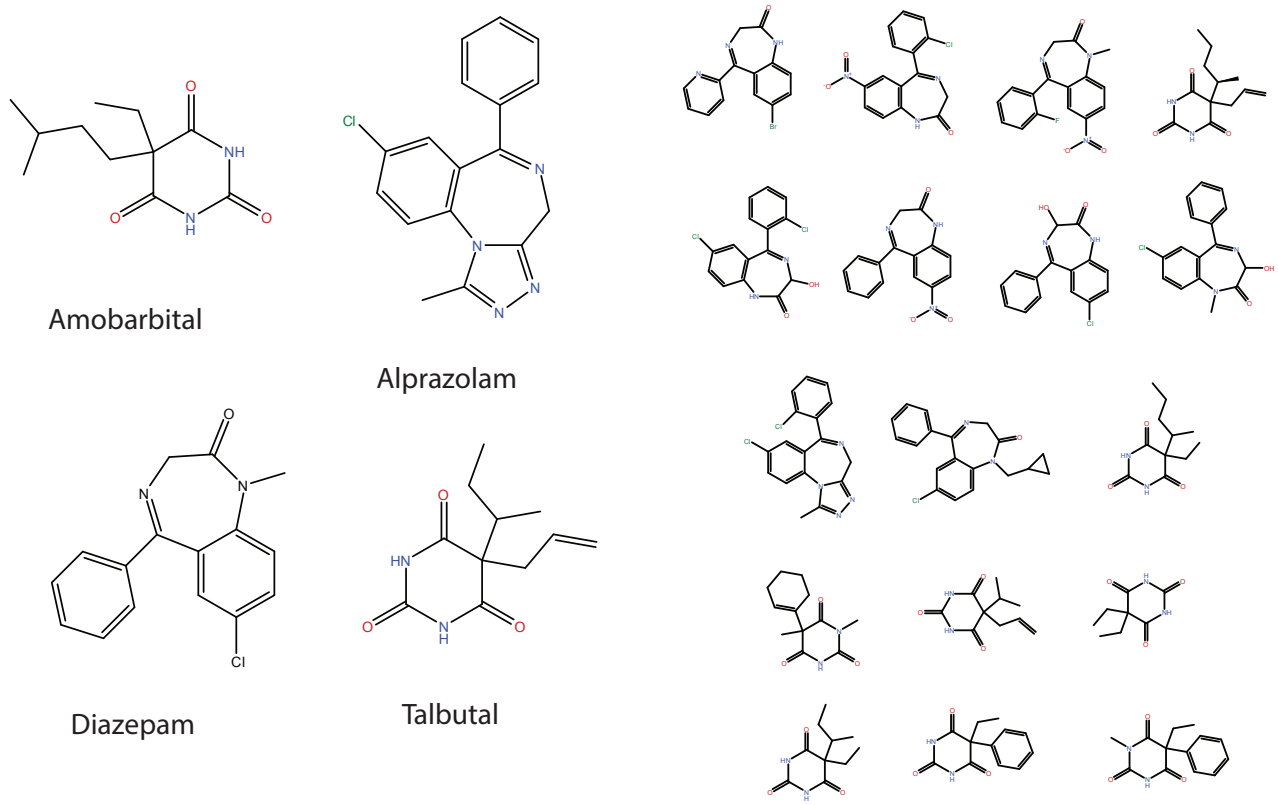


Figure 1: Structures of selected drugs of abuse

Table 1: MRM parameters

Type	Event#	+/-	Compound Name (m/z)	Dwell (msec)	Q1 Pre Bias(V)	CE	Measurement Time
MRM	1	+	Lorazepam 320.7>274.9	2	-30	-25	0-1.25
MRM	2	+	Nitrazepam 282.0>236.05	2	-11	-25	0-1.25
MRM	3	+	Oxazepam 286.7>240.95	2	-30	-25	0-1.25
MRM	4	+	Bromazepam 315.6>182.05	2	-30	-35	0-1.25
MRM	5	+	Diazepam 284.6>193.05	2	-28	-35	0-1.25
MRM	6	+	Temazepam 300.6>255.05	2	-30	-25	0-1.25
MRM	7	+	Prazepam 324.6>271.05	2	-30	-25	0-1.25
MRM	8	+	Alprazolam 308.7>281.0	2	-30	-30	0-1.25
MRM	9	+	Triazolam 342.7>308.0	2	-30	-30	0-1.25
MRM	10	+	Clonazepam 316.0>269.95	2	-16	-30	0-1.25
MRM	11	+	Flunitrazepam 314.05>268.15	2	-15	-30	0-1.25
MRM	12	-	Barbital 183.1>42.0	2	13	15	0-1.25
MRM	13	-	Talbutal 223.1>42.0	2	16	20	0-1.25
MRM	14	-	Pentobarbital 225.1>42.0	2	16	20	0-1.25
MRM	15	-	Hexobarbital 235.1>42.0	2	11	15	0-1.25
MRM	16	-	Butabarbital 211.1>42.0	2	10	20	0-1.25
MRM	17	-	Phenobarbital 231.0>42.0	2	11	15	0-1.25
MRM	18	-	Methylphenobarbital 245.1>42.0	2	11	15	0-1.25
MRM	19	-	Amobarbital 225.1>42.0	2	11	20	0-1.25
MRM	20	-	Secobarbital 237.1>42.0	2	11	20	0-1.25
MRM	21	-	Aprobarbital 209.1>42.0	2	15	15	0-1.25

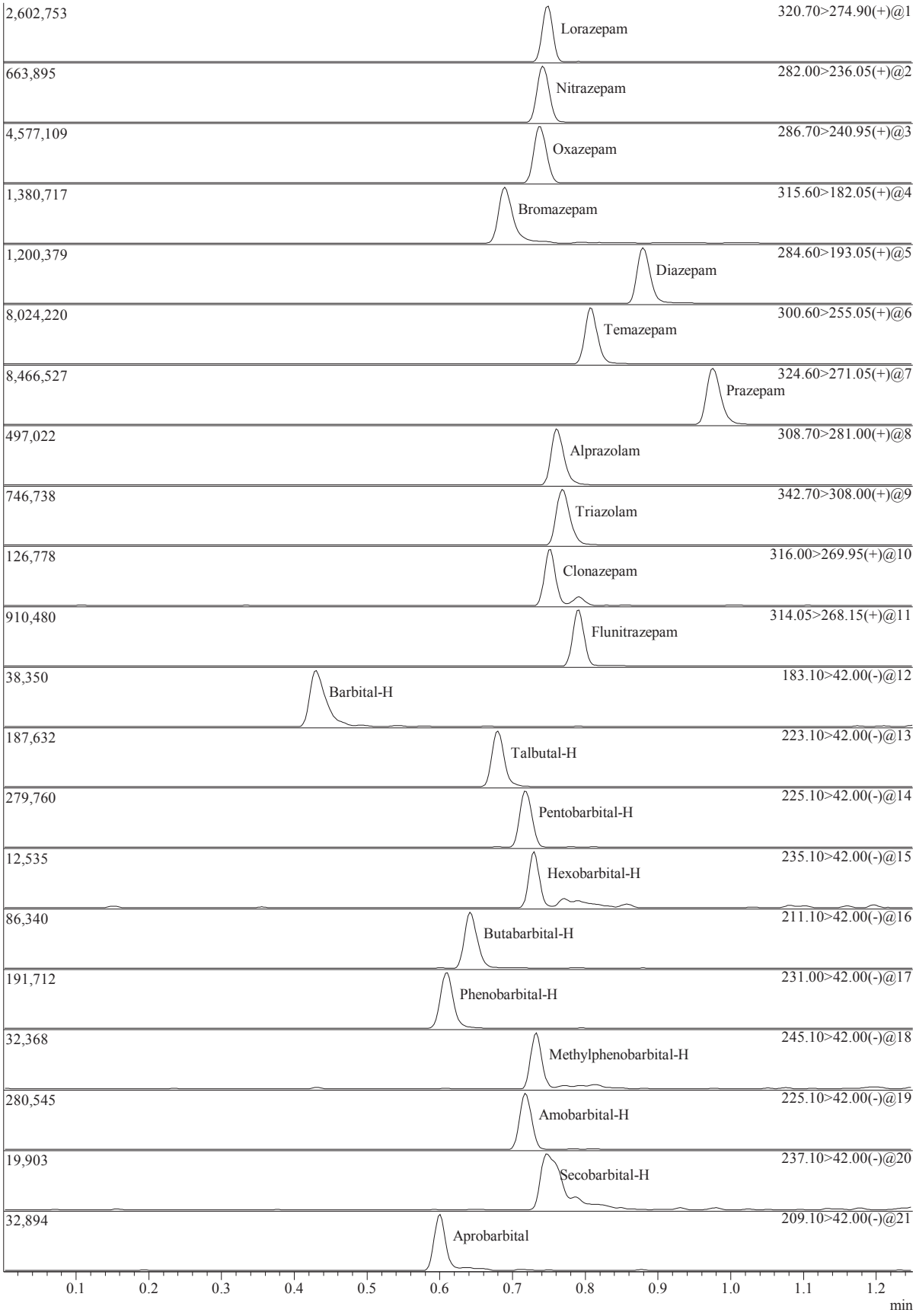


Figure 2: LC-MS-MS Chromatograms of the standard mix in solution

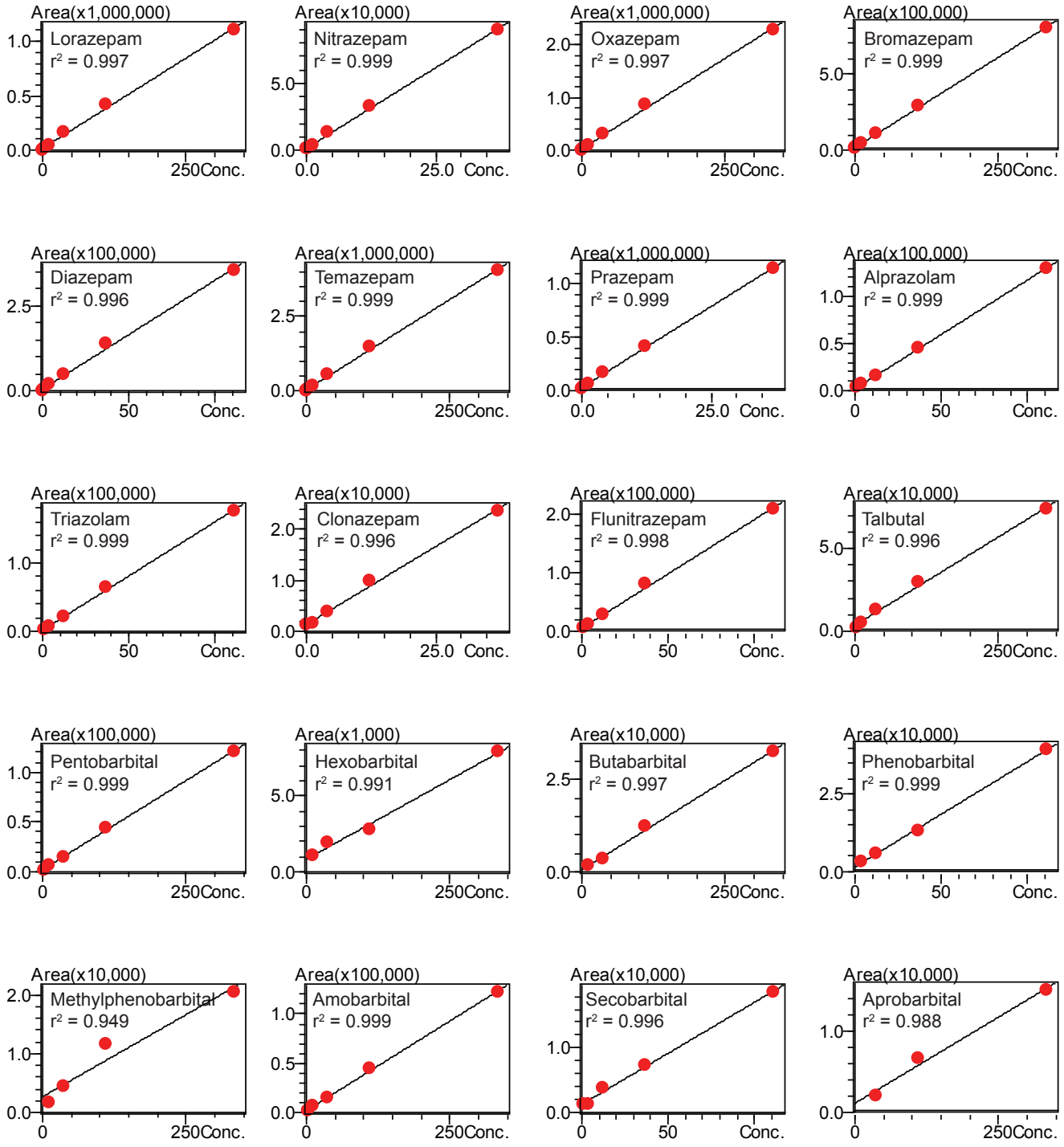


Figure 3: Calibration curves for standards in solution

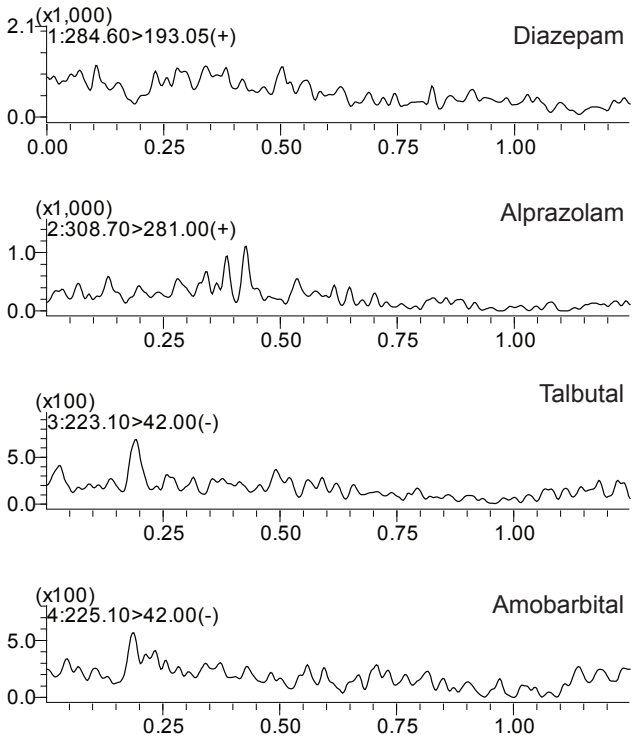


Figure 4: Blank injection immediately following matrix-matched high standard

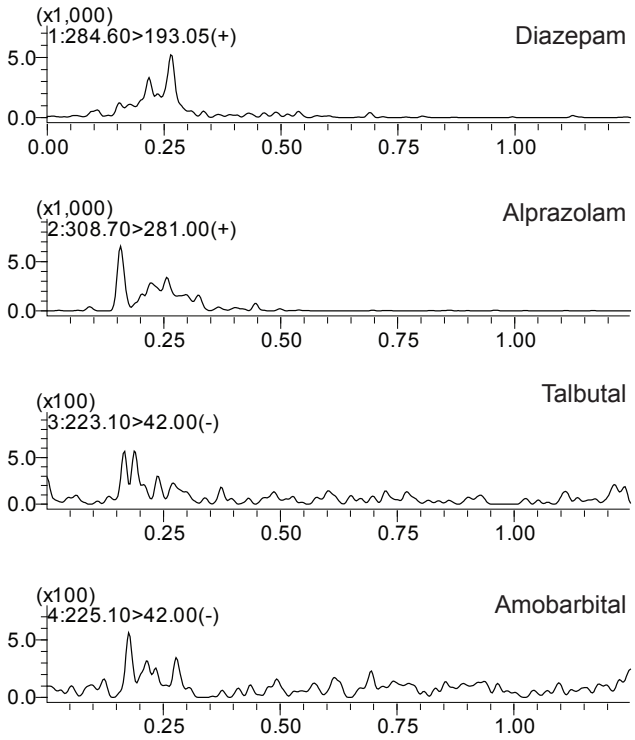


Figure 5: Matrix Blank

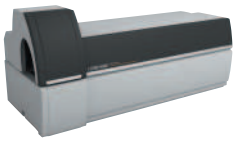
	Diazepam			Alprazolam			Talbutal			Amobarbital		
	Spiked	Measured	RSD%	Spiked	Measured	RSD%	Spiked	Measured	RSD%	Spiked	Measured	RSD%
Lower QC	100.00	106.00	3.11	100.00	100.20	7.09	100.00	101.50	9.72	100.00	120.25	12.72
Upper QC	1000.00	900.42	7.10	1000.00	1015.01	4.70	1000.00	1091.47	7.72	1000.00	1149.72	6.75
Test Sample 1	0.00	ND		0.00	ND		1000.00	1002.28	8.74	380.00	425.61	4.26
Test Sample 2	240.00	220.57	7.55	0.00	ND		0.00	ND		0.00	ND	
Test Sample 3	200.00	193.01	5.60	175.00	169.52	9.18	0.00	ND		0.00	ND	
Test Sample 4	110.00	106.87	6.51	0.00	ND		600.00	626.24	9.93	0.00	ND	
Test Sample 5 (Matrix Blank)	0.00	ND		0.00	ND		0.00	ND		0.00	ND	

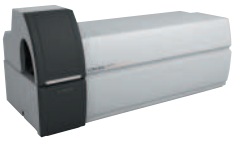
Table 2: Quantitative results of high and low QC, four spiked test samples, and the matrix blank. Each sample was run five times and the RSD% is shown.

Results and Discussion: All 21 compounds eluted within the 1.25 minute UHPLC run time with excellent peak shape and retention time stability. No carryover was detected in the blank injection immediately folowing the upper level matrix-matched calibration curve sample. Calibration curves for all compounds are shown in **Figure 3**. As shown in **Table 2**, the accuracy for all matrix-matched QC and test samples was within ±20% and the repeatability was within ±15% RSD. Higher precision and accuracy could be easily achieved by using an external or internal standard as appropriate. As expected, no analytes were detected in the unspiked matrix sample.

Conclusion: A rapid, accurate, and reproducible UHPLC-MS-MS method for drugs of abuse in urine was developed. Dilution was the only required sample treatment prior to analysis.

ULTRA FAST MASS SPECTROMETRY


LCMS-8040


LCMS-8030


LCMS-8080


LCMS-2020


LCMS-IT-TOF

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Application Note : 1702

Quick turnaround analysis of 8 drugs for driver under influence using LDTD-MS/MS system

Serge Auger, Jonathan Rochon, Jean Lacoursière and Pierre Picard
Phytronix Technologies, Québec, Canada
Keywords: High-throughput, screening, saliva, LDTD-MS/MS

Introduction

Each year, commonly abused drugs, such as Cannabinoids, Amphetamines, Cocaine, or Opioids become more easily available. As a consequence, there are an increasing number of individuals driving under the influence of these drugs. The recent judgment of the French Department of Justice specifies a cut-off (decision point) for the screening of 8 drugs in saliva (**Table 1**). A fast and effective method for sample extraction in saliva could provide a realistic and efficient approach for on-site drug screening using mobile laboratories.

A generic extraction method combined with LDTD®-MS/MS analysis was developed for fast turnaround screening of drugs in saliva. This new method could give police officers rapid and accurate answers in less than 10 minutes allowing on-site screening during a police roadblock. High Throughput capability of 400 samples per hour enable by LDTD-MS/MS run time of 9 seconds.

LDTD-MS/MS System

Figure 1 - LDTD-MS/MS system

Sample Preparation Method

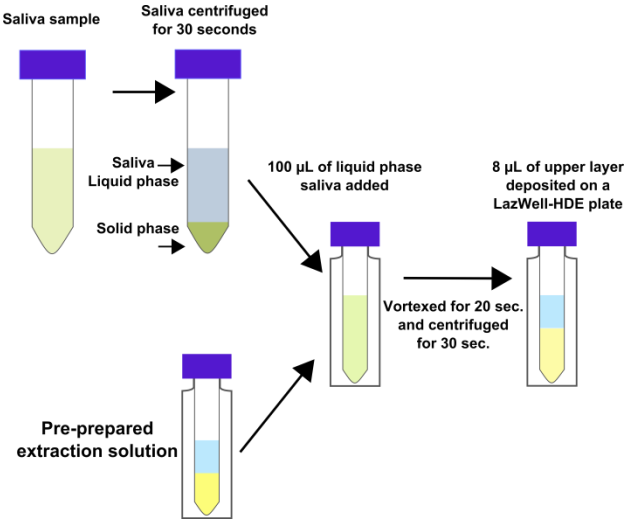

Figure 2 - Workflow from saliva collection to analysis

Saliva sample preparation:

- Approximately 1 mL of whole saliva was collected
- Centrifuge for 1 min. at 3000 rpm
- Spike at 50%, 100% and 150% of decision point concentration.

Pre-prepared extraction solution:

- In a 300 µL fused glass insert vial
- Add 100 µL of internal standard solution in acetonitrile.
- Add 75 µL of Extraction buffer

Saliva sample extraction:

- Add 50 µL of saliva
- Vortex 20 seconds
- Centrifuge 30 seconds at 5000 rpm
- Spot 8 µL of upper layer in a LazWell™ plate HDE
- Evaporate to dryness
- LDTD-MS/MS screening analysis after complete solvent evaporation

LDTD®-MS/MS Parameters

LDTD
Model: Phytronix, SH-960
Carrier gas: 3 L/min (air)
Laser pattern: 6 seconds ramp to 55% power.

MS/MS
Model: Shimadzu LCMS- 8060
Dwell Time: 3 msec
Pause Time: 3 msec
Total run time: 9 seconds per sample
Ionization: APCI
Analysis Method:

- Positive MRM transition

Table 1 - MRM transition

Drugs/Internal standard	Q1	Q3	CE	Cut-off (ng/mL)
Amphetamine	136.10	119.15	-15	50
Amphetamine-d5	141.10	124.10	-15	NA
Methamphetamine	150.15	119.15	-16	50
Methamphetamine-d9	159.15	125.20	-16	NA
MDMA	194.00	163.10	-14	50
MDMA-d5	199.00	165.10	-14	NA
Morphine	286.15	165.15	-40	10
Morphine-d3	289.18	165.15	-40	NA

Benzoylecgonine	290.15	168.15	-20	10
Benzoylecgonine-d8	298.26	171.19	-20	NA
Cocaine	304.15	182.15	-20	10
Cocaine-d3	307.15	185.19	-20	NA
THC	315.25	193.1	-25	15
THC-d3	318.25	196.14	-25	NA
6-AM	328.15	165.15	-36	10
6-AM-d6	334.25	165.15	-38	NA

Results and Discussion

LDTD®-MS/MS screening Method

Drug extract obtained were analyzed using a MRM method in positive mode. After a fast desorption, fortified and blank samples are evaluated using peak area ratio. All samples having a peak area ratio higher than the cut-off standard are classified as drug positive samples.

Precision of Screening Method

Spiked samples at 50%, 100% and 150% of the decision point and blank solutions (one with IS solution and one without) are used to validate the precision of the screening method. Each concentration must not exceed 20% CV and the mean concentration ± 2 times the standard deviation must not overlap with other concentrations at the decision point. The peak area against IS ratio was used to normalize the signal. Triplicate extractions, analyzed twice, are deposited on a LazWell96HDE plate and dried before analysis. No overlapping at the decision point is observed for all curves and the CV% was below 15%. Results using the ± 2 STD overlay are plotted. **Figure 3** shows the results for the all the drugs of abuse tested.

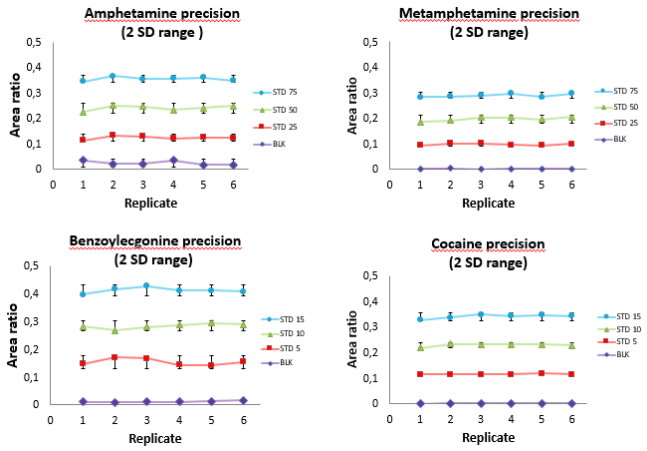

Figure 3 - Precision Screening curve

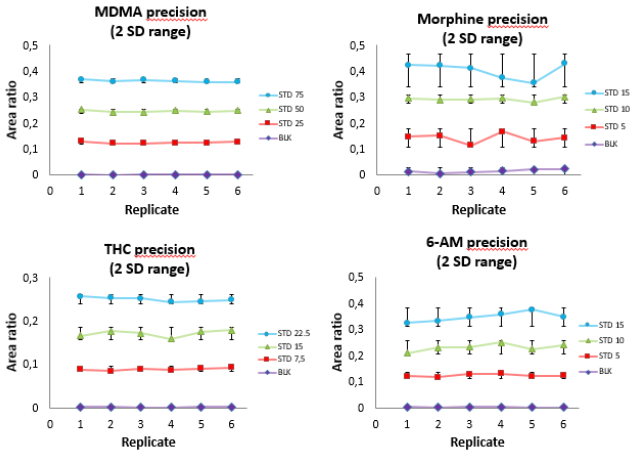

Figure 4 - Precision Screening curve

Matrix effect evaluation

6 different matrices were collected. Each sample was divided in two parts. One part was used as a blank sample to validate **Negative** samples and the other part was spiked at 150% of decision point to validate **Positive** results for the 8 drugs of abuse. Example for the Benzoylecgonine is shown in Table 2 where the ratio of 150% spiked sample (15 ng/mL) clearly discriminates from the value of the cut-off ratio (0.28 at 10 ng/mL). No false negative or false positive results were observed.

Table 2 - Benzoylecgonine area ratio for blank and spiked samples in different matrix

Matrix samples	Area ratio for Blank samples	Area ratio for Spiked samples
M1	0.0074	0.3774
M2	0.0045	0.3636
M3	0.0039	0.3850
M4	0.0040	0.4103
M5	0.0049	0.3868
M6	0.0087	0.4054

Conclusion

LDTD technology combined with the Shimadzu LCMS-8060 mass spectrometer system allows ultra-fast (**9 seconds per sample**) and specific drug screening in saliva samples with single and generic sample preparation.

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Application News
SSI-LCMS-060

Liquid Chromatography Mass Spectrometry

Analysis of 90 Multi-Class Drugs with Polarity Switching in Plasma

Liquid Chromatograph Mass Spectrometer
LCMS-8050

Summary: Ninety drugs and internal standards were prepared in plasma using a Biotage SLE 96-well plate and analyzed on an LCMS-8050 .

Background: Plasma is an important matrix to measure the exposure of the drug prior to elimination. Clinical research often requires that drug concentrations in blood be monitored. The capability of the LCMS-8050 to detect low concentrations of drugs in plasma was evaluated.

Method:
A 200 µL aliquot of plasma was extracted using a Biotage SLE 96-well plate. Samples were diluted with H_2O and eluted using ethyl acetate. The eluent was then dried down under nitrogen gas on an SPE dry 96 evaporation system and reconstituted in mobile phase, all in a 96-well plate. Samples were analyzed on an LCMS-8050 in MRM mode with a Nexera MP front end. Drugs were separated using a Biphenyl column (2.7uM ,100 X 2.1mm) from Restek. All analytes were eluted in under 4 minutes.

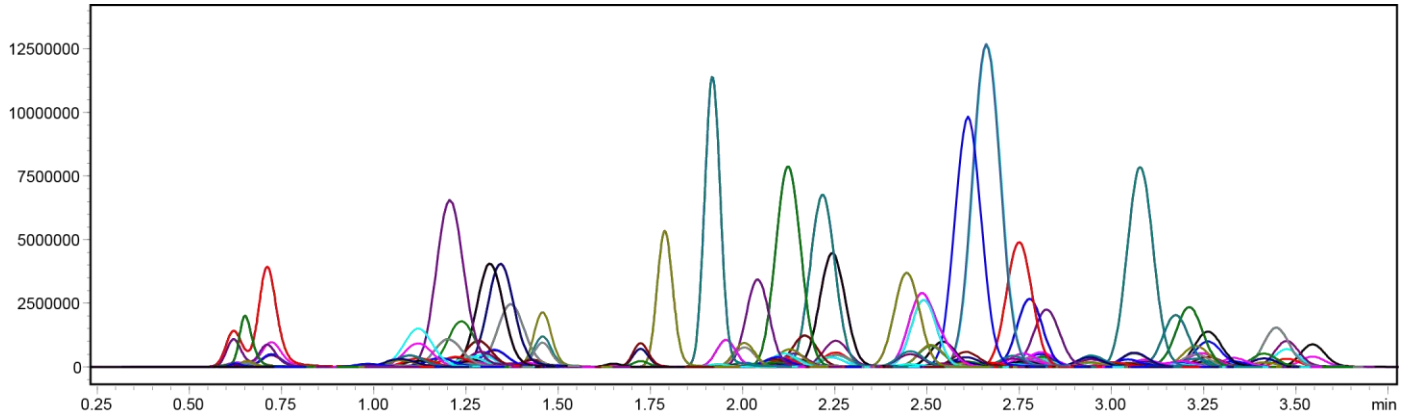

Figure 1. Chromatogram of all analytes eluted in under 4 minutes.

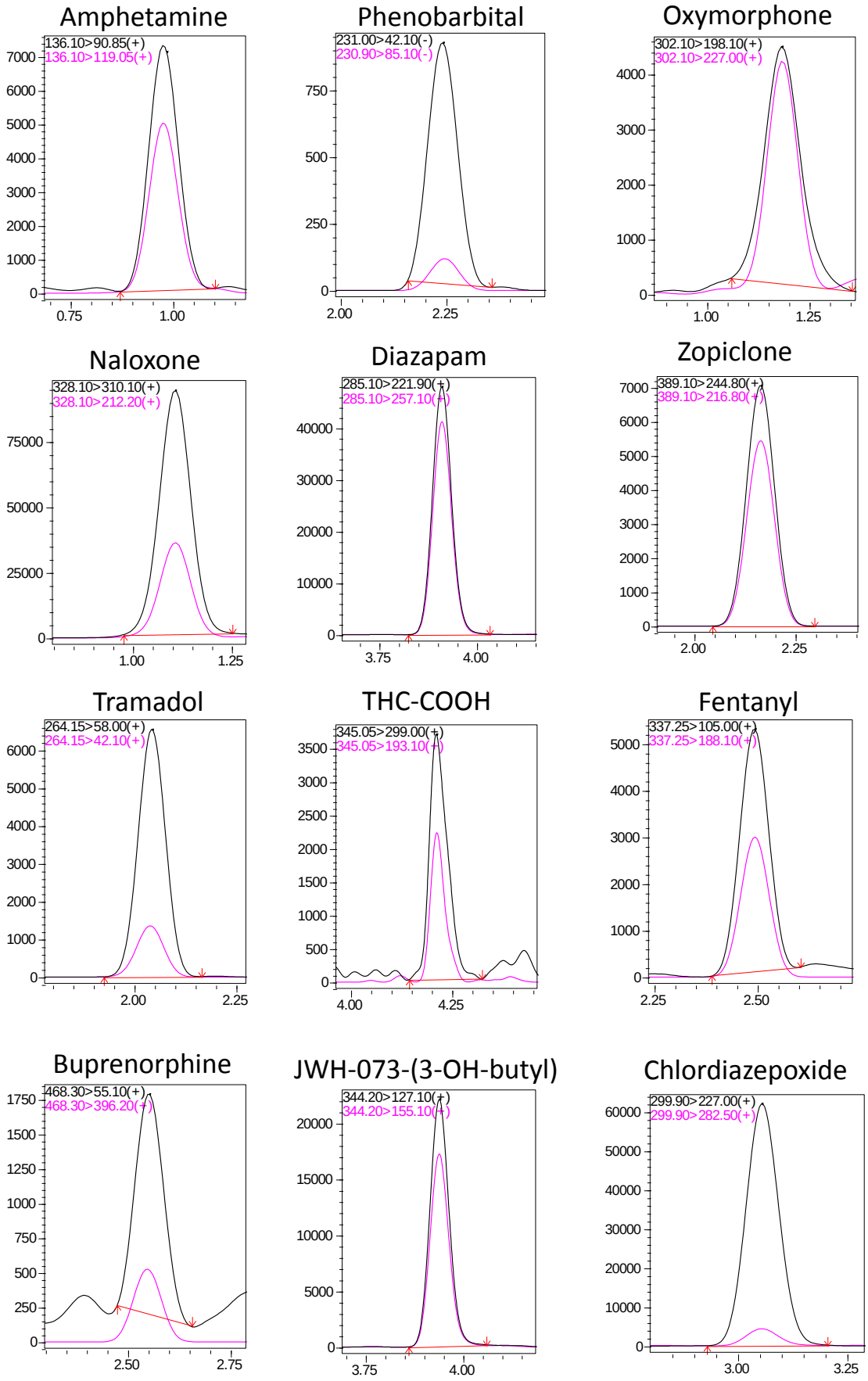

Figure 2. Chromatograms at the LLOQ for representative analytes.

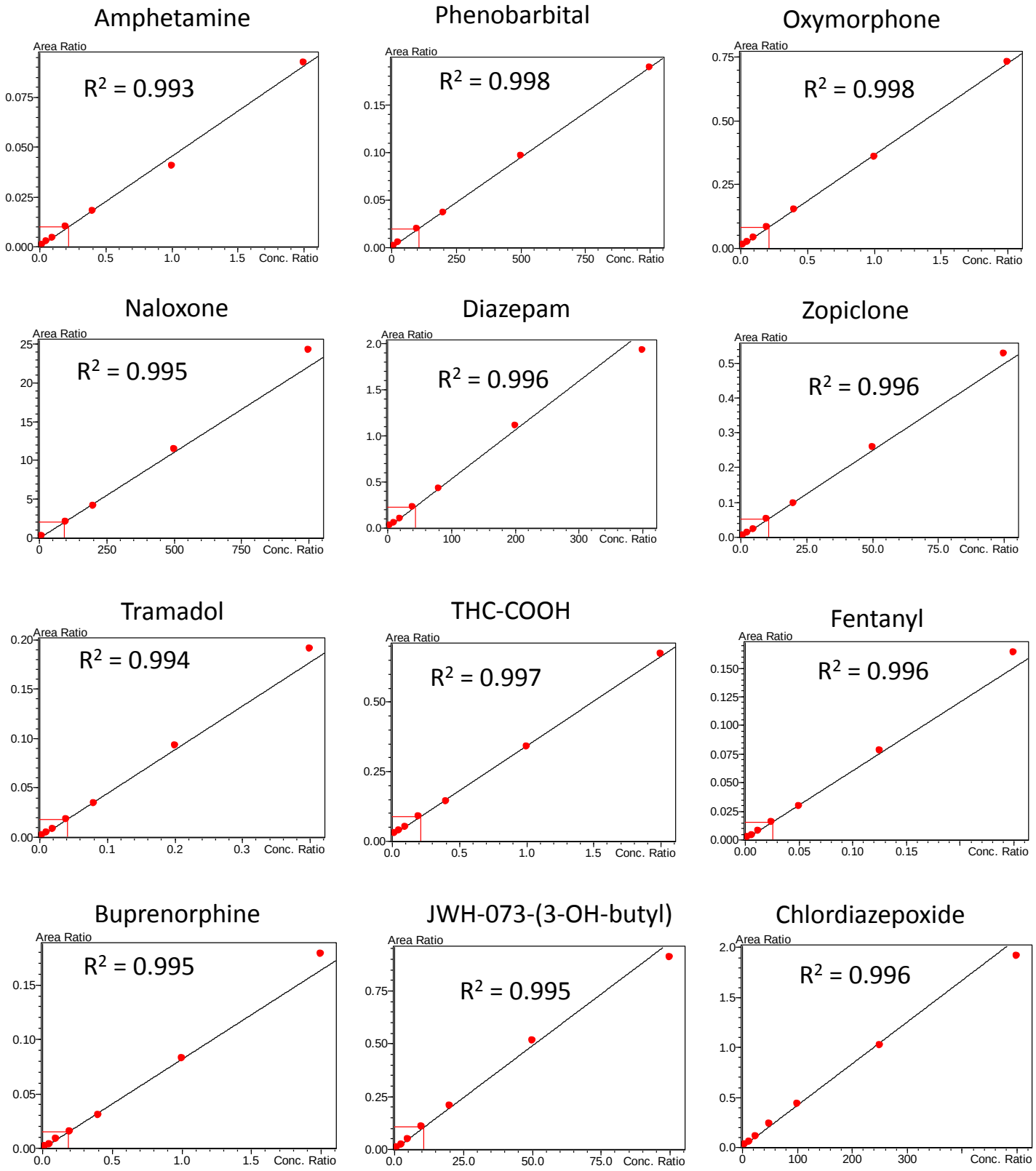

Figure 3. Calibration curves for representative analytes.

Table 1. The linear range and results for the LLOQ of all analytes.

Name	Retention time	Area	Linear Range (ng/mL)	Calc. Con. (ng/mL)	Std. Conc.	Accuracy
Morphine	0.619	135853	25 - 500	24.42	25	97.7
Oxymorphone	1.183	26554	5 - 500	5.02	5	100.4
Noroxycodone	0.649	43649	2.5 - 250	2.63	2.5	105.1
Hydromorphone	0.711	156603	5 - 250	5.08	5	101.5
Amphetamine	0.985	33637	5 - 500	4.88	5	97.5
Methylhexanamine	1.065	101215	5 - 500	5.2	5	103.9
Naloxone	1.108	504071	10 - 1000	10.09	10	100.9
6-MAM	1.278	5731	0.4 - 40	0.39	0.4	98.7
Methamphetamine	1.209	16449	5 - 500	5.13	5	102.6
Phentermine	1.214	7,750	5 - 500	5.18	5	103.6
Codeine	1.249	50918	5 - 500	5.1	5	102
Norhydrocodone	1.259	107,903	5 - 500	5.17	5	103.4
Methylone	1.277	236363	1 - 100	1.02	1	102.3
O-desmethyltramadol	1.307	364833	5 - 500	5.37	5	107.4
MDA	1.21	172644	5 - 500	5.19	5	103.8
4-methylephedrine	1.359	80,290	5 - 500	5.23	5	104.6
Oxycodone	1.414	176617	2.5 - 250	2.54	2.5	101.7
MDMA	1.442	419135	5 - 500	5.06	5	101.2
Hydrocodone	1.493	20536	5 - 500	5.02	5	100.4
Diethylpropion	1.636	13,651	5 - 500	5.09	5	101.8
MDEA	1.711	236144	5 - 500	5.05	5	101
Norfentanyl	1.926	23074	0.4 - 40	0.41	0.4	102.3
Tapentadol	1.954	30340	2.5 - 250	2.58	2.5	103.3
Dextrorphan	1.999	299180	5 - 500	5.28	5	105.5
Tramadol	2.044	29628	1 - 100	1.05	1	104.9
Benzoylecgonine	2.083	407	2.5 - 250	2.53	2.5	101.1
Methylphenidate	2.085	100482	5 - 500	4.64	5	92.9
Normeperidine	2.105	166951	2.5 - 250	2.64	2.5	105.7
alpha-PVP	2.113	85311	1 - 100	1.04	1	104.3
Meperidine	2.119	192731	2.5 - 250	2.55	2.5	102.1
Meprobamate	2.142	90452	5 - 500	5.31	5	106.2
Bupropion	2.151	81502	2.5 - 100	2.56	2.5	102.4
Zopiclone	2.165	36901	1 - 100	1.04	1	103.6
MDPV	2.8	145770	1 - 100	1.02	1	102
Phenobarbital	2.243	4155	10 - 1000	10	10	100
7-aminoclonazepam	2.244	347504	4 - 400	4.04	4	101
Norbuprenorphine	2.256	2335	2.5 - 250	2.45	2.5	97.9
Butalbital	2.305	4730	10 - 1000	10.37	10	103.7
Desmethyldoxepin	2.448	272384	5 - 500	5.31	5	106.2
Zolpidem	2.454	24029	1 - 20	1.04	1	103.8
Pentobarbital	2.461	7623	10 - 200	10.07	10	100.7
Amobarbital	2.464	5103	10 - 200	10.33	10	103.3
Doxepin	2.477	19875	5 - 100	5.14	5	102.9
Fentanyl	2.494	26377	0.05 - 5	0.05	0.05	103.2
Fluoxetine	2.503	474850	2.5 - 50	2.66	2.5	106.6
Buprenorphine	2.553	7540	0.5 - 10	0.5	0.5	100.9
Secobarbital	2.578	4,379	10 - 200	10.23	10	102.3
Flurazepam	2.584	90,697	4 - 80	4.11	4	102.80
Mitragynine	2.78	2624	0.1 - 2	0.1	0.1	102.4
Carisoprodol	2.601	191006	5 - 100	5.13	5	102.6
Phencyclidine	2.615	35040	0.5 - 10	0.5	0.5	100.6
Propoxyphene	2.663	76949	5 - 100	5.04	5	100.7
Protriptyline	2.712	12487	5 - 500	4.9	5	98
Desipramine	2.722	190852	5 - 500	5.14	5	102.8

Table 1.(Continued) The linear range and results for the LLOQ of all analytes.

Name	Retention time	Area	Linear Range(ng/mL)	Calc. Con. (ng/mL)	Std. Conc.	Accuracy
Imipramine	2.774	124106	5 - 100	5.09	5	101.8
Cyclobenzaprine	2.801	292087	5 - 250	5.06	5	101.1
EDDP	2.81	64096	5 - 100	5.21	5	104.2
Nortriptyline	2.82	22163	5 - 500	4.85	5	96.9
Amitriptyline	2.853	517	5 - 500	4.83	5	96.6
Trimipramine	2.934	105597	5 - 500	5.07	5	101.5
N-desmethylflunitrazepam	2.942	156263	4 - 200	3.94	4	98.4
Lorazepam	2.995	68387	4 - 200	4.13	4	103.3
Desmethylclomipramine	3.035	93075	5 - 250	5.25	5	105
Risperidone	2.539	164080	0.5 - 10	0.53	0.5	105.3
Chlordiazepoxide	3.055	335299	5 - 500	4.93	5	98.5
Oxazepam	3.07	61690	4 - 400	4.03	4	100.7
Clonazepam	3.082	123232	4 - 400	3.96	4	99
Methadone	3.086	18359	12.5 - 250	12.76	12.5	102.1
Clomipramine	3.082	162619	5 - 500	5.08	5	101.7
Triazolamx	3.181	47530	4 - 200	4.09	4	102.2
Desalkylflurazepam	3.183	76196	4 - 400	4.13	4	103.1
Midazolamx	3.223	276869	4 - 400	4.2	4	105
Midazolam	3.255	539866	4 - 200	4.17	4	104.2
Alprazolax	3.257	108930	4 - 400	4.16	4	103.9
Methaqualone	3.296	73990	10 - 500	10.44	10	104.4
Zaleplon	3.327	587109	5 - 100	4.57	5	91.5
Nordiazepam	3.406	162996	4 - 400	4.17	4	104.3
Flunitrazepam	3.467	298163	4 - 400	4.22	4	105.5
Triazolam	3.539	263725	4 - 80	4.08	4	102.1
Temazepam	3.825	1422	10 - 400	10.49	10	104.9
Alprazolam	3.628	81689	4 - 400	3.98	4	99.6
AB-PINACA	3.776	413805	1.5 - 150	1.54	1.5	102.6
Diazepam	3.91	176095	4 - 400	4	4	99.9
JWH-073-(3-OH-butyl)	3.939	78981	1 - 100	1.03	1	102.6
AB-FUBINACA	3.64	243101	1.5 - 150	1.55	1.5	103.5
JWH-018-(4-OH-pentyl)	4.093	19287	1 - 100	1.02	1	102.4
THC-COOH	4.212	11899	1.5 - 150	1.47	1.5	97.8
XLR-11	4.35	53481	3.75 - 75	3.86	3.75	103
Aripiprazole	3.23	3625	1 - 50	1	1	99.7
Quetapine	2.698	21954	2.5 - 250	2.49	2.5	99.5

Results and Discussion: Ninety different drugs and internal standards from multiple drug classes including opioids, amphetamines, sympathomimetics, benzodiazepines, barbiturates, tricyclic antidepressants, and cannabinoids were extracted from plasma. With an extraction time under an hour, the SLE extraction in the 96 well format provides a high throughput sample prep technique to compliment the ultra fast separations and scan speeds of the LCMS-8050 coupled to a Nexera LC system.

Figure 1 shows representative chromatograms at the LLOQ for a few analytes demonstrating excellent signal to noise. These analytes represent the many drug classes that can be detected and quantitated by the LCMS-8050. As shown in **Table 1** and **Figure 2.**, the LCMS-8050 demonstrated excellent accuracy and linearity. Accuracy at the LLOQ ranged from 96.6% to 107.4%. The R^2 value for all curves was >0.990.

Conclusion: Using a rapid chromatographic separation, 90 drugs were detected and quantitated in less than five minutes. The LCMS-8050 was demonstrated to provide highly sensitive and accurate detection of drugs in plasma extracts.

ULTRA FAST MASS SPECTROMETRY

LCMS-8030 LCMS-8040 LCMS-8050 LCMS-2020 LCMS-IT-TOF

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Application News

SSI-LCMS-094

Liquid Chromatography Mass Spectrometry

LC-MS/MS Detection of Fentanyl and Related Synthetic Opioids in Biological Matrices Using the Shimadzu LCMS-8060

Liquid Chromatograph Mass Spectrometer
LCMS-8060

Summary:

A single LC-MS/MS method was developed and optimized using the Shimadzu LCMS-8060 to identify and determine a limit of detection (LOD) for several synthetic opioids including fentanyl , and several fentanyl analogues (Figure 1) as well as a few of their major metabolites in blood and urine matrices.

Background:

The potency of these compounds result in small concentrations being detected in real-world samples, thus requiring methods utilized by a forensic or clinical research laboratory to be very sensitive. Hyphenated techniques such liquid chromatography-tandem mass spectrometry (LC-MS/MS) can achieve this needed sensitivity through multiple reaction monitoring (MRM). The Shimadzu LCMS-8060 features a heated dual ionization source (DUIS) coupled with ultrafast MRM acquisition software and polarity switching for increased accuracy, sensitivity and robustness.

DUIS combines electrospray ionization (ESI) and atmospheric-pressure chemical ionization (APCI) by using the ESI source and the corona needle used in APCI.

The development process involved MRM optimization, MS source optimization and finally column selection for a method that successfully separated and identified a mixture of synthetic opioids. This analytical method when used in combination with a validated solid phase extraction procedure, achieved sub-ng/mL detection limits in blood and urine samples.

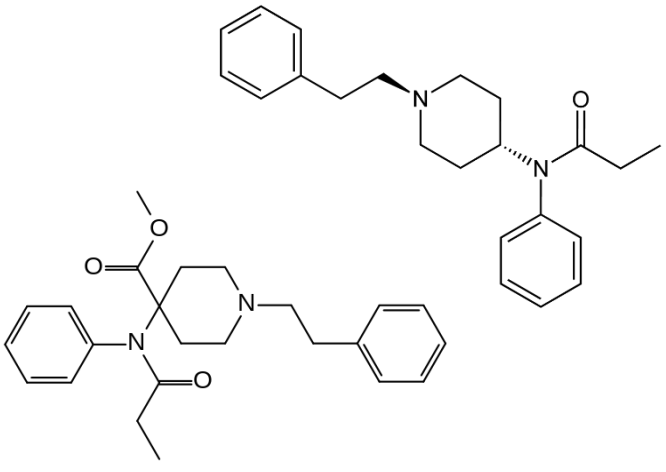

Figure 1: Chemical Structure of Fentanyl (top) and Carfentanyl (bottom)

Method: Method development began with optimizing the compounds on the LCMS-8060 using flow injection analysis and LabSolutions MRM Optimization Wizard. The MRM transitions were fully optimized to enhance sensitivity of all of the compounds in the mixture. The LC flow rate was set at 0.5 mL/min with a 50:50 mixture of mobile phases A and B. The mass spectrometer source parameters and LC conditions are listed in Table 1. The optimized MRM transitions for each compound are listed in Table 2.

Following the MRM optimization, four LC columns were evaluated using a five minute LC gradient starting at 20% mobile phase B increasing to 80%. The Restek Raptor Biphenyl column was chosen for the study after a comparison between peak shape, peak separation, and sensitivity on column was made. The overall chromatograms for the four columns are represented by Figure 2.

Table 1: LC/LCMS Analysis Conditions

LCMS-8060 Source Parameters		LC Conditions	
Ion Source:	DUIS	Flow Rate:	0.5 mL/min
Interface Temp:	400 ℃	Mobile Phase A:	95% Water: 5% Methanol: 0.1% Formic Acid
Desolvation Line Temp:	200 ℃	Mobile Phase B:	100% Methanol: 0.1% Formic Acid
Heat Block Temp:	250 ℃	Injection Solvent:	20% MPB: 80%MPA
Nebulizing Gas Flow:	1.5 L/min	Column Oven:	40 ℃
Heating Gas Flow:	15 L/min	Analytical Column:	Restek Raptor Biphenyl 100 x 2.1 mm (2.7 µm)
Drying Gas Flow:	5 L/min	Injection Volume	30 µL

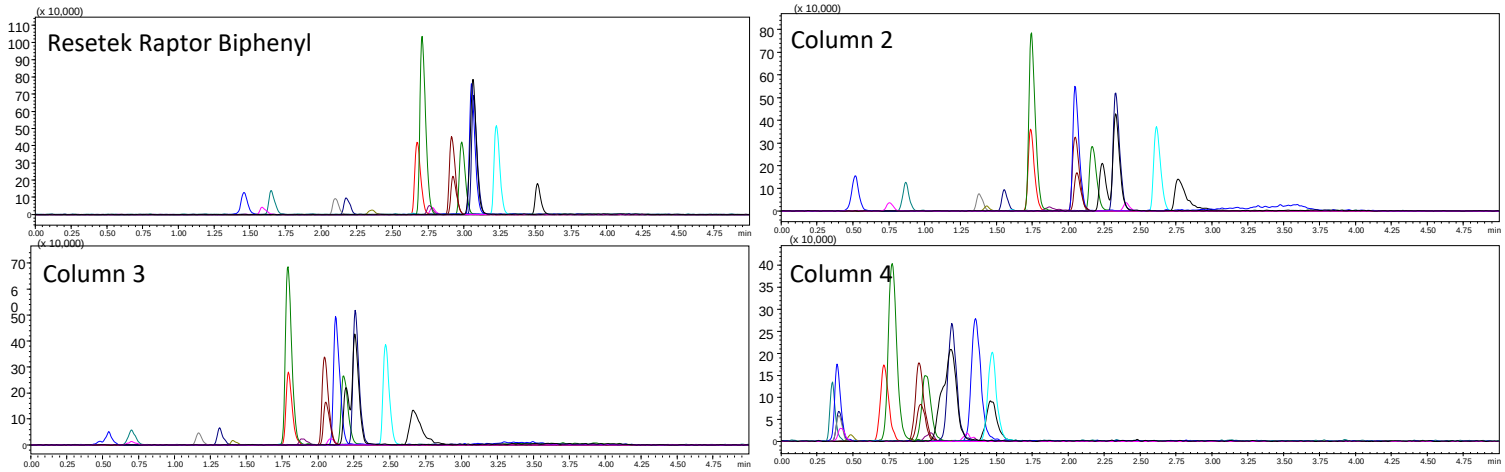

Figure 2: Total ion chromatograms of the fentanyl mixture on four different LC columns. The Restek Raptor Biphenyl column is represented by the top left chromatogram.

Serial dilutions (1:5) of a 1000 pg/mL solution were used to create a 6 point calibration curve in certified blank urine and certified blank whole blood. Fentanyl-d_5 was used as the internal standard.

The samples were extracted for analysis using a simple solid phase extraction. The sample was added to the cartridge after the SPE cartridge was conditioned. The wash solutions were added to the cartridge and the compounds were eluted off using a mixture of $MeCl_2$/IPA/NH_4OH. Once eluted the samples were dried down and reconstituted in 80:20 Water:MeOH mixture.

The calibration curve in matrix was prepared in triplicate along with a certified matrix blank spiked with internal standard, a solvent blank, and a single unknown blood sample. The unknown blood sample was calculated against both matrix curves.

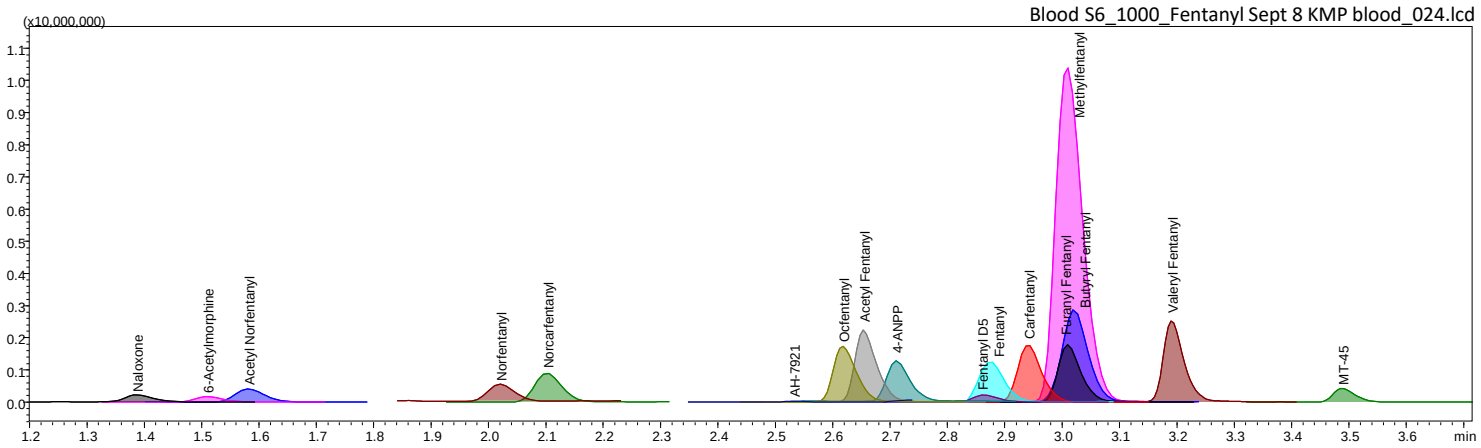

Figure 2: 1000 pg/mL extracted blood sample

Figure 3 shows the peak shape and separation of a 1000 pg/mL extracted blood sample on column.

The limit of detection for each compound in matrix is based on the signal to noise ratio and is listed in Table 2. The high LOD for Naloxone in blood is due to co-eluting peak that interferes with the Naloxone transition at lower concentrations.

Table 2: MRM Transitions and LOD of each compound

Compound	Quant Ion	Qual Ion	Blood LOD (pg/mL)	Urine LOD (pg/mL)
Methylfentanyl	351.10>105.10	351.10>202.25	1.6	40
6-Acetylmorphine	328.00>181.10	328.00>165.10	1.6	40
Naloxone	328.00>310.20	328.00>212.10	200	8
Fentanyl	337.30>188.20	337.30>105.10	8	8
Acetyl Fentanyl	323.10>188.20	323.10>105.15	1.6	40
Butyryl Fentanyl	351.10>105.25	351.10>188.20	8	40
Norfentanyl	233.00>84.05	233.00>55.20	0.32	8
4-ANPP	281.10>188.20	281.10>105.10	8	8
Valeryl Fentanyl	365.10>188.20	365.10>105.05	8	8
Ocfentanyl	371.10>188.20	371.10>355.15	1.6	1.6
MT-45	349.30>181.15	349.30>166.10	1.6	40
Furanyl Fentanyl	375.10>188.20	375.10>105.15	1.6	8
Carfentanyl	395.10>335.30	395.10>113.25	1.6	8
Norcarfentanyl	291.10>231.30	291.10>146.25	0.32	40
Fentanyl D5	342.10>188.20	342.10>105.25		

Results and Discussion: The Shimadzu LCMS-8060 and analytical conditions shown in this application note have demonstrated the ability of the LCMS-8060 to detect Fentanyl, Fentanyl analogs, and metabolites at picogram per milliliter levels in extracted whole blood and urine matrices. The continuation of this study will include linear ranges, limit of quantitation of the compounds as well as precision and accuracy of the method and extraction.

ULTRA FAST MASS SPECTROMETRY


LCMS-8040


LCMS-8045


LCMS-8050


LCMS-8060


LCMS-2020


LCMS-IT-TOF

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Application News

No. GCMS-1403

Gas Chromatograph Mass Spectrometer

Analysis of Blood Alcohol by Headspace with Simultaneous GC-FID and MS Detection

■ Introduction

Determination of Blood Alcohol Content (BAC) has been a standard analytical method in criminal labs for many years. The typical instrument configuration consists of a static headspace instrument for sample introduction, followed by gas chromatography (GC) with two dissimilar capillary columns for separation, and two Flame Ionization Detectors (FIDs) for detection and quantitation. Two sets of data are obtained simultaneously, and the quantitative results from the two FIDs are compared for confirmation of the reported BAC levels.

With the BAC method, compound identification is done by comparing the retention time (RT) of blood alcohol in the unknown sample to the RT obtained from analysis of an analytical standard. Recently however, additional compound identification provided by matching the ethanol mass spectrum to a library spectrum, in addition to RT, has proven to offer an additional level of confirmation. This application note describes BAC analysis using a GC-FID in parallel with a mass spectrometer (MS) for positive compound identification.

■ Experimental

Instrument Configuration

The Shimadzu HS-20 Loop headspace sampler (Figure 1) was used in the static-loop headspace mode for sample introduction. Effluent from the HS-20 was split 20-to-1, and then divided to two identical columns using a 3-way "T" fitting. The outlet ends of the two columns were connected to the FID and MS detectors. Because the MS detector was under vacuum, RTs for the two columns were different and the exact split ratio between the FID and the MS was not determined. Instrument configuration and operating parameters are outlined in Table 1.

Figure 1: Shimadzu HS-20 Loop Headspace Sampler with GCMS-QP2010 SE

Table 1: Instrument Operating Conditions and Method Parameters

Head Space	**HS-20 Loop Model**
Operation Mode	Static headspace with loop
Sample	1-mL sample volume 10-mL headspace vial
Equilibration	15 minutes at 65 °C Agitation level 3 (of 9 levels)
Sample Loop	1-mL loop Vial pressurization 0.5 min, equilibration 0.1 min Loop load time 0.5 min, equilibration 0.1 min Injection time 0.5 min
Sample Pathway Temperature	150 °C
Transfer Line Temperature	150 °C

Gas Chromatograph	**GC-2010 Plus**
Injection	Split injection from HS-20, with 20:1 split ratio to inlet side of SGE SilFlow pre-column splitter ("T" fitting) Nominal 50:50 division to two capillary columns
Column	Pre-column "T" fitting splitter to two columns Rtx-BAC1, 30 m x 0.32 mm x 1.8 µm film (x2) Helium carrier gas Constant linear velocity, 40 cm/second (each column)
Oven Program	Isothermal at 40 °C Total GC run time 5.0 minutes Total cycle time 6.0 minutes

Detector #1	**GCMS-QP2010 SE**
Operating Mode	Scan mode 30-150 m/z
Ion Source	200 °C, EI mode, 70 eV
Solvent Cut Time	0.9 min
MS Interface	200 °C
Detector #2	**Flame Ionization Detector**
FID Temperature	240 °C
FID Gas Flow Rates	H_2 = 40 mL/min Air = 400 mL/min Makeup (He) = 30 mL/min

Sample Preparation

Forensic ethanol solutions were purchased commercially with concentrations of 0.01, 0.05, 0.2, and 0.4 g/dL. An internal standard (IS) solution of n-propanol was prepared at 0.2 g/dL in TOC-grade water. Finally, a control standard (CS) was prepared by mixing methanol, ethanol, acetone, and isopropanol in TOC-grade water at 0.05 g/dL. Aliquots for analyses were prepared by mixing 1.0 mL of the IS solution with 100 µL of the individual calibration or control standard in a 10-mL headspace vial, and sealing immediately with a crimper prior to analysis.

■ Results and Discussion

Chromatography

The FID was at atmosphere and the MS was under vacuum, so the Retention Times (RT) for the 4 target compounds were different in the two chromatograms. The different RTs are inconsequential, since all compounds were individually calibrated on each of the two detectors, and RTs using the standard procedure (i.e., dissimilar columns and two FIDs) would also have been different. No effort was made to adjust the RTs for this project, but this can be done quite easily by adding a restriction to the outlet of the FID column. The FID and MS chromatograms are shown in Figure 2 with the target compounds and internal standard labeled.

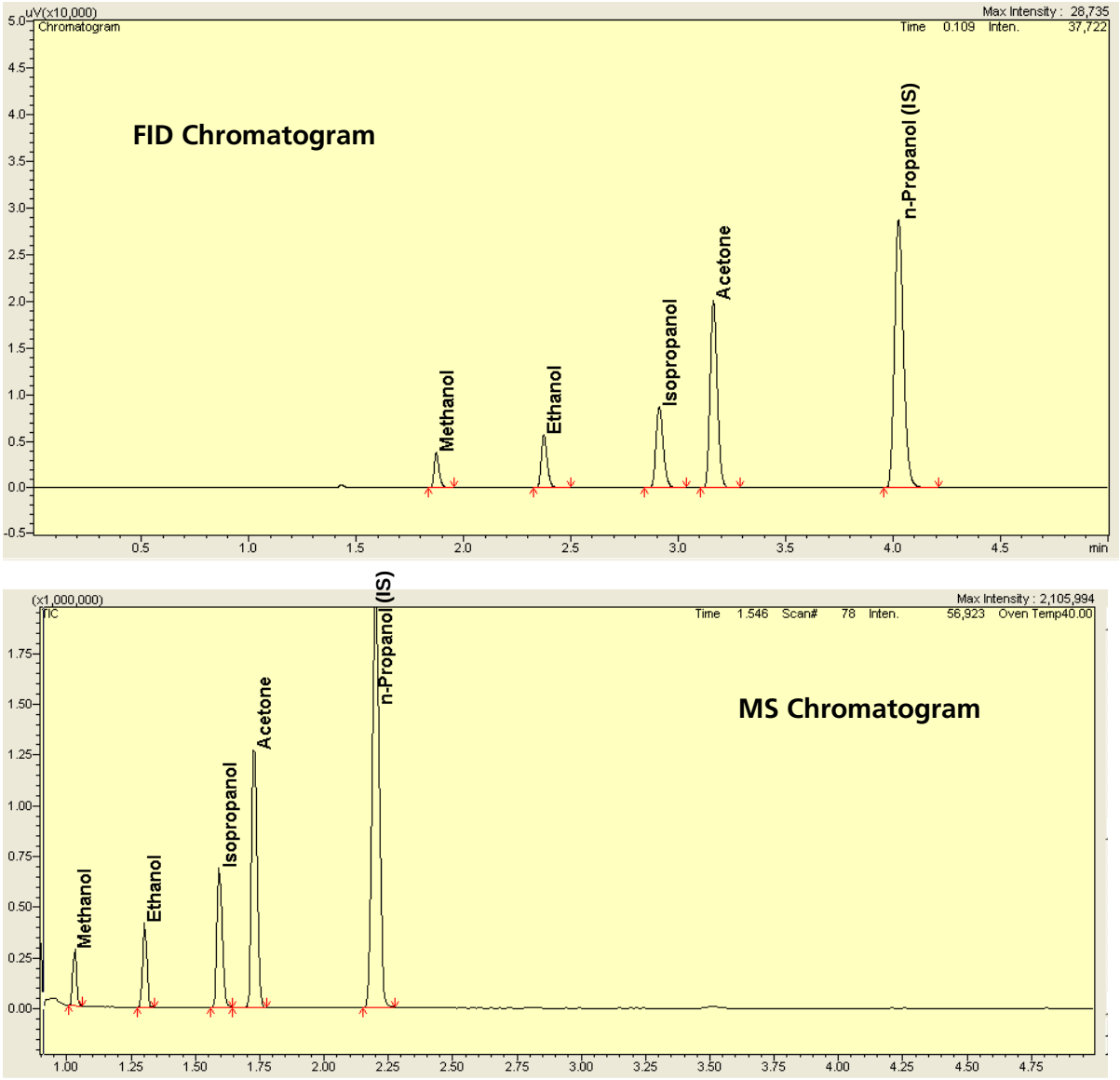

Figure 2: Chromatograms from the FID and MS with Compound Peaks Labeled

Ethanol Confirmation

Identity of the ethanol was confirmed in the MS chromatogram by matching the mass spectrum for the ethanol peak to the standard spectrum in the NIST Library. In all cases the identity of ethanol was confirmed through library matching with a similarity index of 98 or better. Figure 3 illustrates the NIST Library matching and confirmation of ethanol.

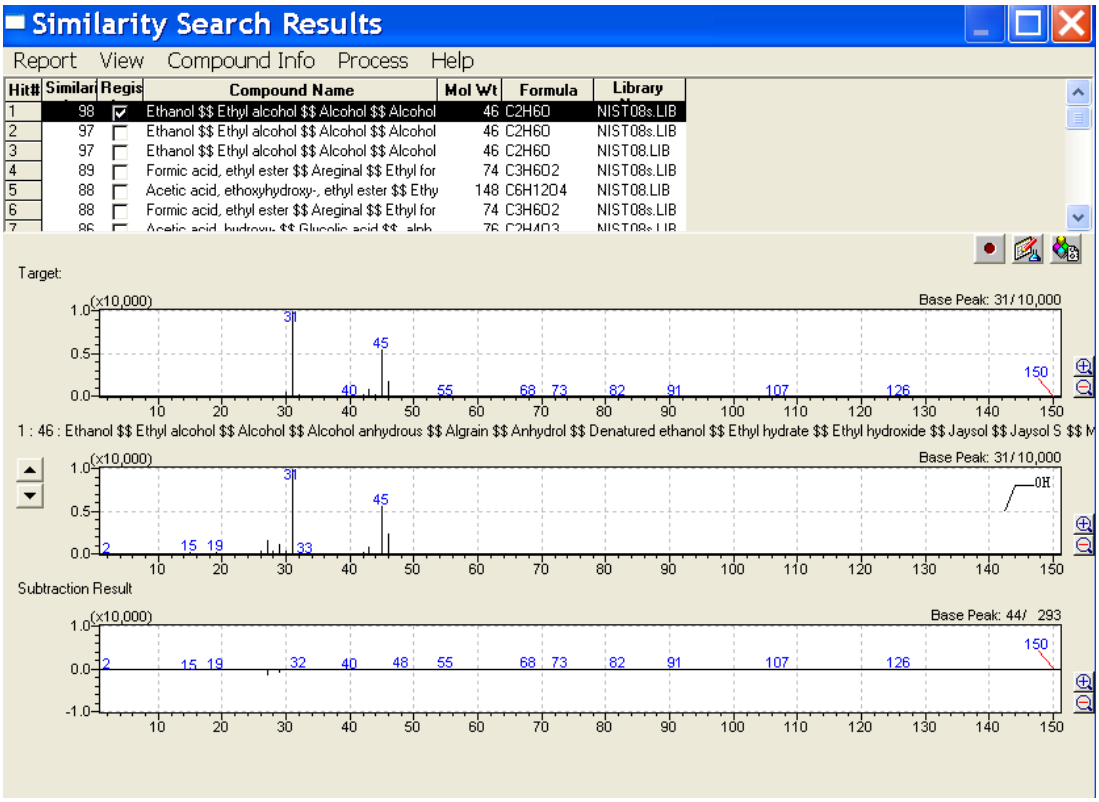

Figure 3: Mass Spectral Library Search Using the NIST11 Library to Confirm the Identity of Ethanol

Calibration

A 4-point calibration curve was generated by analyzing 3 individual aliquots at each calibration level. Data were collected on both the FID and the MS, and individual curves plotted using the internal standard technique. Calibration curves were created using the average of the data collected for the 3 individual standards at each concentration level. Figure 4 is the plotted calibration curves for ethanol on the FID and MS detectors. Table 2 shows the linearity for all 4 compounds in the FID and MS detectors.

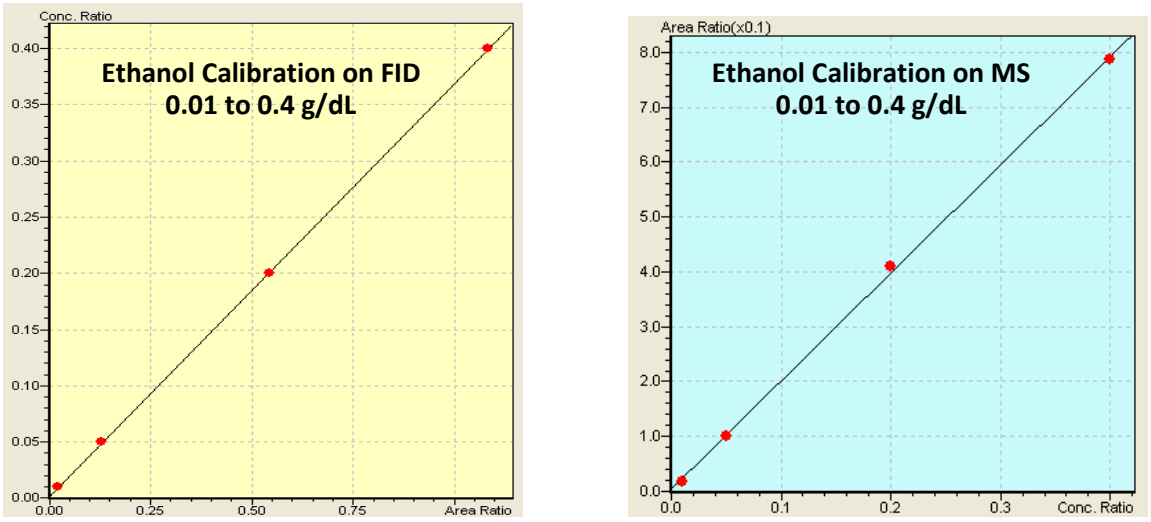

Figure 4: Calibration Curves for Ethanol on the FID and MS Detectors

Table 2: Linearity of Calibration Compounds on the FID and MS Detectors over Range of 0.01 to 0.4 g/dL

Compound	R^2 on FID	R^2 on MS
Methanol	0.9999	0.9995
Ethanol	0.9999	0.9998
Isopropanol	0.9999	0.9991
Acetone	0.9999	0.9992

Precision

Six replicate aliquots of the CS (0.05 g/dL) were prepared and analyzed using the conditions outlined in Table 1 to measure the analytical precision of the system. Overlaid chromatograms from the FID and MS are shown in Figure 5. Table 3 lists the precision results for all 4 target compounds.

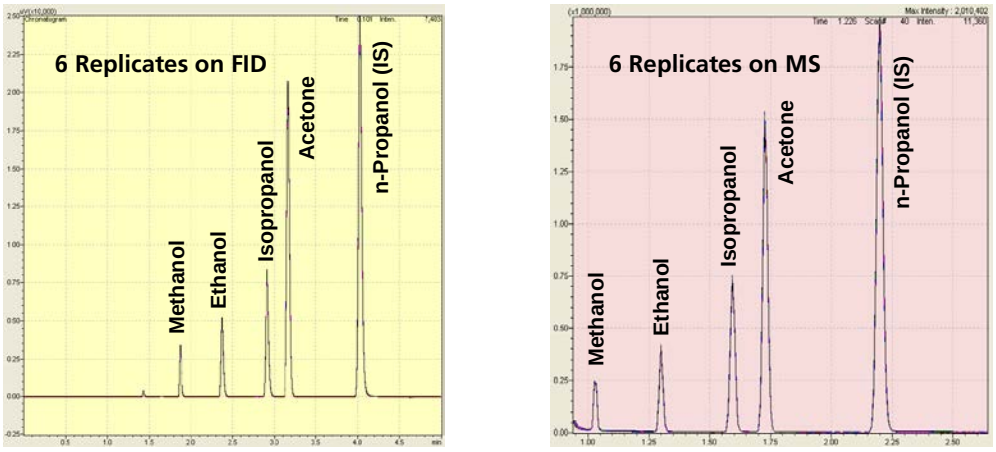

Figure 5: Overlaid Chromatograms from 6 Replicate Analyses of the Control Standard Run on the FID and the MS

Table 3: Precision Results for 6 Replicate Analyses of the Control Standard at 0.05 g/dL

Compound	RSD on FID (n = 6)	RSD on MS (n = 6)
Methanol	1.6%	1.0%
Ethanol	1.4%	0.9%
Isopropanol	1.1%	1.5%
Acetone	0.8%	1.7%

■ Summary and Conclusions

When a mass spectrometer is used in parallel with a GC-FID for analysis of blood alcohol content, the additional compound identification provided by matching the alcohol mass spectrum to an industry-standard library spectrum provides unambiguous, defensible confirmation of the ethanol. Calibration over the target concentration range is linear on both detectors, and precision is demonstrated below 2% for analysis of six replicate standards at the concentration range of interest.

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Application News

No. GCMS-1501

Gas Chromatograph Mass Spectrometer

Analysis and Quantitation of Cocaine on Currency Using GC-MS/MS

Shilpi Chopra, Ph.D., Laura Chambers

■ Introduction

Cocaine (CAS # 50-36-2), a white crystalline alkaloid derived from the *coca* plant, is a popular illegal drug of abuse in the United States and elsewhere. Cocaine is a stimulant that acts on the central nervous system (CNS) causing increased heart rate, tightness in the chest, heightened alertness, numbness, stroke, and even death.

Paper currencies around the world are usually made of a cellulose based paper which can adsorb cocaine onto the surface, and when a person handling cocaine subsequently touches paper money, or uses the bill as a tool to inhale cocaine, the currency easily becomes contaminated. When this contaminated paper money comes into contact with other bills, the cocaine is easily transferred from one bill to the next.

This application note describes a method for extraction, identification, and quantitation of cocaine on paper money from nine different geographical areas around the globe, including five samples from the United States, using the Shimadzu GCMS-TQ8040 triple quadrupole mass spectrometer and the Multiple Reaction Monitoring (MRM) monitoring mode (Figure 1).

Figure 1: Shimadzu GCMS-TQ8040 Triple Quadrupole Mass Spectrometer

■ Experimental

Sample Preparation

Fifteen individual paper notes from nine different countries were each extracted with 10 mL of methanol, and the final volume reduced to 1 mL prior to analysis. No other sample preparation was necessary.

Gas Chromatography Conditions

The capillary column had a 5% phenyl stationary phase, with dimensions of 15 meter x 0.25 mm I.D. x 0.25 µm film thickness. The inlet was maintained at an isothermal temperature of 250 °C, and operated in the splitless mode with a 2.0 minute splitless time. The GC oven was programmed starting at 150 °C (1 minute hold), and ramped to 290 °C at 10 °C per minute, with a final hold time of 1 minute. The GC-to-MS transfer line temperature was held constant at 280 °C. The solvent delay was 4.0 minutes, and cocaine eluted at 8.26 minutes (Figure 2).

MRM Conditions

The GCMS-TQ8040 was operated in the MRM mode to take full advantage of the enhanced selectivity for the target compound. There were two options for developing the MRM method: individual MRM transitions for cocaine can be selected from the Shimadzu Smart Forensics Database[1], or they can be optimized individually using the MRM Optimization Tool[2]. For cocaine, the Smart Forensic Database provides a suite of seven fully optimized MRM transitions with collision energies, empirically derived peak ratios for QA, and retention indices for predicting retention times. Any combination of the seven transitions can be selected for analysis depending on what types of matrix interferences may be present.

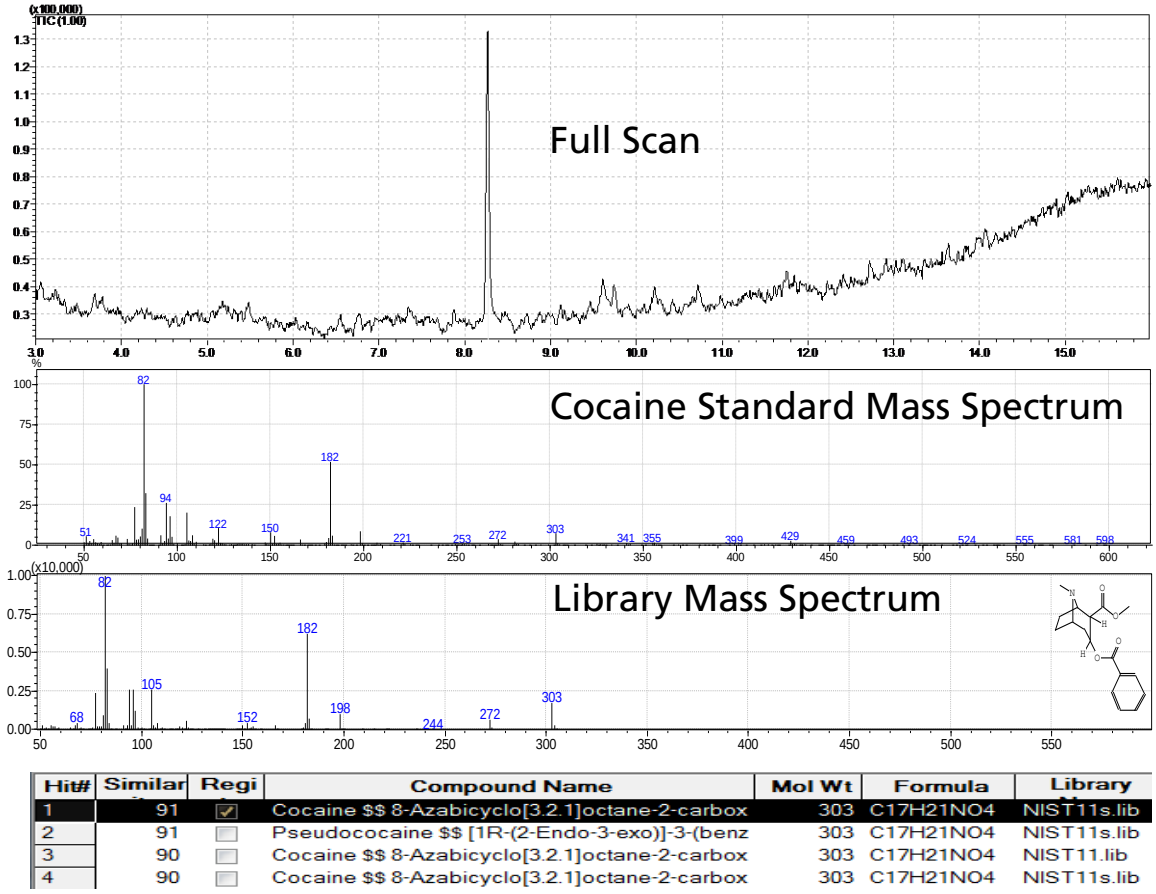

Figure 2: Full-scan TIC of Cocaine with Spectrum and Library Search Results

Because there was only one compound of interest for this study, the required MRM transitions were selected and optimized easily and quickly at the point-of-use. Three transitions were selected based on the sensitivity of their response, and using the *m/z* 182 dominant high mass ion fragment as the precursor in all cases. The three selected transitions are shown in Table 1.

Table 1: MRM Transitions Used for Identification and Quantitation of Cocaine

Transition	Precursor > Product	Collision Energy	Ratio
Primary Tx	182.0 > 82.0	15	100 %
Confirmation Tx #1	182.0 > 122.0	15	43 %
Confirmation Tx #2	182.0 > 93.0	15	37 %

■ Results and Discussion

Method Validation

Several statistical tests were run to validate the method, including establishing a linear calibration, repeatability, limit of detection, and percent recovery. A 9-point calibration curve was prepared (external standard method) from 0.005 to 100 µg/mL (part-per-million, ppm). The method was determined to be linear over this range with an R^2 value of 0.9966, as shown in Figure 3. Repeatability was tested by analyzing six aliquots of the mid-range, 1.0 µg/mL standard, and resulted in a relative standard deviation (RSD) of < 6% for the peak area counts using the primary MRM transition. Figure 4 shows chromatograms of the three overlaid MRM transitions from analysis of the 1.0 µg/mL cocaine standard.

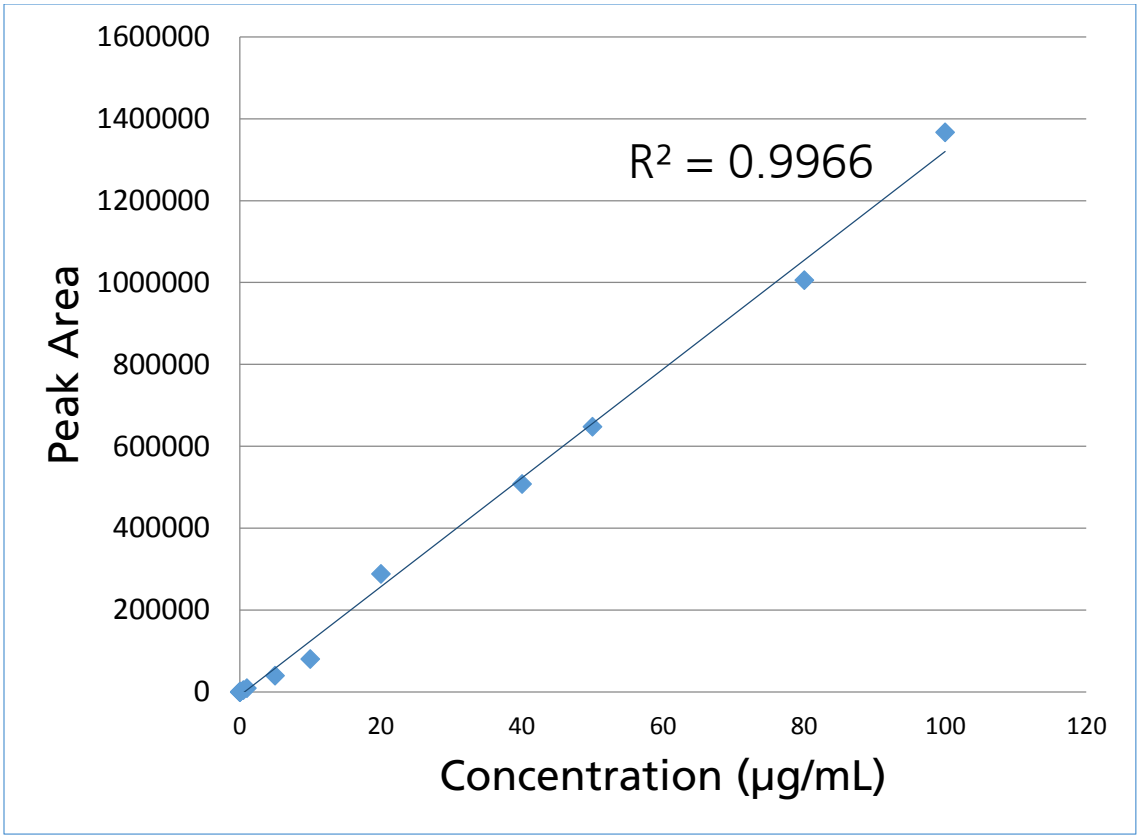

Figure 3: Linear Calibration Curve for Cocaine from 0.005 to 100 µg/mL

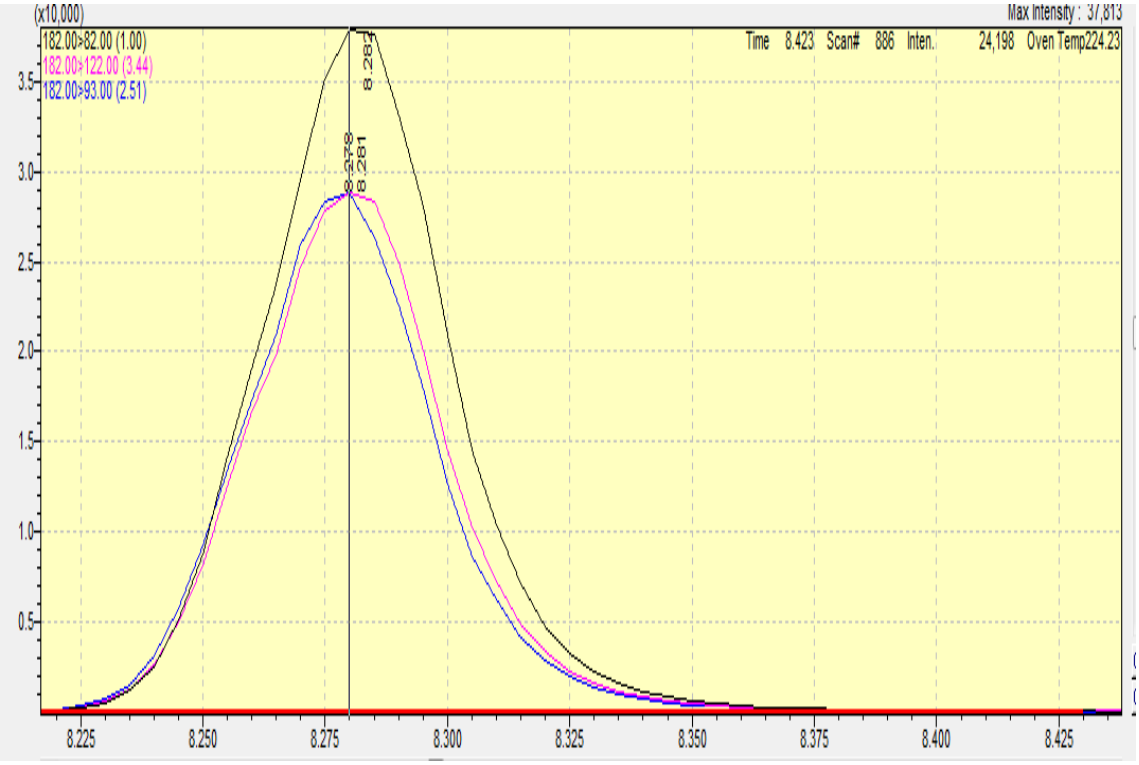

Figure 4: Three Overlaid Transitions for the mid-level, 1.0 µg/mL Cocaine Standard

The percent recovery from each sample was determined by first analyzing a 0.004 µg/mL sample and using that response and the calibration curve to estimate the expected response of a 0.006 µg/mL sample. This was then compared to the response of an actual 0.006 µg/mL sample, and the ratio expressed as a percentage. The recovery was estimated to be 98% at 0.006 µg/mL.

LOQ and LOD was determined using the IUPAC method, using the equation shown below:

$$LOD = ks_B / m$$

Where:

- *k* is the S/N threshold required to define a peak, using 3 for the LOD and 10 for the LOQ
- s_B is the standard deviation of the blank, which was determined taking the standard deviation of the noise readings from 10 data points adjacent to the peak at S/N between 2 and 3
- *m* is the slope of calibration curve

The Limit of Quantitation (LOQ) and Limit of Detection (LOD) were found to be 0.01 µg/mL and 0.005 µg/mL respectively.

Real-World Samples

Fifteen individual paper currency notes were extracted using the procedure described above, analyzed using the MRM method, and quantified against the calibration curve. Calculated concentrations were converted to nanograms (ng) of cocaine per paper note, to illustrate how much of the illegal drug was found on the currencies from different countries. Results are shown in Table 2.

Table 2: Amount of Cocaine Detected on Currency from Different Countries

Origin of Currency Tested	Denomination	Amount of Cocaine Found
USA, Florida	20 Dollars ($20)	1.76 ng
USA, Florida	20 Dollars ($20)	8.25 ng
USA, New Jersey	1 Dollar ($1)	2.85 ng
USA, New Jersey	1 Dollar ($1)	19.6 ng
USA, New Jersey	1 Dollar ($1)	1.1 ng
China	10 Yuan	0.84 ng
Indonesia	1 Rupiah	0.68 ng
France	10 Euro	ND
Brazil	1 Real	ND
Mexico	1 Peso	ND
Mexico	10 Peso	ND
Canada	1 Canadian Dollar	ND
Britain	5 Pounds	ND
India	100 Rupee	ND
India	500 Rupee	0.69 ng
ND = Not Detected		

■ Conclusion

The MRM analysis method for cocaine was quickly and easily developed at point-of-use, and was used to detect and quantify the amount of cocaine found on different currencies from around the world. The $1 and $20 bills from the United States easily had the highest amount of cocaine, and in at least one case it was 20-times higher than the amount found on the paper currency from the next highest country, China.

■ Acknowledgement

The authors wish to acknowledge Professor Nicholas H. Snow and Ramkumar Dhandapani at Seton Hall University, NJ where these analyses were conducted.

■ References

1. The Smart Forensic Database, Shimadzu Corporation (Japan), is part of the Smart Database Series. It is registered with 201 forensic toxicological substances such as poisons, drugs of abuse, psychotropic drugs, pharmaceuticals, and pesticides. It includes over 1200 fully optimized MRM transitions with collision energies, with ion ratios and retention indices, and full GC-MS/MS operating conditions. The Smart Forensic Database is used to create fully optimized MRM methods automatically for analysis of forensic toxicological substances.

2. The MRM Optimization Tool, Shimadzu Corporation (Japan), works with Shimadzu's unique Smart MRM function to automatically find and optimize up to 10 transitions for each compound in a list. The process is fully automated so that even a TQ novice can be successful the first time, and comes standard with all Shimadzu triple quadrupole instruments.

3. Chopra, Shilpi, "Extending the Limits of Solid Phase Microextraction" (2014). Seton Hall University Dissertations and Theses (ETDs). Paper 1990.

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SHIMADZU
Excellence in Science

Gas Chromatograph Mass Spectrometer

Determination of Drugs of Abuse in Oral Fluids

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The center also features high-definition state-of-the-art video and conferencing equipment to facilitate customer interactions both within the center and in collaboration with manufacturing, software, and R&D groups.

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Your chromatography work touches peoples' lives every day. From drug screens to criminal evidence to post-mortem analyses, your results are more than just numbers on paper; they can forever alter the course of a person's life. Fast, accurate, verifiable data is everything. For that, Shimadzu's GCMS-QP2010 Series gas chromatograph/mass spectrometers provide unsurpassed performance, accuracy, speed and flexibility of data reporting – all at a favorable cost/performance ratio to meet every lab's needs.

The methods in this booklet are a compilation of work collected from working forensics laboratories. Each of these labs is a Shimadzu customer and all of the data was generated using the GCMS-QP2010 series of instruments.

Table of Contents

Opiates

Recommended Federal Cut-off: *Morphine, codeine: 40 ng/mL;*
6-acetylmorphine: 4 ng/mL

i. Extraction Procedure

1. From Quantisal™ device, remove 1 mL of oral fluid + buffer
2. Add 100 µL of deuterated internal standard to the calibrator and controls

Standards:

a) D_3-codeine, D_3-morphine; D_3-6-AM at a concentration of 200 ng/mL
b) Codeine, morphine; 6-AM at a concentration of 200 ng/mL

100 µL in 1 mL of oral fluid sample gives an internal standard concentration of 20 ng/mL (= 80 ng/mL without buffer)

Note: Acetonitrile (ACN) is a better storage solvent than methanol due to stability issues with 6-AM

Calibration Curve:

i. Negative:	100 µL of deuterated stock solution (200 ng/mL)
ii. 2 ng/mL:	100 µL of deuterated stock solution (200 ng/mL) 25 µL of 20 ng/mL stock solution (dilute 200 ng/mL 1:10)
iii. 4 ng/mL:	100 µL of deuterated stock solution (200 ng/mL) 5 µL of 200 ng/mL stock solution
iv. 10 ng/mL:	100 µL of deuterated stock solution (200 ng/mL) 12.5 µL of 200 ng/mL stock solution
v. 20 ng/mL:	100 µL of deuterated stock solution (200 ng/mL) 25 µL of 200 ng/mL stock solution
vi. 40 ng/mL:	100 µL of deuterated stock solution (200 ng/mL) 50 µL of 200 ng/mL stock solution
vii. 80 ng/mL:	100 µL of deuterated stock solution (200 ng/mL) 100 µL of 200 ng/mL stock solution

3. Add 0.1 M sodium phosphate buffer (pH 6.0, 1 mL). Vortex
4. Condition solid phase extraction columns (Part # 691-0353T, SPEWare, San Pedro, CA):
    - Methanol (2 mL)
    - 0.1 M phosphate buffer (pH 6.0; 2mL)
5. Add sample and allow to drain through the column
6. Wash column with:
    - Deionized water (1 mL)
    - Acetate buffer (pH 4.2; 1 mL)
    - Methanol (1 mL)
    - Ethyl acetate (1 mL)

7. Place glass collection tubes into the sample rack and elute drugs with ethyl acetate: ammonium hydroxide (98:2 v/v, 2 mL)
8. Evaporate the sample to dryness under nitrogen

Derivatization

- Reconstitute in ethyl acetate (25 µL); add BSTFA + 1% TMCS (25 µL)
- Transfer to autosampler vials, cap and heat at 70°C/20 min
- Analyze using GC/MS

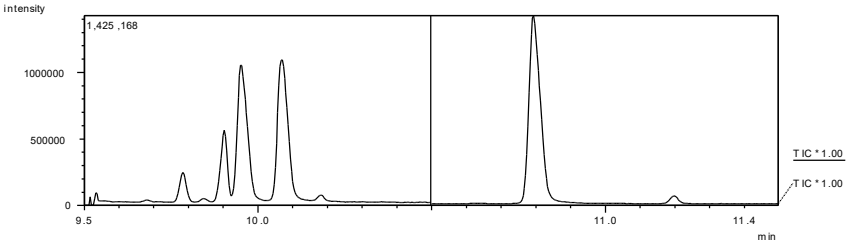
ii. Analytical Procedure

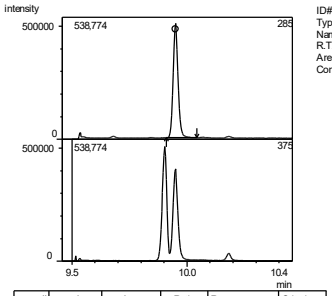
Instrument:	Shimadzu GCMS-QP2010
Column:	RTX-XLB (Ultra low bleed, proprietary low polarity phase) 30 m length x 0.25 mm diameter x 0.25 µm film thickness
Injection volume:	2 µL
Injection Temp:	250°C
Injection mode:	Splitless
Column flow:	1.3 mL/min
Linear velocity	43.3 cm/sec
Purge flow:	3 mL/min
Total flow:	49.7 mL/min.
Oven program:	150°C for 1.5 min ramp at 20°C/min to 290°C, hold for 3 min
Ion source temperature:	230°C
Interface temperature:	250°C
Mode of operation:	Standard CI mode (positive ion)
Reagent gas:	Methane
Detector gain:	0.8kV above tune
Derivative:	BSTFA
Ions monitored:	375, 285 for deuterated codeine (d3); 372, 282 for codeine; 433, 417 for deuterated morphine (d3); 430, 414 for morphine 402, 343 for deuterated 6-acetylmorphine (d3) 399, 340 for 6-acetylmorphine

In Electron Impact mode:

Ions monitored:	374.1, 237.1 for deuterated codeine (d3); 371.1, 234.1 for codeine 432.1, 417.1 for deuterated morphine (d3); 429.1, 414.1 for morphine 402.2, 343.2 for deuterated 6-acetylmorphine (d3); 399.2, 340.2 for 6-acetylmorphine
Linearity:	0 – 80 ng/mL; limit of quantitation: 2 ng/mL
Correlation coefficients:	Codeine $r^2 = 0.991$ Morphine: $r^2 = 0.996$ 6-acetylmorphine $r^2 = 0.994$

Multi Compound Quantitation Report





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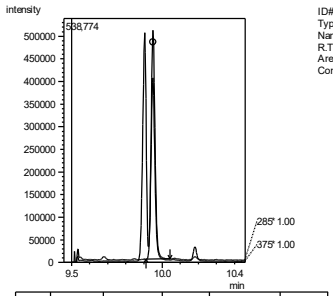

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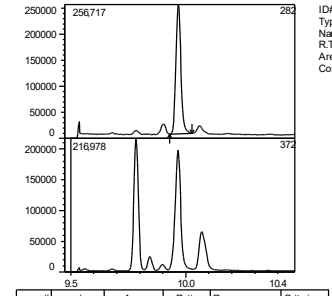

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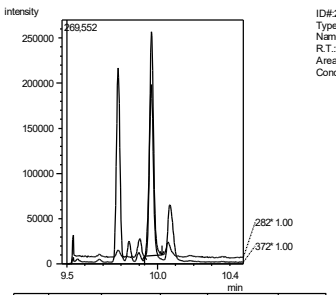

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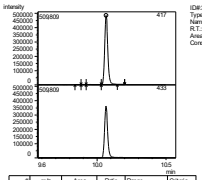

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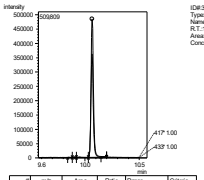

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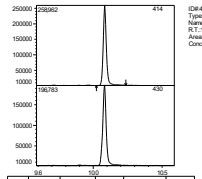

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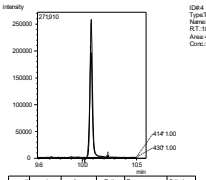

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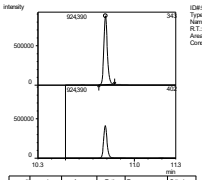

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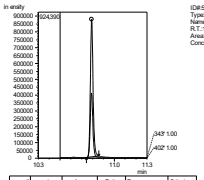

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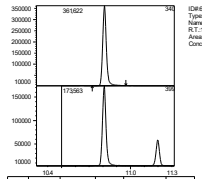

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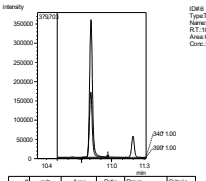

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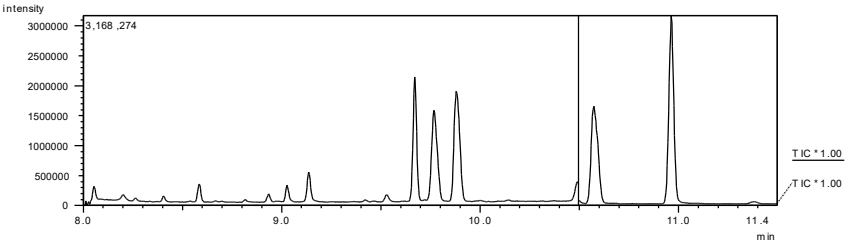
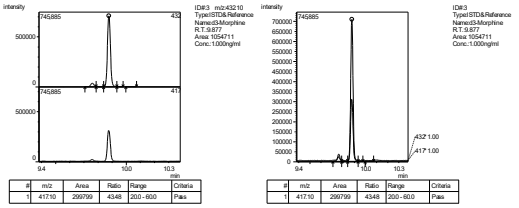
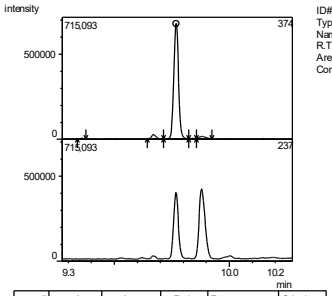
1	399.00	165803	47.50	32.0 - 48.0	Pass

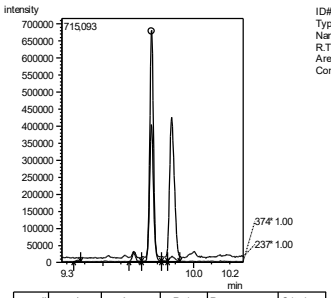
Multi Compound Quantitation Report







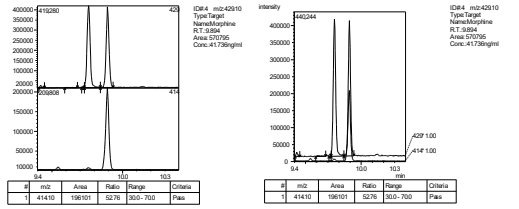
ID#:1 m/z:374.10
Type:ISTD&Reference
Name:d3Codeine
R.T.:9.768
Area: 962479
Conc.:1.000ng/ml

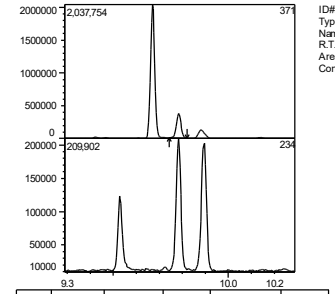


ID#:1
Type:ISTD&Reference
Name:d3Codeine
R.T.:9.768
Area: 962479
Conc.:1.000 ng/ml

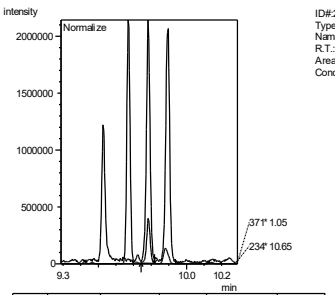
#	m/z	Area	Ratio	Range	Criteria
1	237.10	369229	57.61	30.0 - 70.0	Pass

#	m/z	Area	Ratio	Range	Criteria
1	237.10	369229	57.61	30.0 - 70.0	Pass





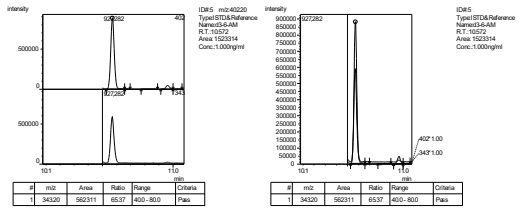
ID#:2 m/z:371.10
Type:Target
Name:Codeine
R.T.:9.784
Area: 522228
Conc.:40.232ng/ml

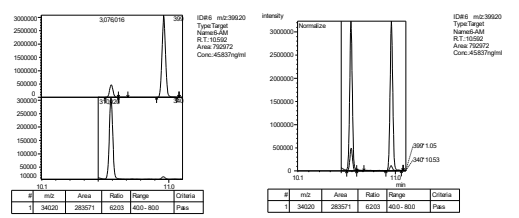


ID#:2 m/z:371.10
Type:Target
Name:Codeine
R.T.:9.784
Area: 522228
Conc.:40.232ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	234.10	187358	53.23	30.0 - 70.0	Pass

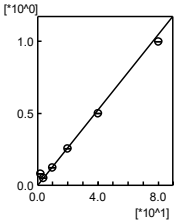
#	m/z	Area	Ratio	Range	Criteria
1	234.10	187358	53.23	30.0 - 70.0	Pass





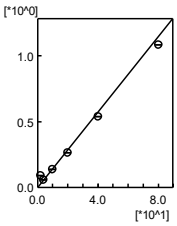
Calibration

ID#:2 Mass:282.00 Name:codeine
f(x)=0 .013081*x+0 .000000
rr2=0 .877100



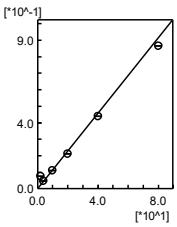
#	Conc. Ratio (ng/ml)	Mean Area Ratio
1	2.000	0.08
2	4.000	0.05
3	10.000	0.12
4	20.000	0.25
5	40.000	0.50
6	80.000	1.00

ID#:4 Mass:414.00 Name:morphine
f(x)=0 .014337*x+0 .000000
rr2=0 .880423



#	Conc. Ratio (ng/ml)	Mean Area Ratio
1	2.000	0.09
2	4.000	0.06
3	10.000	0.14
4	20.000	0.27
5	40.000	0.54
6	80.000	1.08

ID#:6 Mass:340.20 Name:6-AM
f(x)=0 .011437*x+0 .000000
rr2=0 .868939



#	Conc. Ratio (ng/ml)	Mean Area Ratio
1	2.000	0.08
2	4.000	0.05
3	10.000	0.11
4	20.000	0.21
5	40.000	0.44
6	80.000	0.87

Cocaine and BZE

Recommended Federal Cut-off: Cocaine or benzoylecgonine: 8 ng/mL

i. Extraction Procedure

1. From Quantisal specimen, remove 1 mL of oral fluid + buffer
2. Add 40 µL of deuterated internal standard to the calibrator and controls

Standards:

c) D_3-cocaine, D_3-benzoylecgonine at a concentration of 100 ng/mL
d) Cocaine and benzoylecgonine at a concentration of 100 ng/mL

40 µL in 1mL of oral fluid gives an equivalent internal standard concentration of 16 ng/mL (4 ng/ml diluted)

Calibration Curve:

i. Negative:	40 µL of deuterated stock solution (100 ng/mL)
ii. 4 ng/mL:	40 µL of deuterated stock solution (100 ng/mL) 10 µL of 100 ng/mL stock solution
iii. 8 ng/mL:	40 µL of deuterated stock solution (100 ng/mL) 20 µL of 100 ng/mL stock solution
iv. 16 ng/mL:	40 µL of deuterated stock solution (100 ng/mL) 40 µL of 100 ng/mL stock solution
v. 32 ng/mL:	40 µL of deuterated stock solution (100 ng/mL) 80 µL of 100 ng/mL stock solution

3. Add 0.1 M potassium phosphate buffer (pH 6.0, 1 mL). Vortex
4. Condition solid phase extraction columns (Part # 691-0353T, SPEWare, San Pedro, CA):
    - Methanol (2 mL)
    - 0.1 M phosphate buffer (pH 6.0; 2mL)
5. Add sample and allow to drain through the column
6. Wash column with:
    - Deionized water (2 mL)
    - 0.1M hydrochloric acid (2 mL)
    - Methanol (3 mL)
    - Ethyl acetate (3 mL)
7. Place glass collection tubes into the sample rack and elute drugs with methylene chloride: isopropanol: ammonium hydroxide (78:20:2 v/v. 3 mL)
8. Evaporate the sample to dryness under nitrogen

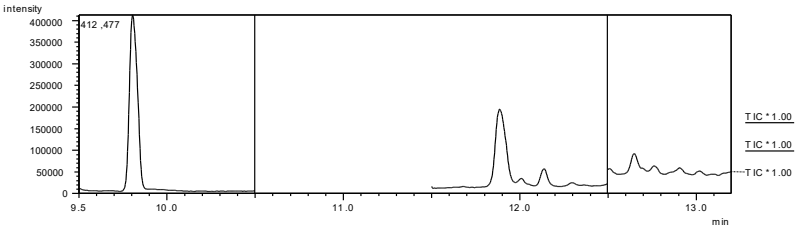
Derivatization

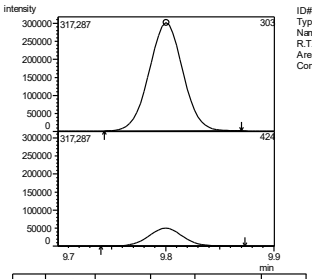
- Add methylene chloride (40 µL), trifluoroethanol (20 µL), and heptafluorobutyric anhydride (HFBA, 20 µL) to dried extract
- Cap; allow to equilibrate for 10 minutes
- Evaporate to dryness in a vacuum oven; reconstitute in ethyl acetate (50 µL)
- Transfer to autosampler vials for analysis using GC/MS

ii. Analytical Procedure

Instrument:	Shimadzu GCMS-QP2010
Column:	RTX-XLB (Ultra low bleed, proprietary low polarity phase) 30 m length x 0.25 mm diameter x 0.25 µm film thickness
Injection volume:	2 µL
Injection Temp:	260°C
Injection mode:	Splitless
Column flow:	1.32 mL/min
Linear velocity	43.3 cm/sec
Purge flow:	3 mL/min
Total flow:	50.4 mL/min
Oven program:	130°C for 1 min ramped at 25°C/min to 250°C, held for 3 min ramped at 30°C/min to 310°C
Ion source temperature:	230°C
Interface temperature:	250°C
Mode of operation:	Standard CI mode (positive ion)
Reagent gas:	Methane
Detector gain:	0.8kV above tune
Ions monitored:	307.15,185.15 for deuterated cocaine (d3); 304.15, 182.15 for cocaine; 375.1, 253,1 for deuterated benzoylecgonine (d3); 372.1, 250.1 for benzoylecgonine
Linearity:	0 – 32 ng/mL; Limit of quantitation: 2 ng/mL
Correlation coefficients:	BZE $r^2 = 0.9998$ Cocaine: $r^2 = 0.9985$

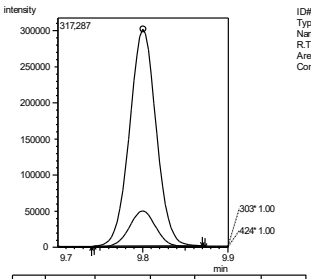
Multi Compound Quantitation Report





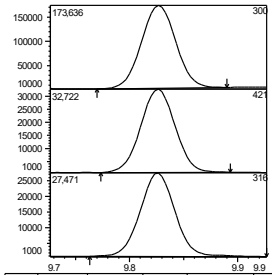
ID#:1 m/z:303.00
Type:ISTD
Name:BZE D3
R.T.:9.800
Area:651489
Conc.:16.000ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	423.95	109542	16.81	14.4 - 21.6	Pass



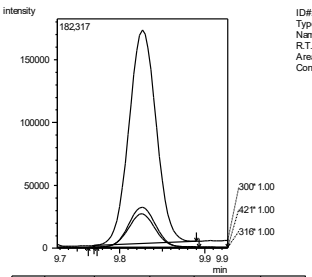
ID#:1
Type:ISTD
Name:BZE D3
R.T.:9.800
Area:651489
Conc.:16.000 ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	423.95	109542	16.81	14.4 - 21.6	Pass



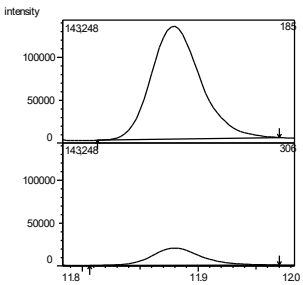
ID#:2 m/z:300.00
Type:Target
Name:BZE
R.T.:9.827
Area:377463
Conc.:7.778ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	420.95	70785	18.75	14.0 - 26.0	Pass
2	316.00	59634	15.80	12.0 - 18.0	Pass



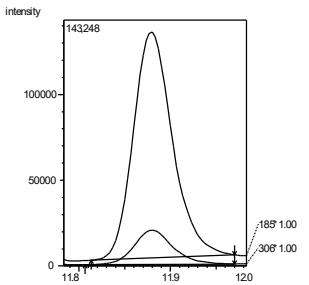
ID#:2 m/z:300.00
Type:Target
Name:BZE
R.T.:9.827
Area:377463
Conc.:7.778ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	420.95	70785	18.75	14.0 - 26.0	Pass
2	316.00	59634	15.80	12.0 - 18.0	Pass



ID#:3 m/z:185.00
Type:ISTD
Name:COC D3
R.T.:11.879
Area:393342
Conc.: N.D.(Ref) ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	306.05	59467	*15.12	9.6 - 14.4	Fail

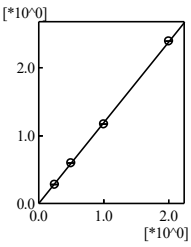


ID#:3
Type:ISTD
Name:COC D3
R.T.:11.879
Area:393342
Conc.: N.D.(Ref) ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	306.05	59467	*15.12	9.6 - 14.4	Fail

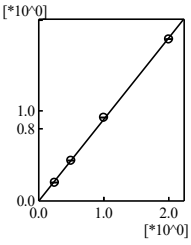
Calibration

ID#:2 Mass:300.00 Name:BZE
f(x)=1.191832*x+0.000000
rr2=0.999841

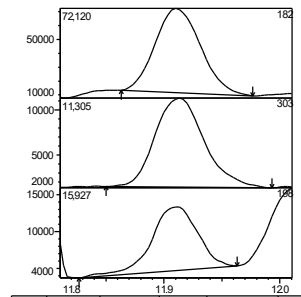


#	Conc.	Ratio (ng/ml)	Mean Area Ratio
1	0.250		0.29
2	0.500		0.60
3	1.000		1.17
4	2.000		2.40

ID#:4 Mass:182.00 Name:COC
f(x)=0.898234*x+0.000000
rr2=0.999324

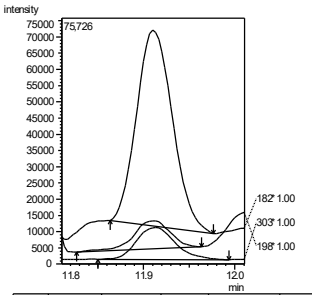


#	Conc.	Ratio (ng/ml)	Mean Area Ratio
1	0.250		0.21
2	0.500		0.45
3	1.000		0.92
4	2.000		1.79



ID#:4 m/z:182.00
Type:Target
Name:COC
R.T.:11.910
Area: 165058
Conc.:0.000ng/ml

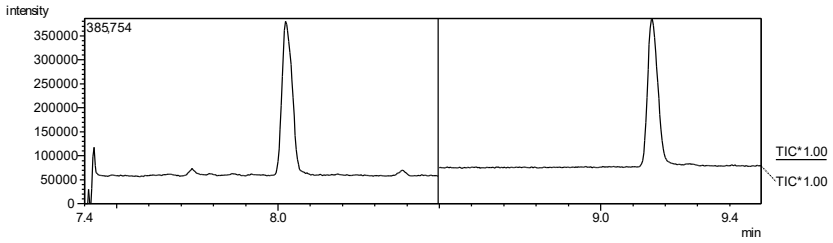
#	m/z	Area	Ratio	Range	Criteria
1	303.05	29240	17.72	14.4 - 21.6	Pass
2	198.00	24216	14.67	10.4 - 15.6	Pass

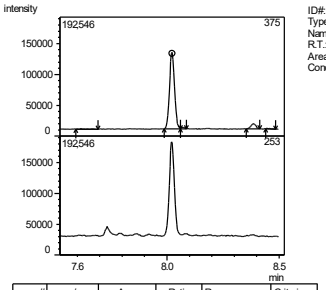


ID#:4 m/z:182.00
Type:Target
Name:COC
R.T.:11.910
Area: 165058
Conc.:0.000ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	303.05	29240	17.72	14.4 - 21.6	Pass
2	198.00	24216	14.67	10.4 - 15.6	Pass

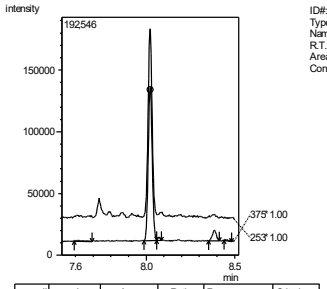
Multi Compound Quantitation Report





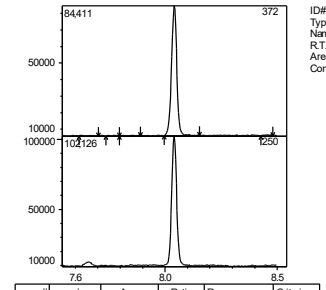
ID#:1 m/z:375.10
Type:ISTD
Name:d3-BZE
R.T.:8.022
Area: 193477
Conc.:1.000 ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	25310	144052	121.59	96.0 - 1440	Pass



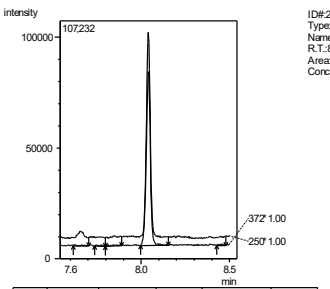
ID#:1
Type:ISTD
Name:d3-BZE
R.T.:8.022
Area: 193477
Conc.:1.000 ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	25310	144052	121.59	96.0 - 1440	Pass



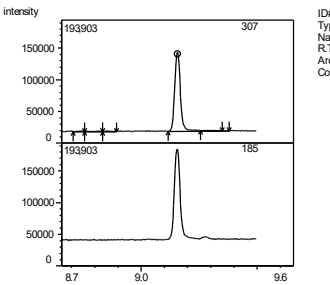
ID#:2 m/z:372.10
Type:Target
Name:BZE
R.T.:8.041
Area: 129609
Conc.:2.959 ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	25010	88836	117.72	96.0 - 1440	Pass



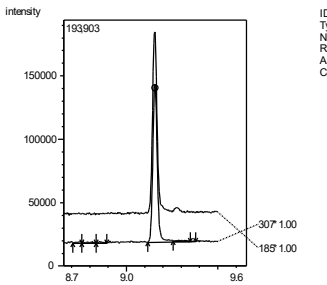
ID#:2 m/z:372.10
Type:Target
Name:BZE
R.T.:8.041
Area: 129609
Conc.:2.959 ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	25010	88836	117.72	96.0 - 1440	Pass



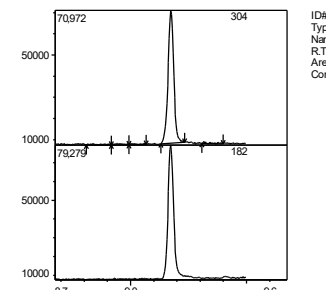
ID#:3 m/z:307.10
Type:ISTD
Name:d3-Cocaine
R.T.:9.156
Area:216169
Conc.:1.000 ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	18510	138469	113.86	97.6 - 146.4	Pass



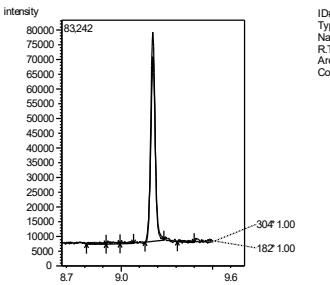
ID#:3
Type:ISTD
Name:d3-Cocaine
R.T.:9.156
Area:216169
Conc.:1.000 ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	18510	138469	113.86	97.6 - 146.4	Pass



ID#:4 m/z:304.10
Type:Target
Name:Cocaine
R.T.:9.174
Area: 106855
Conc.:7.297 ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	18210	68176	113.18	96.0 - 144.0	Pass

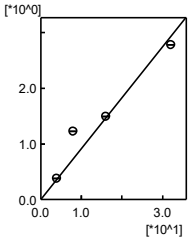


ID#:4 m/z:304.10
Type:Target
Name:Cocaine
R.T.:9.174
Area: 106855
Conc.:7.297 ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	18210	68176	113.18	96.0 - 144.0	Pass

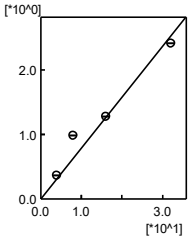
Calibration

ID#:2 Mass:372.10 Name:BZE
f(x)=0 .091169*x+0 .000000
rr2=0 .950493



#	Conc. Ratio (ng/ml)	Mean Area Ratio
1	4.000	0.38
2	8.000	1.22
3	16.000	1.49
4	32.000	2.78

ID#:4 Mass:304.10 Name:Cocaine
f(x)=0 .078733*x+0 .000000
rr2=0 .970739



#	Conc. Ratio (ng/ml)	Mean Area Ratio
1	4.000	0.37
2	8.000	0.99
3	16.000	1.28
4	32.000	2.41

Phencyclidine

Recommended Federal Cut-off: PCP: 10 ng/mL

i. Extraction Procedure

1. Measure out 1 mL of Quantisal buffer (or appropriate amount of collection buffer corresponding to 250 µL oral fluid)
2. Add 20 µL of deuterated internal standard to the calibrator and controls

Standards:

a. D5-phencyclidine at a concentration of 250 ng/mL
b. Phencyclidine at a concentration of 250 ng/mL

20 µL in 1 mL of oral fluid gives an equivalent internal standard concentration of 20 ng/mL (5 x 4)

Calibration Curve:

i. Negative:	20 µL of deuterated stock solution (250 ng/mL)
ii. 5 ng/mL:	20 µL of deuterated stock solution (250 ng/mL) 5 µL of 250 ng/mL stock solution
iii. 10 ng/mL:	20 µL of deuterated stock solution (250 ng/mL) 10 µL of 250 ng/mL stock solution
iv. ng/mL:	20 µL of deuterated stock solution (250 ng/mL) 20 µL of 250 ng/mL stock solution
v. ng/mL:	20 µL of deuterated stock solution (250 ng/mL) 40 µL of 250 ng/mL stock solution

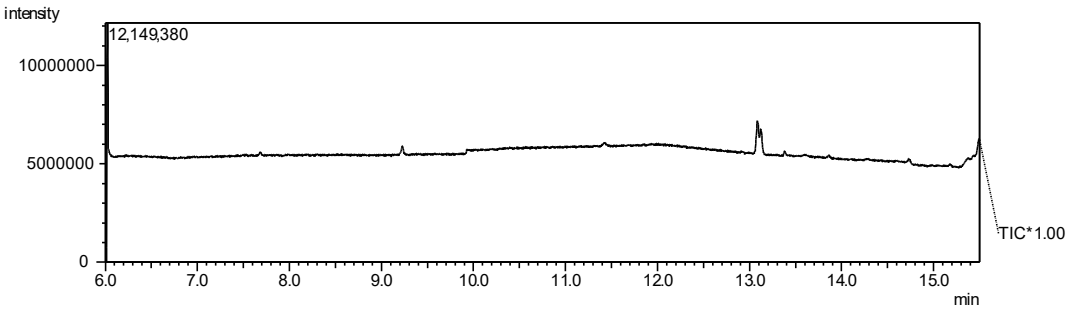
3. Add 0.1M sodium bicarbonate buffer (pH 8.0, 1mL); vortex
4. Place extraction tubes (SPEWare 691-0353T) onto the vacuum manifold
5. Label columns. Condition each column:
   - Methanol (2 mL)
   - 0.1M phosphate buffer (pH 6.0, 2 mL)

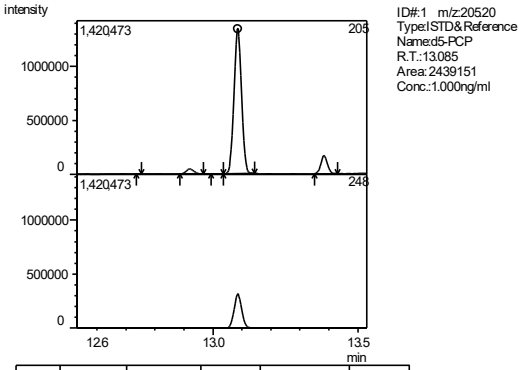
   Important: Do not allow the column bed to go dry.
6. Pour each sample through extraction column. Allow the sample to flow through the column. Dry.
7. Rinse each column with:
   - DI water (1 mL), dry for 1 min
   - 0.1M acetate buffer (pH 4.5, 1 mL), dry for 1 min
   - Methanol (1 mL), dry for 5 min
   - Ethyl acetate (1 mL)
8. Place labeled glass tubes into manifold; wipe the tips.
9. Elute drugs: ethyl acetate + 2% ammonium hydroxide (2 mL)
10. Evaporate to dryness under nitrogen (20 psi/37°C)
11. Reconstitute in ethyl acetate (30 µL); Vortex.
12. Transfer to auto-sampler vials

ii. Analytical Procedure

Instrument:	Shimadzu GCMS-QP2010
Column:	RTX-XLB (Ultra low bleed, proprietary low polarity phase) 30 m length x 0.25 mm diameter x 0.25 µm film thickness
Injection volume:	2 µL
Injection Temp:	250°C
Injection mode:	Splitless
Column flow:	1 mL/min
Linear velocity	36.5 cm/sec
Purge flow:	3 mL/min
Total flow:	38.9 mL/min
Oven program:	60°C for 1 min ramp at 25°C/min to 200°C, hold for 5.2 min ramp at 25°C/min to 300°C
Ion source temperature:	230°C
Interface temperature:	250°C
Mode of operation:	Electron Impact
Detector gain:	0.8kV above tune
Ions monitored:	205.2, 248.2 for deuterated PCP (d5); 200.2, 243.2, 242.2 for PCP
Linearity:	0 – 40 ng/mL; Limit of quantitation: 5 ng/mL
Correlation coefficients:	PCP r^2 = 0.9998

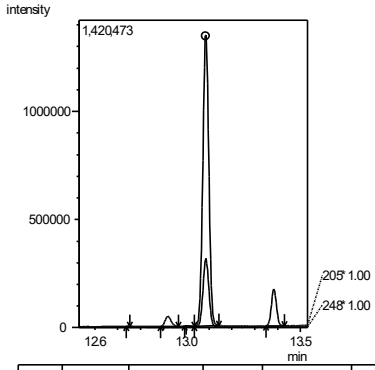
Multi Compound Quantitation Report





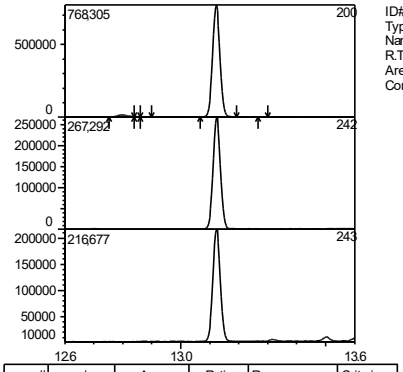
ID#:1 m/z:205.20
Type:ISTD&Reference
Name:d5-PCP
R.T.:13.085
Area:2439151
Conc.:1.000ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	248.20	299976	23.18	5.0 - 45.0	Pass



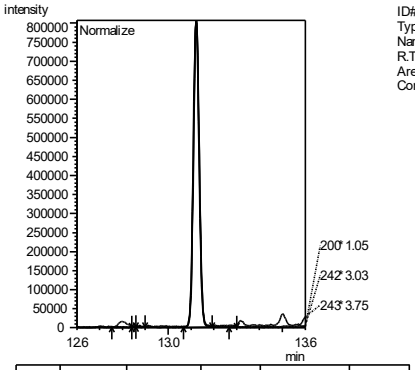
ID#:1
Type:ISTD&Reference
Name:d5-PCP
R.T.:13.085
Area:2439151
Conc.:1.000ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	248.20	299976	23.18	5.0 - 45.0	Pass



ID#:2 m/z:200.20
Type:Target
Name:PCP
R.T.:13.123
Area:1364559
Conc.:10.264ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	242.20	259866	34.76	15.0 - 55.0	Pass
2	243.20	211155	28.24	0.0 - 60.0	Pass



ID#:2 m/z:200.20
Type:Target
Name:PCP
R.T.:13.123
Area:1364559
Conc.:10.264ng/ml

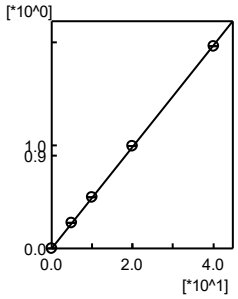
#	m/z	Area	Ratio	Range	Criteria
1	242.20	259866	34.76	15.0 - 55.0	Pass
2	243.20	211155	28.24	0.0 - 60.0	Pass

Calibration

ID#:2 Mass:200.20 Name:PCP

f(x)=0 .049240*x+0 .000000

rr2=0 .999932



#	Conc. Ratio (ng/ml)	Mean Area Ratio
1	0.000	0.00
2	5.000	0.25
3	10.000	0.50
4	20.000	1.00
5	40.000	1.96

Amphetamines

Recommended Federal Cut-off:
Methamphetamine, amphetamine, MDMA, MDA, MDEA: 50 ng/mL

Cannot report methamphetamine as positive without amphetamine present above the limit of detection

i. Extraction Procedure

1. Measure out 1 mL of Quantisal buffer (or appropriate amount of collection buffer corresponding to 250 µL oral fluid)
2. Add 50 µL of deuterated internal standard to the calibrator and controls

Standards:

a) D5-amphetamine, D5-methamphetamine, D5-MDMA, D5-MDA and D5-MDEA at a concentration of 250 ng/mL
b) Amphetamine, methamphetamine, MDMA, MDA and MDEA at a concentration of 250 ng/mL

50 µL of (A) in 1 mL of oral fluid gives an equivalent internal standard concentration of 50 ng/mL (12.5 x 4)

Calibration Curve:

i. Negative:	50 µL of deuterated stock solution (250 ng/mL)
ii. 25 ng/mL:	50 µL of deuterated stock solution (250 ng/mL) 25 µL of 250 ng/mL stock solution
iii. 50 ng/mL:	50 µL of deuterated stock solution (250 ng/mL) 50 µL of 250 ng/mL stock solution
iv. 100 ng/mL:	50 µL of deuterated stock solution (250 ng/mL) 100 µL of 250 ng/mL stock solution
v. 200 ng/mL:	50 µL of deuterated stock solution (250 ng/mL) 200 µL of 250 ng/mL stock solution

3. To specimens, add 0.1M potassium phosphate buffer (pH 6.0, 1 mL); vortex
4. Place extraction tubes (SPEWare 691-0353T) onto the vacuum manifold
5. Label columns. Condition each column:
   - Methanol (2 mL)
   - 0.1M phosphate buffer (pH 6.0, 2 mL)

   Important: Do not allow the column bed to go dry.
6. Allow the sample to flow through the column. Dry.
7. Rinse each column with:
   - DI water (1mL)
   - 0.1M acetate buffer (pH 4, 1 mL)
   - Methanol (1 mL)
   - Ethyl acetate (1 mL); dry for 5 min; 30 psi
8. Place labeled glass tubes into manifold; wipe the tips.
9. Elute drugs: ethyl acetate + 2% ammonium hydroxide (2 mL)

10. Dry samples under nitrogen to dryness.
11. After 5 min add one drop of 0.35M H_2SO_4:acetone (10:90 v,v); After 10 min add another drop.
12. Add heptafluorobutyric anhydride (HFBA, 20 µL); heat at 60°C/20 min
13. Evaporate to dryness in vacuum oven
14. Reconstitute in ethyl acetate (60 µL); Vortex
15. Transfer to auto-sampler vials; analyze by GC/MS

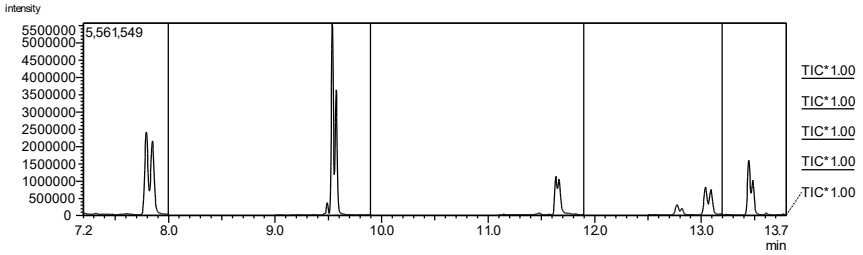
ii. Analytical Procedure

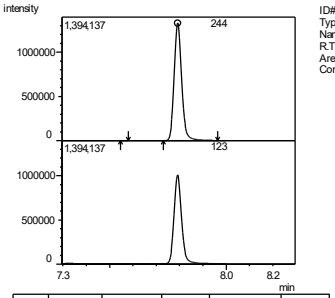
Instrument:	Shimadzu GCMS-QP2010
Column:	RTX-XLB (Ultra low bleed, proprietary low polarity phase) 30 m length x 0.25 mm diameter x 0.25 µm film thickness
Injection volume:	2 µL
Injection Temp:	150°C
Injection mode:	Splitless
Column flow:	1.3 mL/min
Linear velocity	41.6 cm/sec
Purge flow:	0 mL/min
Total flow:	46.7 mL/min
Oven program:	60°C for 1 min ramp at 25°C/min to 140°C, hold for 4 min ramp at 30°C/min to 200°C, hold for 3 min ramp at 40°C/min to 300°C
Ion source temperature:	220°C
Interface temperature:	250°C
Mode of operation:	Electron Impact
Detector gain:	0.8kV above tune
Linearity:	0 – 200 ng/mL; Limit of quantitation: 25 ng/mL
Correlation coefficients:	r2 = 0.9998

Acquisition Parameter File: Amphetamine Acquisition
Group Entries: Number of Groups: 5

Ions *Quantifying ion	Retention Time (min)
Group 1: 244.1*, 123.2 (d5 Amphetamine); 240.1*, 118.15, 91.1 (Amphetamine)	7.7 min
Group 2: 258.05*, 213.05 (d5 Methamphetamine); 254.1*, 210.05*, 118.15 (Methamphetamine)	9.5 min
Group 3: *136.15, 380.2 (d5 MDA); 135.15*, 162.15, 375.15 (MDA)	11.6 min
Group 4: 258.1*, 213.05 (d5 MDMA); 254.05*, 210.05, 162.15 (MDMA)	13.0 min
Group 5: *273.15, 241.1 (d5 MDEA); 268.1*, 240.1, 162.1 (MDEA)	13.4 min

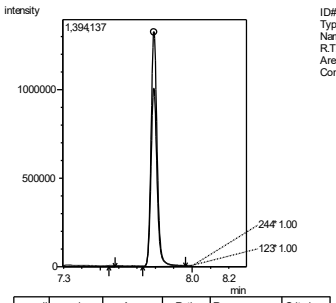
Multi Compound Quantitation Report





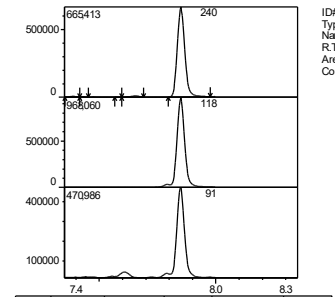
ID#:1 m/z:244.10
Type:ISTD&Reference
Name:Amp d5
R.T.:7.792
Area: 3003557
Conc.:100000ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	123.20	986579	75.20	56.0 - 84.0	Pass



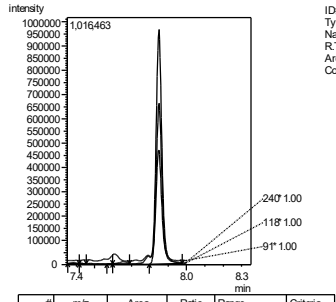
ID#:1
Type:ISTD&Reference
Name:Amp d5
R.T.:7.792
Area: 3003557
Conc.:100000ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	123.20	986579	75.20	56.0 - 84.0	Pass



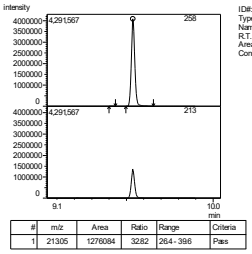
ID#:2 m/z:240.10
Type:Target
Name:Amp
R.T.:7.850
Area: 1490283
Conc.:48.413ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	118.15	917804	142.04	104.0 - 156.0	Pass
2	91.10	432410	66.92	48.0 - 72.0	Pass



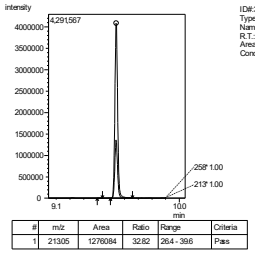
ID#:2 m/z:240.10
Type:Target
Name:Amp
R.T.:7.850
Area: 1490283
Conc.:48.413ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	118.15	917804	142.04	104.0 - 156.0	Pass
2	91.10	432410	66.92	48.0 - 72.0	Pass



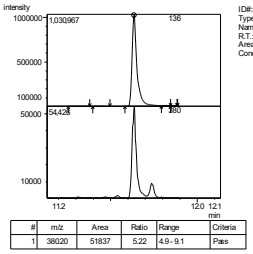
ID#:3 m/z:258.05
Type:ISTD&Reference
Name:Meth d5
R.T.:9.538
Area: 5727452
Conc.:100000ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	213.05	1276084	32.82	26.4 - 39.6	Pass



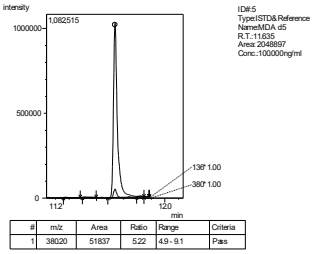
ID#:3
Type:ISTD&Reference
Name:Meth d5
R.T.:9.538
Area: 5727452
Conc.:100000ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	213.05	1276084	32.82	26.4 - 39.6	Pass



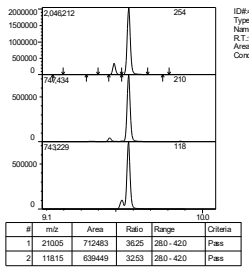
ID#:5 m/z:136.15
Type:ISTD&Reference
Name:MDA d5
R.T.:11.635
Area: 2048897
Conc.:100000ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	380.20	51837	5.22	4.9 - 9.1	Pass



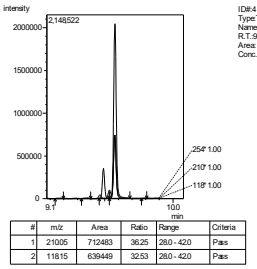
ID#:5
Type:ISTD&Reference
Name:MDA d5
R.T.:11.635
Area: 2048897
Conc.:100000ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	380.20	51837	5.22	4.9 - 9.1	Pass



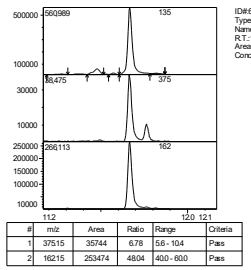
ID#:4 m/z:254.10
Type:Target
Name:Meth
R.T.:9.575
Area: 2716094
Conc.:48.700ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	210.05	712483	36.25	28.0 - 42.0	Pass
2	118.15	639449	32.53	28.0 - 42.0	Pass



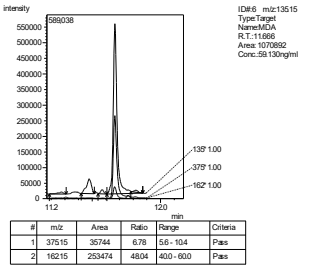
ID#:4 m/z:254.10
Type:Target
Name:Meth
R.T.:9.575
Area: 2716094
Conc.:48.700ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	210.05	712483	36.25	28.0 - 42.0	Pass
2	118.15	639449	32.53	28.0 - 42.0	Pass



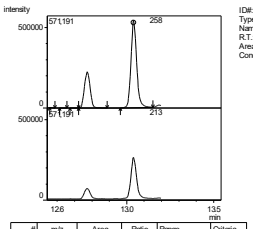
ID#:6 m/z:135.15
Type:Target
Name:MDA
R.T.:11.686
Area: 1070892
Conc.:59.130ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	375.15	35744	6.78	5.6 - 10.4	Pass
2	162.15	253474	48.04	40.0 - 60.0	Pass

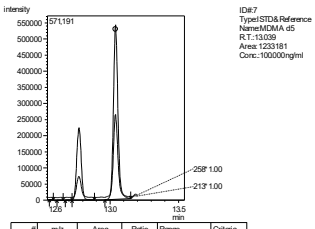


ID#:6 m/z:135.15
Type:Target
Name:MDA
R.T.:11.686
Area: 1070892
Conc.:59.130ng/ml

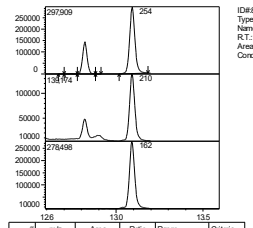
#	m/z	Area	Ratio	Range	Criteria
1	375.15	35744	6.78	5.6 - 10.4	Pass
2	162.15	253474	48.04	40.0 - 60.0	Pass



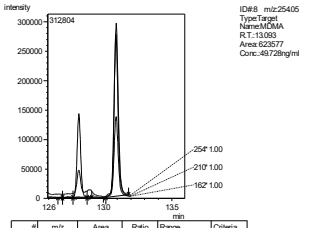
#	m/z	Area	Ratio	Range	Criteria
1	213.05	249995	47.39	360 - 540	Pass



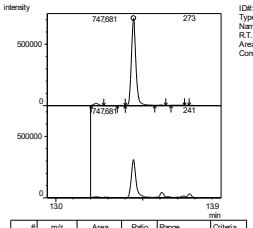
#	m/z	Area	Ratio	Range	Criteria
1	213.05	249995	47.39	360 - 540	Pass



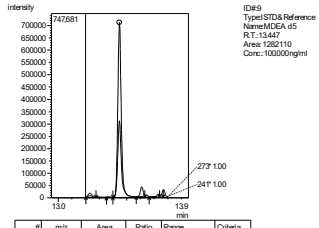
#	m/z	Area	Ratio	Range	Criteria
1	210.05	132363	46.52	360 - 540	Pass
2	162.15	263071	92.47	720 - 1080	Pass



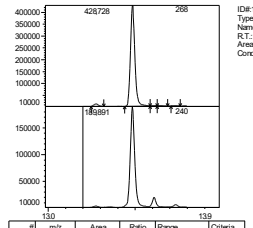
#	m/z	Area	Ratio	Range	Criteria
1	210.05	132363	46.52	360 - 540	Pass
2	162.15	263071	92.47	720 - 1080	Pass



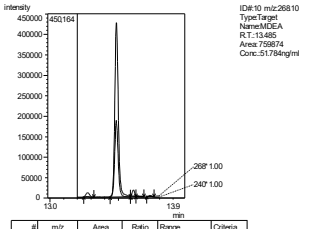
#	m/z	Area	Ratio	Range	Criteria
1	241.10	296445	43.15	320 - 480	Pass



#	m/z	Area	Ratio	Range	Criteria
1	241.10	296445	43.15	320 - 480	Pass



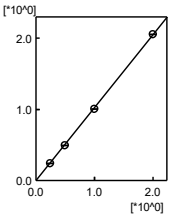
#	m/z	Area	Ratio	Range	Criteria
1	240.10	179213	43.35	344 - 516	Pass



#	m/z	Area	Ratio	Range	Criteria
1	240.10	179213	43.35	344 - 516	Pass

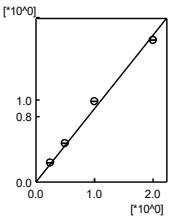
Calibration

ID#:2 Mass:240.10 Name:Amp
f(x)=1 .020487*x+0 .000000
rr2=0 .999958



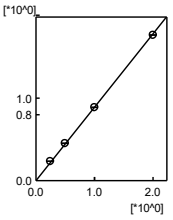
#	Conc. Ratio (ng/ml)	Mean Area Ratio
1	0.250	0.24
2	0.500	0.50
3	1.000	1.01
4	2.000	2.05

ID#:4 Mass:254.10 Name:Meth
f(x)=0 .890418*x+0 .000000
rr2=0 .994056



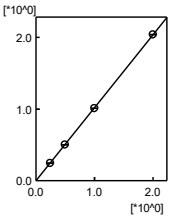
#	Conc. Ratio (ng/ml)	Mean Area Ratio
1	0.250	0.24
2	0.500	0.47
3	1.000	0.98
4	2.000	1.73

ID#:6 Mass:135.15 Name:MDA
f(x)=0 .883944*x+0 .000000
rr2=0 .999984



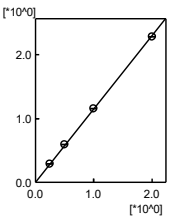
#	Conc. Ratio (ng/ml)	Mean Area Ratio
1	0.250	0.24
2	0.500	0.45
3	1.000	0.89
4	2.000	1.76

ID#:8 Mass:254.05 Name:MDMA
f(x)=1 .016862*x+0 .000000
rr2=0 .999971



#	Conc. Ratio (ng/ml)	Mean Area Ratio
1	0.250	0.25
2	0.500	0.51
3	1.000	1.01
4	2.000	2.04

ID#:10 Mass:268.10 Name:MD EA
f(x)=1 .144507*x+0 .000000
rr2=0 .999914



#	Conc. Ratio (ng/ml)	Mean Area Ratio
1	0.250	0.29
2	0.500	0.59
3	1.000	1.16
4	2.000	2.28

THC

Recommended Federal Cut-off: THC 2 ng/mL

i. Extraction Procedure

1. Aliquot 1 mL of Quantisal new buffer (=0.25 mL neat oral fluid)
2. Add 20 µL of 1000 ng/mL solution of deuterated (d3-THC) (20 ng; 80 ng/mL)

Standards:

a) D3-THC at a concentration of 1000 ng/mL
b) THC at a concentration of 100 ng/mL

Calibration Curve:

i. Negative: 20 µL of deuterated stock solution (1000 ng/mL)

ii. 1 ng/mL: 20 µL of deuterated stock solution (1000 ng/mL)
50 µL of 10 ng/mL stock solution (1:10 of 100 ng/mL)

iii. 2 ng/mL: 20 µL of deuterated stock solution (1000 ng/mL)
100 µL of 10 ng/mL stock solution (1:10 of 100 ng/mL)

iv. 4 ng/mL: 20 µL of deuterated stock solution (1000 ng/mL)
20 µL of 100 ng/mL stock solution

v. 8 ng/mL: 20 µL of deuterated stock solution (1000 ng/mL)
40 µL of 100 ng/mL stock solution

3. Add 0.1M acetate buffer (pH 4.5, 1 mL)
4. Condition SPEWare columns:
   - Methanol (0.5 mL),
   - 0.1M acetic acid (100 µL)
5. Pour sample into column and pass through at a flow rate of 1ml / min
6. Wash column 80 : 20 D.I. H_20 : acetic acid (1ml)
   40 : 60 D.I. H_20: methanol (1ml)
7. Dry column (5 min; 30 psi)
8. Elute samples: hexane: glacial acetic acid (98:2, 0.8 mL)
9. Evaporate sample to dryness
10. Add ethyl acetate (50 µL); transfer into auto sampler vial
11. Add BSTFA (20 µL); heat at 60°C /15 min

ii. Analytical Procedure

Instrument:	Shimadzu GCMS-QP2010
Column:	RTX-XLB (Ultra low bleed, proprietary low polarity phase) 30 m length x 0.25 mm diameter x 0.25 µm film thickness
Injection volume:	2 µL
Injection Temp:	250°C
Injection mode:	Splitless
Column flow:	1.39 mL/min
Linear velocity	44.4 cm/sec
Total flow:	50 mL/min
Oven program:	125°C for 0.2 min ramp at 20°C/min to 250°C, hold for 3 min ramp at 30°C/min to 300°C
Ion source temperature:	220°C
Interface temperature:	280°C
Mode of operation:	Electron impact
Detector gain:	0.8kV above tune
Ions monitored:	389, 374 for deuterated THC (d3); 386, 371 for THC

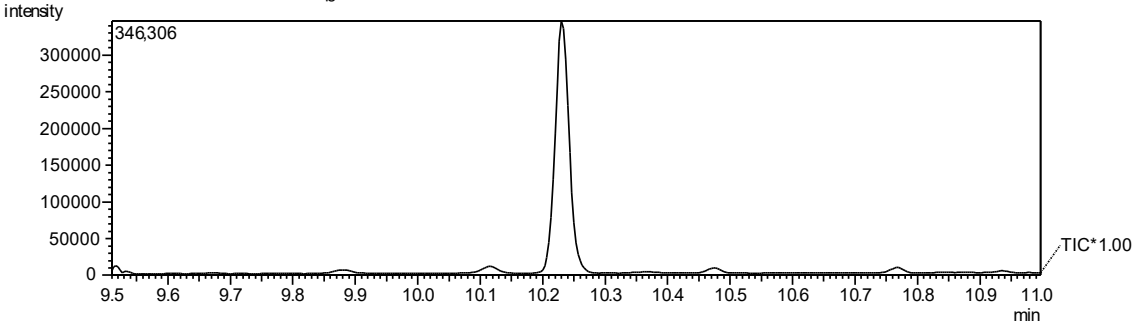
The limit of quantitation of the method was 1 ng/mL

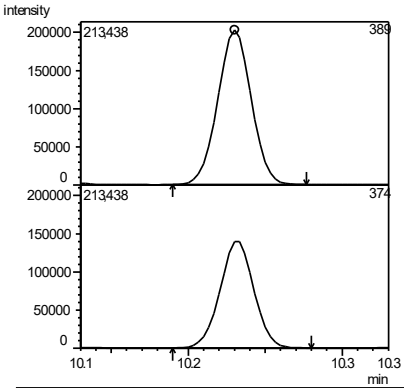
Reference

http://www.shimadzu.com/apps/appnotes/GCMS%20QP-2010%20THC%20Saliva.pdf

Multicompound Quantiation Report

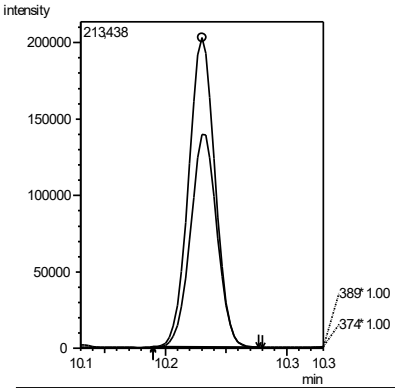
Data Acquired by : Admin
Acquisiton Date : 2/17/20054:18:34 PM
Sample Name : 2 ng cal
Data File : D:\Oral fluid THC\03.qgd
Method File : D:\Oral fluid THC\THCOFDT.qgm





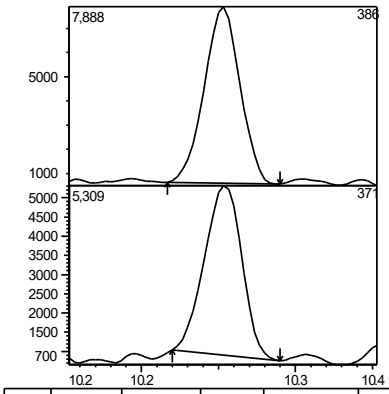
ID#:1 m/z:389.00
Type:ISTD
Name:THC D3
R.T.:10.230
Area:305438
Conc.:1.000ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	374.00	213028	69.75	55.2 - 82.8	Pass



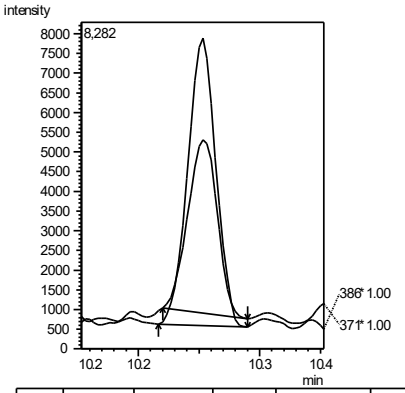
ID#:1
Type:ISTD
Name:THC D3
R.T.:10.230
Area:305438
Conc.:1.000ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	374.00	213028	69.75	55.2 - 82.8	Pass



ID#:2 m/z:386.00
Type:Target
Name:THC
R.T.:10.253
Area:11824
Conc.:2.044ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	371.00	7448	62.99	48.3 - 89.7	Pass



ID#:2 m/z:386.00
Type:Target
Name:THC
R.T.:10.253
Area:11824
Conc.:2.044ng/ml

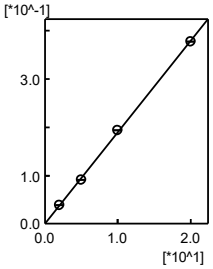
#	m/z	Area	Ratio	Range	Criteria
1	371.00	7448	62.99	48.3 - 89.7	Pass

Analyst _____Date _____

Approved by _____Date _____

Printed on:22 Oct 2007 14:42

Calibration
ID#:2 Mass:386.00 Name:THC
f(x)=0 .018938*x+0 .000000
rr2=0 .999598



#	Conc. Ratio (ng/ml)	Mean Area Ratio
2	2.000	0.04
3	5.000	0.09
4	10.000	0.19
5	20.000	0.38

Methadone

i. Extraction Procedure

1. From Quantisal specimen, remove 1 mL of oral fluid + buffer
2. Add 100 µL of deuterated internal standard to the calibrator and controls

Standards:

a) D9-methadone at a concentration of 200 ng/mL
b) Methadone at a concentration of 200 ng/mL

100 µL in 1 mL of oral fluid sample gives an internal standard concentration of 20 ng/mL (= 80 ng/mL neat oral fluid)

Calibration Curve:

i. Negative:	100 µL of deuterated stock solution (200 ng/mL)
ii. 10 ng/mL:	100 µL of deuterated stock solution (200 ng/mL) 12.5 µL of 200 ng/mL stock solution
iii. 20 ng/mL:	100 µL of deuterated stock solution (200 ng/mL) 25 µL of 200 ng/mL stock solution
iv. 40 ng/mL:	100 µL of deuterated stock solution (200 ng/mL) 50 µL of 200 ng/mL stock solution
v. 80 ng/mL:	100 µL of deuterated stock solution (200 ng/mL) 100 µL of 200 ng/mL stock solution

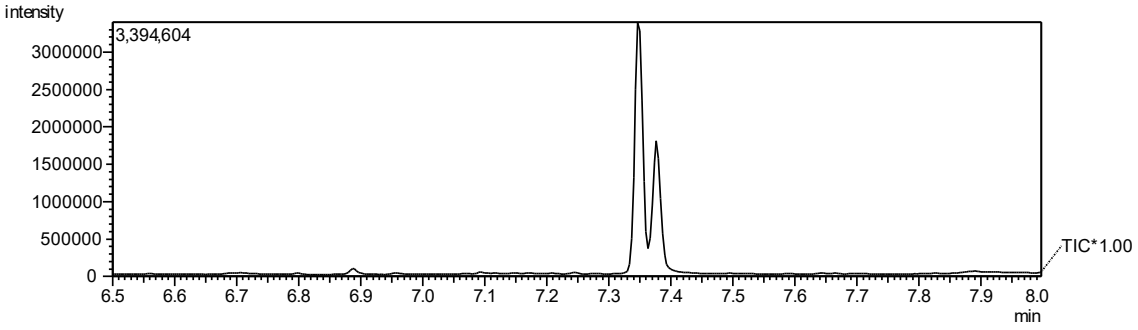
3. Add 0.1 M sodium bicarbonate buffer (pH 8.0, 1 mL). Vortex
4. Condition solid phase extraction columns (Part # 691-0353T, SPEWare, San Pedro, CA):
    - Methanol (2 mL)
    - 0.1 M phosphate buffer (pH 6.0; 2mL)
5. Add sample and allow to drain through the column
6. Wash column with:
    - Deionized water (1 mL)
    - 0.1 M Acetate buffer (pH 4.2; 1 mL)
    - Methanol (1 mL)
    - Ethyl acetate (1 mL); dry for 2 min
7. Place glass collection tubes into the sample rack
8. Elute drugs with ethyl acetate: ammonium hydroxide (98:2 v/v, 2 mL)
9. Evaporate the sample to dryness under nitrogen
10. Reconstitute in ethyl acetate (40 µL); Transfer to autosampler vials
11. Analyze using GC/MS

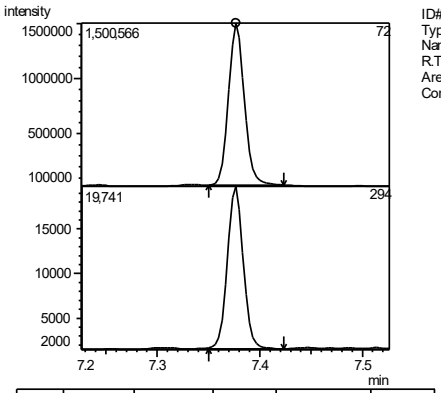
ii. Analytical Procedure

Instrument:	Shimadzu GCMS-QP2010
Column:	RTX-XLB (Ultra low bleed, proprietary low polarity phase) 30 m length x 0.25 mm diameter x 0.25 µm film thickness
Injection volume:	2 µL
Injection Temp:	250°C
Injection mode:	Splitless
Column flow:	1.2 mL/min
Linear velocity	40.8 cm/sec
Total flow:	46.4 mL/min
Oven program:	110°C for 1 min ramp at 30°C/min to 290°C, hold for 1.5 min
Ion source temperature:	230°C
Interface temperature:	300°C
Mode of operation:	Standard electron impact (EI) mode
Detector gain:	0.8kV above tune
Ions monitored:	303.1, 78.15 for deuterated methadone (d9) 294.0, 72.1 for methadone
Correlation coefficient:	Methadone r^2 = 0.9999446
Linearity:	0 – 80 ng/mL; limit of quantitation 10 ng/mL

Multicompound Quantiation Report

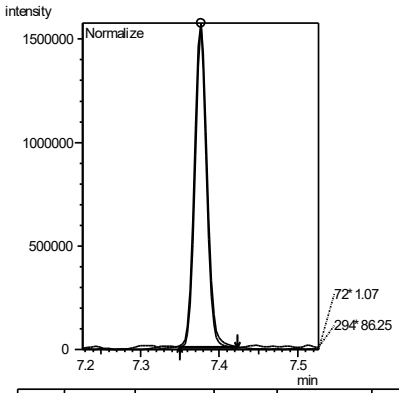
Data Acquired by : Admin
Acquisition Date : 7/19/20054:07:58 PM
Sample Name : 20ng/ml
Data File : D:\Methadone\MED082.qgd
Method File : C:\GCMSsolution\Data\Methadone\METHADONE4.qgm





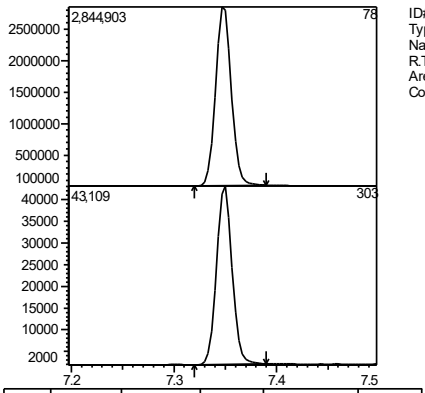
ID#:1 m/z:72.15
Type:Target
Name:Methadone
R.T.:7.377
Area:1573550
Conc.:19.415ng/mL

#	m/z	Area	Ratio	Range	Criteria
1	294.00	19499	1.24	0.7 - 1.3	Pass



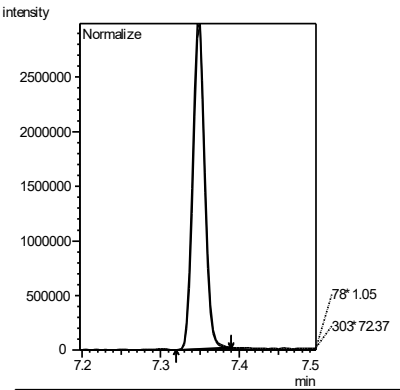
ID#:1
Type:Target
Name:Methadone
R.T.:7.377
Area:1573550
Conc.:19.415ng/mL

#	m/z	Area	Ratio	Range	Criteria
1	294.00	19499	1.24	0.7 - 1.3	Pass



ID#:2 m/z:78.15
Type:ISTD
Name:IMethadone d9
R.T.:7.348
Area:3020157
Conc.:40.000ng/mL

#	m/z	Area	Ratio	Range	Criteria
1	303.10	43406	1.44	1.4 - 2.6	Pass



ID#:2 m/z:78.15
Type:ISTD
Name:IMethadone d9
R.T.:7.348
Area:3020157
Conc.:40.000ng/mL

#	m/z	Area	Ratio	Range	Criteria
1	303.10	43406	1.44	1.4 - 2.6	Pass

Analyst _____Date _____

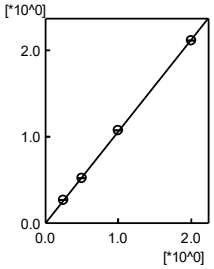
Approved by _____Date _____

Printed on:22 Oct 2007 14:50

Calibration

ID#:1 Mass:72.15 Name:Methadone
f(x)=1 .056983*x+0 .000000
rr2=0 .999854

ID#:2 Mass:78.15 Name:IMethadone d9
f(x)=?
rr2=0 .000000



#	Conc. Ratio (ng/mL)	Mean Area Ratio
1	0.250	0.27
2	0.500	0.52
3	1.000	1.07
4	2.000	2.11

ISTD

Shimadzu Corporation
www.shimadzu.com/an/

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7102 Riverwood Drive, Columbia, Maryland 21046, U.S.A.
Phone: 800-477-1227/410-381-1227, Fax: 410-381-1222
www.ssi.shimadzu.com

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Technical Report

Automatic Identification and Semi-quantitative Analysis of Psychotropic Drugs in Serum Using "GC/MS Forensic Toxicological Database"

Hitoshi Tsuchihashi[1]

Abstract:

A sample consisting of serum extract from a patient administered with psychotropic drugs was analyzed by GC/MS; identification and semi-quantitation of the substances were conducted using the "GC/MS Forensic Toxicological Database". All three administered substances were identified, and their semi-quantitative values were calculated. The results indicated that it is possible to qualitatively and semi-quantitatively determine drugs using this database.

Keywords: GC/MS, psychotropic drug, phenobarbital, semi-quantitation, forensic medicine, toxicology

Introduction

Accidental poisoning due to the abuse and excessive intake of stimulants and other illegal drugs or psychotropic drugs continues to be a troublesome social issue. In particular, when death occurs due to acute drug poisoning, identifying the specific drug responsible and determining its concentration in the blood are essential subjects in university forensic classrooms and critical activities in prefectural police department forensic laboratories.

Identification and quantitative analysis of a drug substance in the blood can be very time-consuming, requiring a calibration curve for verification of retention times using a standard drug sample, and determination of the appropriate preparation and pretreatment of the actual sample. In addition to the automatic search algorithm built into the GC/MS Forensic Toxicological Database, a semi-quantitative analysis feature is also included which uses relative response factors for drugs that often lead to poisoning. These features allow identification of drugs for which standard samples are difficult to obtain, and evaluation based on estimated quantitation values (via semi-quantitation) for these substances.

In this Technical Report, we utilized the substance identification and semi-quantitation features included in the GC/MS Forensic Toxicological Database to identify 3 substances, including the barbiturate, Phenobarbital (used to treat epilepsy), the antipsychotic, chlorpromazine, and the antihistamine antiemetic, promethazine (used to treat Parkinson's disease), in a spiked sample of actual serum. In addition, we verified the results using a serum sample from an actual patient administered these 3 substances.

Experiment

Reagents

The phenobarbital sodium, chlorpromazine hydrochloride, and promethazine hydrochloride were obtained from Wako Chemicals, and each was adjusted to a free-state concentration of 10 mg/mL (by using methanol for phenobarbital sodium and distilled water for chlorpromazine hydrochloride and promethazine hydrochloride). After preparing a mixed solution of these, each at a concentration of 100 µg/mL, the mixture was added to blank serum, and the concentration was adjusted to 10 µg/mL. This was then used as the analytical sample (spiked serum). In addition, after receiving informed consent from a psychiatric patient who had been administered these 3 substances, we used the received blood serum as the actual sample. In addition, two custom standard solutions were obtained from Shimadzu GLC, one an n-alkane C7 – C33 sample (Custom Retention Time Index Standard, Restek Corp.) for retention time correction, and the other an internal standard (Custom Internal Standard, Restek Corp.) sample necessary for the semi-quantitation.

1 Department of Legal Medicine, Osaka Medical College

Pretreatment Procedure

Into 500 µL of each serum sample (spiked serum and actual sample), 20 µL of 10% hydrochloric acid was titrated to acidify, 1000 µL of a chloroform-isopropanol mixture (3:1, v/v) was added, and after vigorously mixing, centrifuging was conducted for 15 minutes, and the organic layer (acidic fraction) was collected. After conducting this operation twice in succession, 20 µL of 28% aqueous ammonia was added to the aqueous layer to make the mixture basic. Then, 1000 µL of a chloroform-isopropanol mixture (3:1, v/v) was added, and after conducting the mixing / centrifuging process described above, the organic layer (basic fraction) was collected.

After combining the acidic and basic fractions, dewatering of the mixture was conducted using anhydrous sodium sulfate, and evaporative drying was performed at 40°C under nitrogen. The obtained residue was dissolved in 250 µL ethyl acetate, and this was used as the sample for GC/MS analysis.

Equipment

For the GC/MS analysis, the GCMS-QP2010 Ultra was used, and GCMSsolution software was used for data processing. Table 1 shows the analytical conditions that were used for the analyses. For retention time adjustment, the AART (Automatic Adjustment of Retention Time) feature included in GCMSsolution Postrun Analysis was used to calculate the retention times of the 162 substances (included in the free-state substance analytical method for psychiatric drugs) from the retention indices, and these were used as the standard retention times for identification. The retention time windows were set to ±0.2 minutes, and the substances included in the samples were identified using the automatic identification feature. In addition, the internal standard used for quantitation was introduced automatically into the GC injection port simultaneously with the sample using the internal standard automatic addition feature of the AOC-20i+s.

Table 1 Analytical Conditions

Instruments	
GC-MS	: GCMS-QP2010 Ultra
Auto-injector	: AOC-20i + s
Column	: Rxi®-5Sil MS (30 m × 0.25 mm I.D. df=0.25 µm, Restek Corporation)
GC condition	
Column Temp.	: 60°C (1 min)-10°C/min-320°C (10 min)
Carrier Gas	: He (Constant Linear Velocity Mode)
Carrier Gas Velocity	: 45.6 cm/sec
Injection Mode	: Splitless
Sample injection volume	: 1 µL
IS injection volume	: 1 µL
MS condition	
Interface Temp.	: 280°C
Ion Source Temp.	: 200°C
Scan Interval	: 0.3 sec
Monitor ion for semi-quantitation	: *m/z* 204 for phenobarbital
	: *m/z* 318 for chrolpromazine
	: *m/z* 72 for promethazine

Results and Discussion

Semi-Quantitation Results of Spiked Serum

The total ion current chromatogram (TIC) obtained from analysis of the spiked serum using the abovementioned procedure is shown in Fig. 1. the 3 added substances were detected within ±0.03 minutes of the expected calculated retention time, and accurate identification was achieved based on the retention times (Fig. 2). Since each substance is detected based on the extracted ion chromatogram (EIC) of multiple *m/z* values set beforehand, the EIC chromatogram can be detected even at trace concentrations or when a discrete chromatographic peak in the TIC cannot be detected due to matrix interferences. The detected chromatographic peak can be accurately identified automatically through confirmation of the degree of similarity with the standard mass spectrum (Fig. 3). In addition, the quantitation values were calculated from the obtained peak intensity ratio of each target substance and internal standard substance, and the relative response factor.

Thus, utilizing the automatic search feature of the GC/MS Forensic Toxicological Database, phenobarbital, chlorpromazine, and promethazine were all detected automatically, and semi-quantitation values were obtained for these 3 substances. The semi-quantitation values obtained using the semi-quantitation feature of the GC/MS Forensic Toxicological Database and the relative response factors (obtained from analysis of the standard solution) are shown in Table 2.

As shown in Table 2, fairly good quantitative results were obtained for chlorpromazine and promethazine, but the quantitative results for phenobarbital indicate a value 1.8 times that of the added amount. The semi-quantitation feature of the GC/MS Forensic Toxicological Database generates a semi-quantitation value which is a rough estimate of "the drug concentration in the final sample" based on the response factor obtained from the previously-analyzed standard sample. Therefore, the semi-quantitation value can vary considerably depending on the drug recovery ratio and sample concentration during sample pretreatment, as well as the matrix effect. Especially, in the case of a serum sample, the recovery of a drug with high lipid solubility will decrease greatly, increasing the likelihood that the difference between the obtained semi-quantitation value and the true value will widen considerably. Thus, since the calculated quantitation value can be expected to vary depending on the pretreatment procedure, the GC injection port, and the column condition, it is necessary to regard it only as an approximate estimated value. For quantitative analysis requiring great accuracy, standard samples must be used.

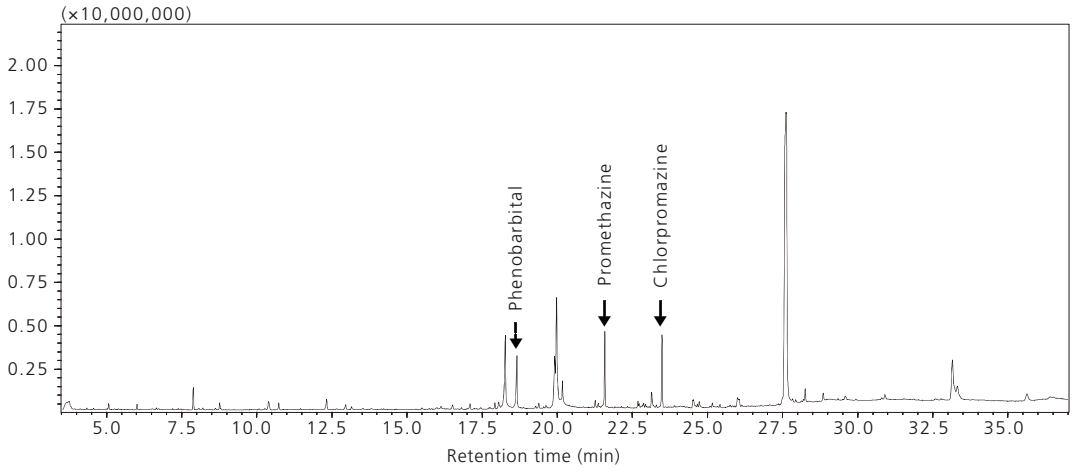


Fig. 1 Total Ion Current Chromatogram Obtained from Spiked Serum

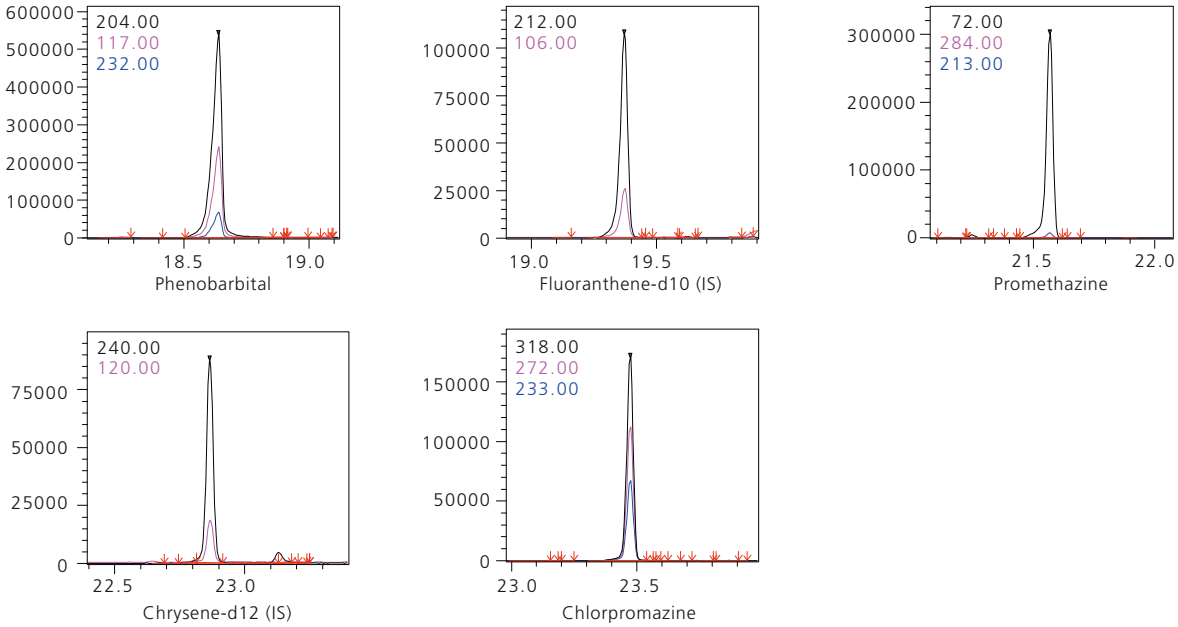


Fig. 2 Mass Chromatograms of 3 Added Substances and Principal Internal Standard Samples

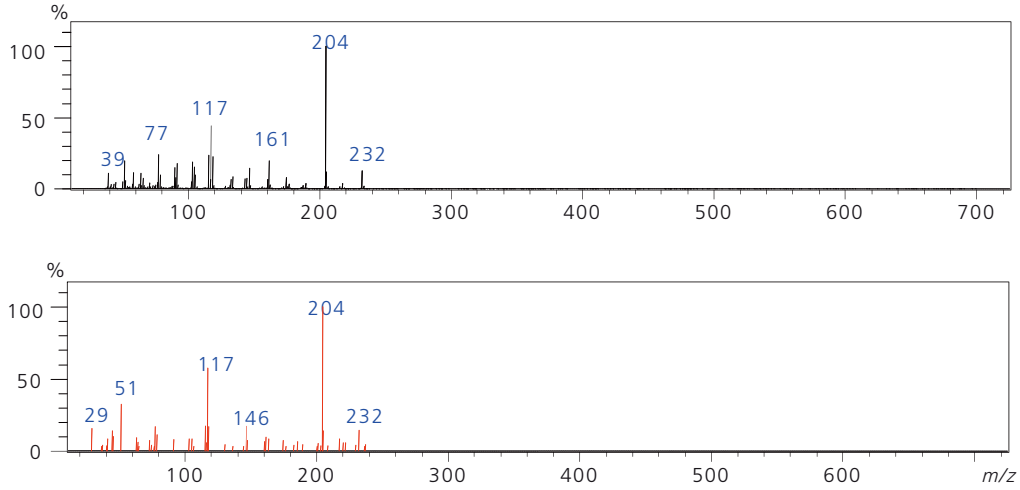


Fig. 3 Comparison of Measured Mass Spectrum of Phenobarbital (above) and Standard Mass Spectrum (below)

Table 2 Semi-Quantitated Results of Spiked Plasma

Compounds	Additive Amount (μg/mL)	Semi-quantitated (μg/mL)	Response Factor
Phenobarbital	10.0	17.6	0.175
Chlorpromazine	10.0	11.3	0.144
Promethazine	10.0	11.4	1.782

Semi-Quantitation Results for Actual Sample

In the case of the actual sample, just as with the spiked serum, the 3 substances were automatically detected by the automatic search algorithm, and the semi-quantitative results were obtained. The chromatogram obtained from analysis of the actual sample is shown in Fig. 4. Also, with respect to the actual sample, the quantitation values obtained using the internal standard method were compared with the semi-quantitation values calculated from the GC/MS Forensic Toxicological Database. Those data are shown in Table 3.

As with the spiked serum, the semi-quantitation values obtained for chlorpromazine and promethazine were relatively close to the values obtained from the calibration curve, but the semi-quantitation value for phenobarbital indicated a value that was about 3 times higher.

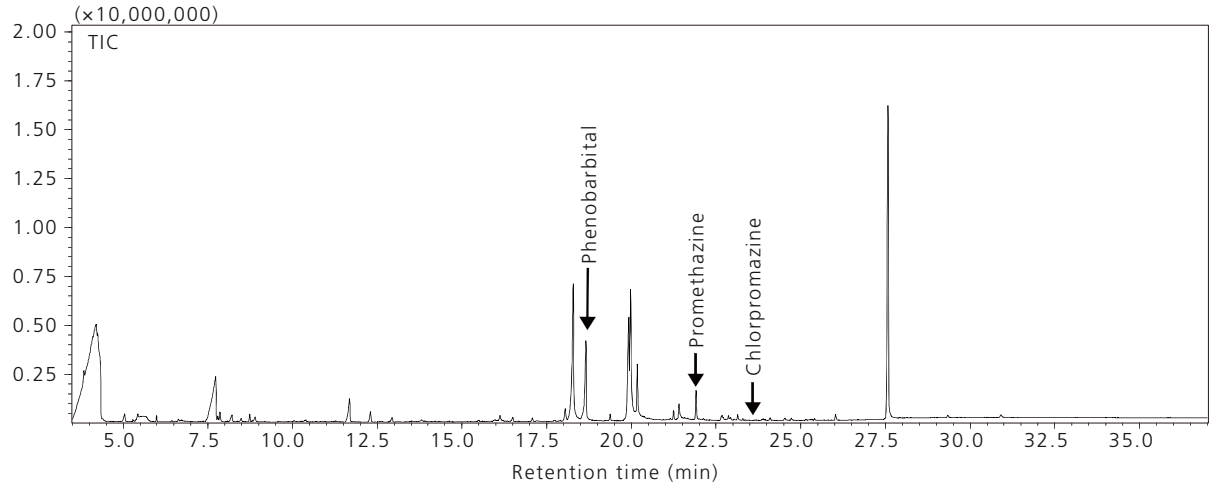


Fig. 4 Total Ion Current Chromatogram Obtained Using Actual Sample

Table 3 Comparison of Quantitated Results and Semi-Quantitated of Real Specimen

Compounds	Quantitation* (µg/mL)	Semi-Quantitation (µg/mL)
Phenobarbital	11.6	33.2
Chlorpromazine	0.03	0.02
Promethazine	0.13	0.15

* Determined from calibration curve generated using spiked serum.

Conclusion

Automatic qualitative and semi-quantitative analyses were conducted for 3 psychotropic drug substances (phenobarbital, chlorpromazine, and promethazine) in serum using the GC/MS Forensic Toxicological Database. Using a method which incorporated information on 162 psychiatric drugs, automatic identification of phenobarbital, chlorpromazine, and promethazine was possible using retention time correction via the AART feature of the GCMSsolution software. Relatively accurate quantitative results were obtained for chlorpromazine and promethazine, but the results obtained for phenobarbital tended to be 2 to 3 times higher than the spiked levels. Since the semi-quantitative values obtained using this feature of the GC/MS Forensic Toxicological Database are just quantitative estimates of the concentrations in the final sample, they should be considered as values subject to great variation, depending on such factors as the error in the drug recovery ratio or sample concentration in pretreatment, matrix effects, and instrument condition. However, since automatic quantitative analysis using this database can be conducted simultaneously with an automatic database search, it can certainly be useful for obtaining a rough estimate of a drug's concentration while conducting qualitative analysis, or for quickly estimating concentration of a specific drug when there is no time for preparing calibration standards.

Gas Chromatograph Mass Spectrometer

GCMS-QP2010 Ultra

Features

1. High sensitivity
2. Easy maintenance
3. Identification of compounds using retention indices

The Shimadzu GCMS-QP2010 series has optimum functions and performance for forensic toxicology.

1. The Shimadzu GCMS-QP2010 series features an extremely high sensitivity and capability to measure forensic toxicology-related compounds down to low concentrations.
2. Urine and blood samples contain lots of contaminants. When samples such as these are measured by GC/MS, contamination of the ion source becomes problematic.The GCMS-QP2010 series is less likely to become dirty, and, moreover, can be easily cleaned even if the ion source is contaminated.
3. It is difficult to obtain standard samples for forensic toxicology-related compounds. However, in the GC/MS Forensic Toxicological Database for the GCMS-QP2010 series, the information of more than 500 medicinal toxicants is registered to method files together with optimum analysis conditions.

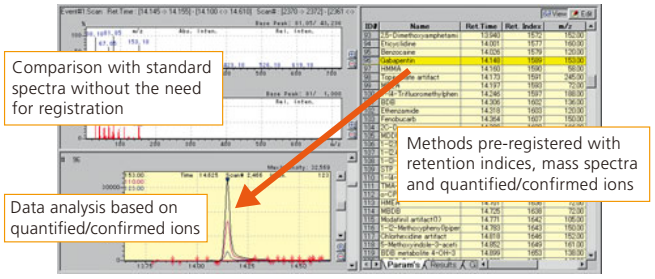
GC/MS Forensic Toxicological Database (Drugs of Abuse / Medicines / Pesticides)

The "GC/MS Forensic Toxicological Database" is exclusively for the GCMSsolution workstation software for GCMS-QP2010 series gas chromatograph mass spectrometers. It is pre-registered with 1011 mass spectra including free-, TMS- and TFA- body types for 502 compounds that are required in forensic toxicological analysis of drugs of abuse, drugs for psychiatric and neurological disease, and other medicines and pesticides.

This database comprises the following: method files pre-registered with analytical conditions, mass spectra, retention indices, etc., compound information including CAS numbers, etc., libraries containing mass spectra and retention indices, and a handbook (printed version of library information).

Spectra for 591 drugs of abuse, 274 drugs for psychiatric and neurological disease, 110 medicines, and 36 pesticides are registered to the methods and libraries.

Use of this database enables high-precision identification of compounds based on the AART (Automatic Adjustment of Retention Time) that uses retention indices, and based on mass chromatograms compared with standard mass spectra and quantified/confirmed ions.



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Application News

Gas Chromatograph Mass Spectrometer

No. GCMS-1401

Analysis of Opioids Using Isotope Dilution with GCMS-TQ8030 GC/MS/MS

■ Introduction

Development of methods for analysis of drugs of abuse has become a high priority for both forensic toxicology and law enforcement. The large numbers of individual drugs and new "designer drugs" has made method development for these compounds a significant undertaking.

Gas chromatography mass spectrometry (GCMS) has been used extensively for analysis of drug residues and trace-level drugs in biological fluids. The most significant challenges have been matrix interference and achievement of meaningful detection limits for the compounds of interest. Triple quadrupole GC/MS/MS has emerged as a powerful technique for trace-level analysis in these complex biological matrices. Operation of the triple quadrupole GC/MS/MS in the Multiple Reaction Monitoring (MRM) mode provides exceptional sensitivity, selectivity, and specificity for detection and quantitation of targeted drugs in the presence of background interferences.

The isotope dilution technique, using isotopically-labeled analogs of target compounds as internal standards, is a widely used analytical approach for precise quantitation in drug assays. However, in many cases, when using deuterium-labeled analogs the mass spectra differ only slightly from the corresponding unlabeled compounds. The challenge is complicated when the native and labeled compounds completely or partially co-elute, as they often do, and the spectra overlap. Combining the specificity of unique MRM transitions for close-eluting native and labeled analogues, with the sensitivity of triple quadrupole MRM transitions is a powerful technique for unambiguous, quantitative determination of this important compound class.

This application note presents instrument configuration, operating parameters, and analytical results for analysis of a common narcotic, hydrocodone, using the isotope dilution technique paired with the specificity of the MRM analysis mode of the Shimadzu GCMS-TQ8030 triple quadrupole GC/MS/MS (Figure 1). Internal standard calibration of codeine and oxycodone was also included in the study.

Figure 1: Shimadzu GCMS-TQ8030 Triple Quadrupole GC/MS/MS

■ Experimental

The analyses were conducted using a Shimadzu GCMS-TQ8030 triple quadrupole GC/MS/MS operated in the multiple reaction monitoring (MRM) mode, with optimized collision energy (CE) for each MRM transition providing ultimate sensitivity. The instrument configuration and operating conditions are shown in Table 1.

Table 1: Instrument Configuration and Operating Conditions for Analysis of Opioid Drugs

Instrument	GCMS-TQ8030
Inlet	270 °C Splitless liner with glass wool (Shimadzu 221-48876-02) Splitless injection, sampling time 1 minute
Column	RXI-5MS, 30 m x 0.25 mm x 0.25 µm (Restek 13423) Helium carrier gas Constant linear velocity 37 cm/second
Oven Program	100 °C, hold 1.0 minute 20 °C/minute to 250 °C, hold 3.0 minutes 10 °C/minute to 300 °C, (no final hold) MS interface 250 °C Analysis time 20 minutes
Ion Source	200 °C Electron ionization (EI) mode, 70 eV
Operation Mode	Multiple Reaction Monitoring (MRM) Argon gas, 200 kPa
Detector	Electron multiplier 1.0 kV

Six MRM transitions were selected for both hydrocodone-d_3 and hydrocodone, most of which had unique precursor ions paired with common product ions. (Refer to Table 2.) This approach allowed evaluation of any potential mass spectral interference, or cross-talk, between the transition pairs of these two co-eluting compounds. Three transitions were selected for codeine and oxycodone, since they were chromatographically resolved from the other compounds, and there were no isotopically labeled internal standards used.

Table 2: MRM Transition Details with Optimized Collision Energies (CE)

Compound	Transition #1 (CE)	Transition #2 (CE)	Transition #3 (CE)	Transition #4 (CE)	Transition #5 (CE)	Transition #6 (CE)
Hydrocodone-d_3 (IS)	302 > 242 (11V)	302 > 214 (19V)	302 > 185 (27V)	302 > 273 (19V)	302 >245 (27V)	302 > 231 (27V)
Hydrocodone	299 > 242 (11V)	299 > 214 (19V)	299 > 185 (27V)	299 > 270 (19V)	299 > 242 (23V)	299 > 228 (23V)
Codeine	299 > 162 (11V)	299 > 229 (19V)	299 > 280 (15V)			
Oxycodone	315 > 258 (11V)	315 > 230 (19V)	315 > 201 (19V)			

Calibration standards were prepared in methanol, and data for a 5-point calibration were acquired over the range of 25-200 ng/mL (parts-per-billion, ppb). The calibration curve for hydrocodone was generated using the isotope dilution technique. The concentration of the internal standard, hydrocodone-d_3 was held constant at 100 ng/mL. The concentration range of the calibration was sufficient to satisfy the requirements of the specific application. The chromatographic conditions chosen were intended to fit into a larger scheme for analysis of numerous drug classes, so optimization of the chromatographic conditions for efficiency was not considered in this study.

■ Results and Discussion

Chromatography

The total ion chromatogram (TIC) acquired in the MRM mode for the opioid drug mix is shown in Figure 2. The chromatographic peaks for hydrocodone-d_3 and hydrocodone partially overlap, with the deuterium labeled analog eluting first. In the MRM mode, the TIC is the sum of the signal for each MRM transition for that particular analyte, so the appearance of the chromatogram is slightly different than the typical TIC chromatogram from full scan analysis.

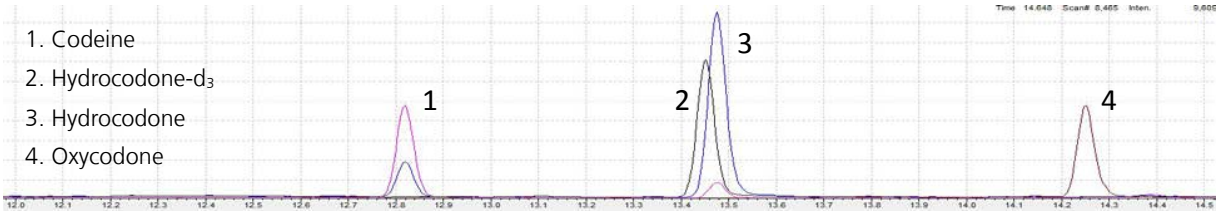


Figure 2: Total Ion Chromatogram (TIC) of Opioid Standard

Mass Spectral Results in the Full Scan ("Q3 Scan") Mode

The full scan mass spectra of hydrocodone-d_3 and hydrocodone are shown in Figures 3A and 3B. Notable features of these mass spectra are the prominent molecular ions for the labeled and unlabeled compounds at *m/z* 302 and 299, respectively, with the difference of 3 *m/z* units associated with the isotopically labeled n-methyl group on hydrocodone-d_3.

Common fragment ions are present in both spectra at *m/z* 242, 214, 199, 185, and 115 (indicated with an ↓ in figures 3A and 3B). These fragments represent loss of a fragment which includes the labeled n-methyl group from hydrocodone-d_3, and the corresponding unlabeled n-methyl group from hydrocodone, to form identical fragment ions from the two compounds.

Fragment ion pairs in the spectra for the labeled/unlabeled compounds can be seen at *m/z* 287 and 284, 273 and 270, 231 and 228, 99 and 96, 62 and 59 (indicated with an * in figures 3A and 3B). In this case, the corresponding fragments are offset by a difference of 3 *m/z* units (e.g. 287 and 284), and represent the loss of the same non-labeled group from hydrocodone-d_3 and hydrocodone, respectively.

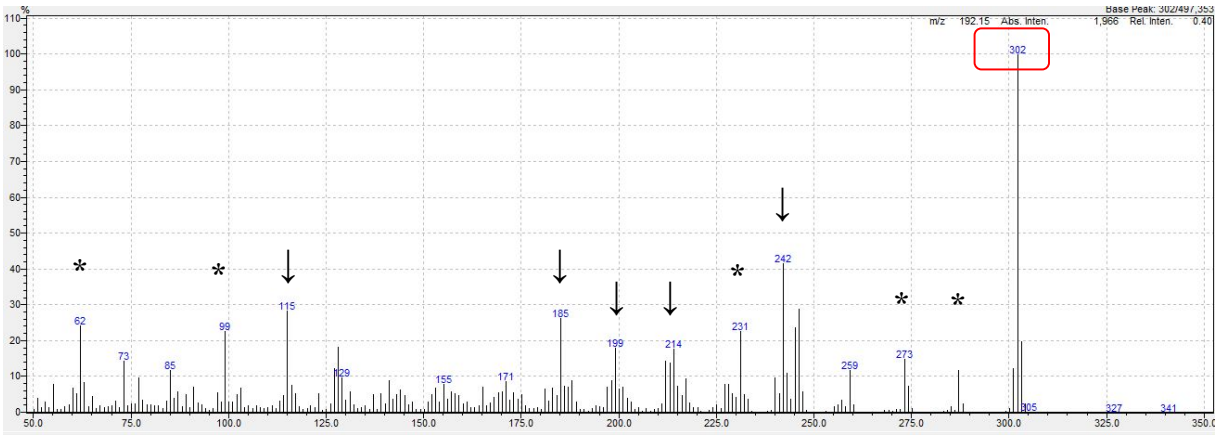


Figure 3A: Total Ion Mass Spectrum of Hydrocodone-d_3

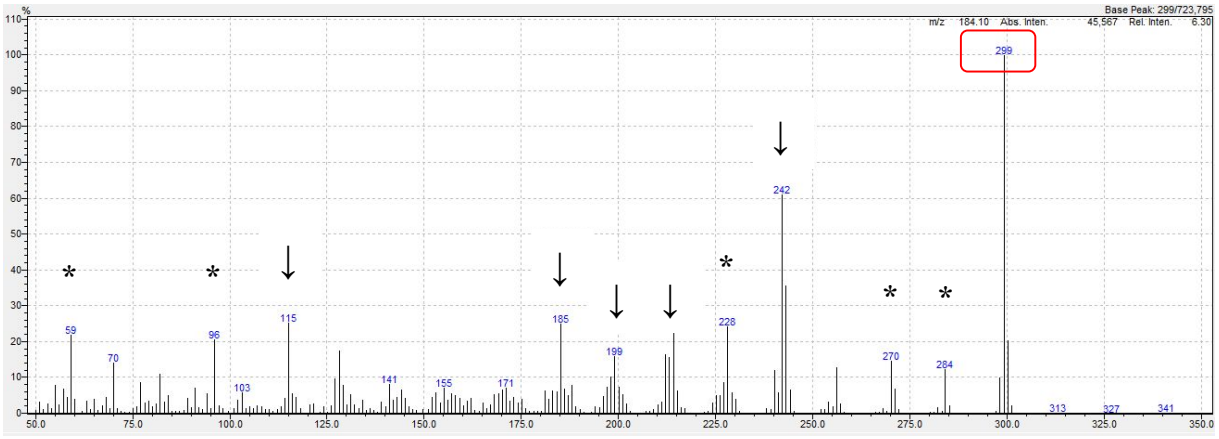


Figure 3B: Total Ion Mass Spectrum of Hydrocodone

The full scan spectra of hydrocodone-d_3 and hydrocodone were used to select precursor and product ions for the MRM transitions. Three transitions were selected for each compound based on their unique molecular ions and common product ions (e.g. 302 → 242 and 299 → 242). To illustrate the unique specificity of the MRM mode, a second set of three transitions was defined using the molecular ions and unique product ions for hydrocodone-d_3 and hydrocodone (e.g. 302 → 273 and 299 → 270). The ions selected for MRM transitions are tabulated in Table 2 above.

Mass Spectral Results in the Multiple Reaction Monitoring (MRM) Mode

Operation of the GCMS-TQ8030 in the MRM mode provides enhanced selectivity for analysis of trace-level contaminants in complex matrices, such as drugs of abuse in biological fluids, because the co-extracted matrix interferences are significantly minimized. The compound specificity that can be achieved by using unique MRM transitions for each compound, even when they have common product ions, is illustrated in Figure 4. Figure 4 includes six overlaid MRM chromatograms for hydrocodone-d_3 and six for hydrocodone, as described above. Note that the chromatograms corresponding to the MRM transitions for hydrocodone-d_3 and hydrocodone are uniquely defined for each of the analytes and do not interfere with one another, even for the three transitions that have common MRM product ions. The non-interfering chromatograms illustrate the power of the MRM mode, and the specificity that can be achieved when unique transitions are selected for close-eluting compounds with similar mass spectra.

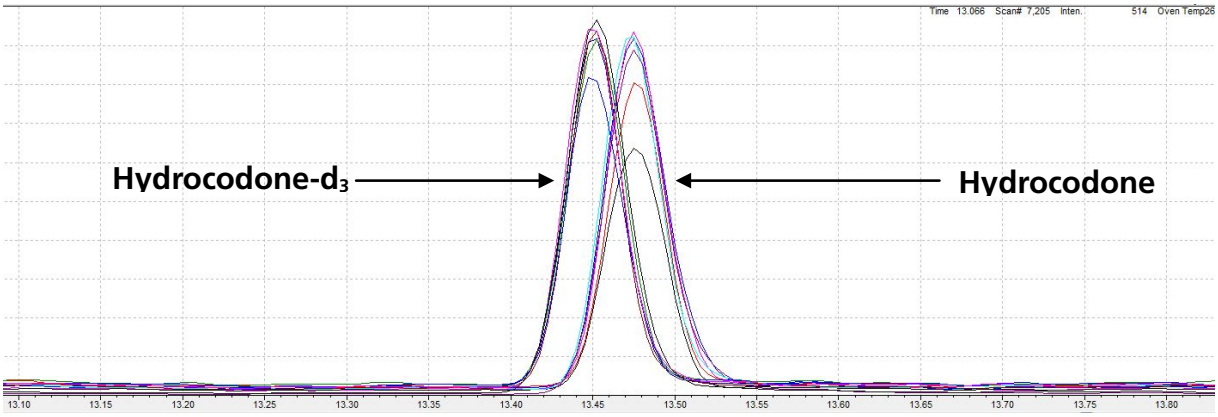


Figure 4: Six Overlaid MRM Chromatograms for Hydrocodone-d_3 and Six for Hydrocodone

Cross-Talk

"Cross-talk" is a phenomenon unique to triple quadrupole mass spectrometry. It occurs when residual ion fragments are not fully swept from the collision cell at the end of a cycle; they remain in the collision cell and are detected as "ghost fragments" in subsequent transitions. Cross-talk is depicted graphically in Figures 5A and 5B below. Figure 5A depicts slowing down of product ions in the collision cell, which results from interactions with the CID gas. In some cases, a small portion of the residual product ions have slowed down, and may not be completely swept from the collision cell during the transition, resulting in cross-talk. Figure 5B illustrates the results of cross-talk as "ghost" mass spectral fragment peaks that can appear in subsequent transitions.

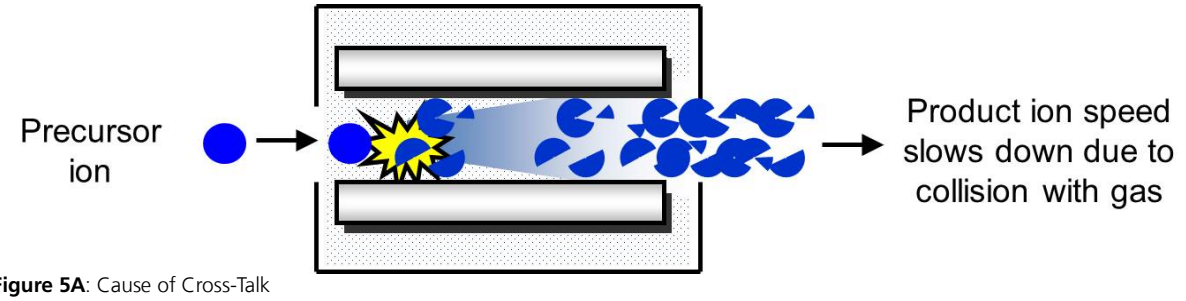


Figure 5A: Cause of Cross-Talk

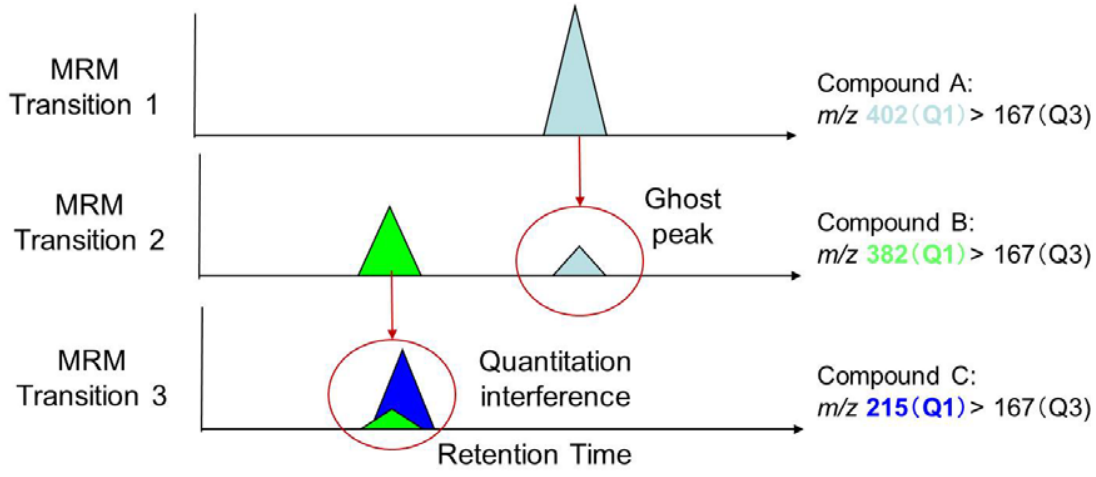
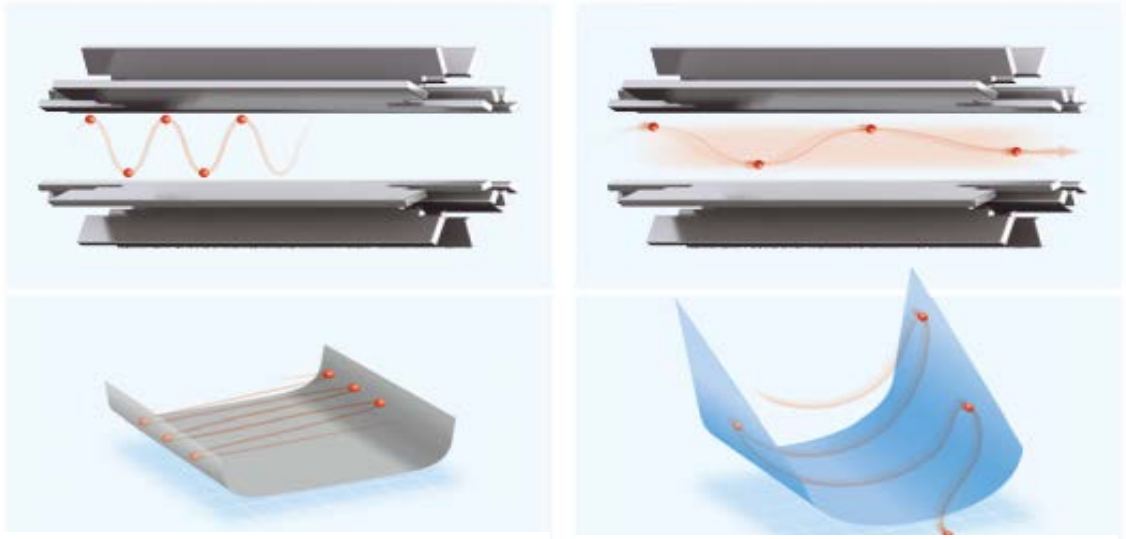


Figure 5B: Results of Cross-Talk

Cross-talk is virtually eliminated in the GCMS-TQ8030 using UFsweeper® technology. The UFSweeper design alters the pseudo-potential surface within the collision cell, shortening the path which ions must travel and accelerating them through the cell and toward Q3. This process completely clears the collision cell with each transition, and eliminates cross-talk from one transition to the next. The pseudo-potential surface of the GCMS-TQ8030 UFsweeper® technology is illustrated in Figure 6 below. The overlaid chromatograms in Figure 4 clearly show that there was no indication of cross-talk present.



With conventional technology, ions lose momentum and have a longer travel path due to collision with CID gas

The UFSweeper® pseudo-potential surface shortens the pathway and accelerates ions through the collision cell without losing momentum

Figure 6: Graphic Depiction of UFSweeper® Technology

Calibration Results

Five calibration standards were prepared for the opioids over the range of 25-500 ng/mL (ppb) and transferred to autosampler vials with limited-volume inserts for analysis; hydrocodone-d_3 was used as the the internal standard and was held at a constant concentration of 100 ng/mL. The calibration standards were analyzed using the instrument conditions outlined above. The electron multiplier was adjusted to give acceptable response at the lowest calibration level and avoid saturation at the highest calibration level.

Response factors were calculated and percent relative standard deviation (%RSD) determined using the GCMSsolution software. The precision of the calibration is evaluated using the %RSD of the response factors and the correlation coefficient (r) for each of the calibration analytes. The %RSD and correlation coefficient values for the multi-point calibration are shown in Table 3. The linear, multi-point calibration curve for hydrocodone is illustrated in Figure 7. Calibration results demonstrate linearity for each of the analytes.

Table 3: Results of the 5-Point Calibration for Three Opioids From 25 to 200 ng/mL using the MRM Analysis Mode

Compound	Calibration Type	Mean RF	RF %RSD	r
Codeine	Internal Standard	0.643	12.1	0.9995
Hydrocodone	Isotope Dilution	1.011	15.6	0.9999
Oxycodone	Internal Standard	0.376	14.8	0.9999

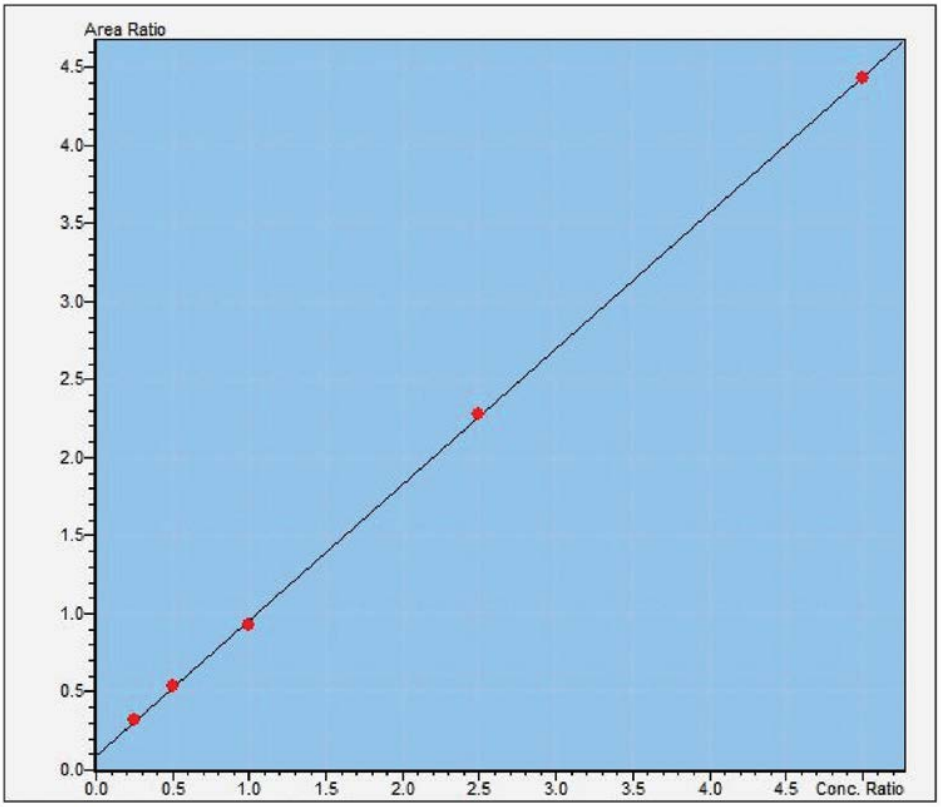


Figure 7: Linear, Multi-point Calibration for Hydrocodone from 25 to 200 ng/mL

■ Summary and Conclusion

The results demonstrate the power and specificity of the MRM analysis mode when using unique transitions for close-eluting compounds such as hydrocodone-d_3 and hydrocodone, even when they have similar mass spectra and common product ions. This experiment also illustrates the effectiveness of the Shimadzu GCMS-TQ8030 fast scanning and UFsweeper technologies for completely clearing the collision cell with each transition and eliminating any cross-talk. The multi-point calibration for hydrocodone was linear and passes thru zero, further supporting that there was no interference from cross-talk or the close-eluting deuterium-labeled internal standard.

■ Acknowledgement

The authors wish to acknowledge the collaboration of chemists from the Niagara County Sherriff's Department Laboratory, Lockport, NY for suggesting the experiment and for providing analytical standards described in this application note.

First Edition: January 2014

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Application Data Sheet

No.100

GC-MS
Gas Chromatograph Mass Spectrometer

Analysis of Toxicological Substances in Whole Blood Using Smart Forensic Database (1)

By providing mass separation in two stages, GC-MS/MS is capable of separating out interferences in biological samples and toxicological substances. Therefore, it is simple to determine whether toxicological substances are present, significantly reducing the time required for data analysis. In order to analyze toxicological substances in MRM mode, however, MRM transitions and collision energies (CE) must be optimized, which is very labor intensive.

Smart Forensic Database is an MRM database containing retention indices, MRM transitions, collision energies, and quantitation/confirmation ion ratios for 201 toxicological substances often involved in poisonings. The retention times for the registered toxicological substances are accurately estimated simultaneously from low-boiling point components to high-boiling point components, using measurement data from a standard n-alkane mixture via the GCMSsolution AART function. Smart MRM, which is provided with the GCMS-TQ8040, can then create MRM analysis methods automatically using the database.

This article introduces an example of applying Smart Forensic Database to the analysis of toxicological substances in a whole-blood sample.

Experimental

Liquid-liquid extraction via EXtrelut® NT3 was used to pretreat the whole-blood sample. The collected whole-blood sample was measured into 1 mL portions for acidic fractionation and basic fractionation, and each portion was diluted with 1 mL of Milli-Q water. The acidic fraction was adjusted to a pH 5 using 10 % hydrochloric acid, and the basic fraction was adjusted to a pH 9 using 10 % ammonia water. The respective solutions were added to the EXtrelut® NT3 columns and left to stand for 30 minutes, after which each was eluted with a 10 mL chloroform:isopropanol (3:1) mixture. The extracted solutions of acidic fraction and basic fraction were then mixed, and after dessication with silica gel and drying in a nitrogen airflow, the sample solution was re-dissolved in a 200 μL chloroform:isopropanol (3:1) mixture. To check the MRM sensitivity, the sample obtained was spiked with promethazine, phenobarbital, chlorpromazine, and triazolam so that the concentration of each compound becomes 50 ng/mL in whole blood.

Analytical Conditions

The conditions registered in Smart Forensic Database were used as the GC-MS/MS analysis conditions. For the compounds subject to MRM measurement, a simultaneous Scan/MRM analysis method was created, in which the 201 components registered in the database were set.

Table 1: Analytical Conditions

GC-MS:	GCMS-TQ8040
Column:	Rxi® -5SilMS (Length 30 m, 0.25 mm I.D., df=0.25 μm)
Glass liner:	Splitless insert with wool (PN: 221-48876-03)

[GC]	
Injection temp.:	260 °C
Column oven temp.:	60 °C (2 min) → (10 °C /min) → 320 °C (15 min)
Injection mode:	Splitless
Flow control mode:	Linear velocity (45.6 cm/sec)
Injection volume:	1 μL

[MS]	
Interface temp.:	280 °C
Ion source temp.:	200 °C
Acquisition mode:	Scan/MRM
Scan event time:	0.1 sec
Scan mass range:	*m/z* 43 – 600
Scan speed:	10,000 u/sec
MRM event time:	0.3 sec
Total loop time:	0.4 sec

Results

The extracted whole-blood sample was spiked with four toxicological substances so that the concentration of each substance becomes 50 ng/mL in whole blood, and then measured using Scan/MRM mode. Fig. 1 shows the mass chromatograms obtained, and Fig. 2 shows the repeatability obtained by repeating analyses five times. With the Scan mode analysis, confirmation ions were not detected, there was an overlap with cholesterol, and the peak for triazolam could not be confirmed. With the MRM mode, however, each component was clearly detected, and favorable repeatability results of 4.29 % max. were obtained.

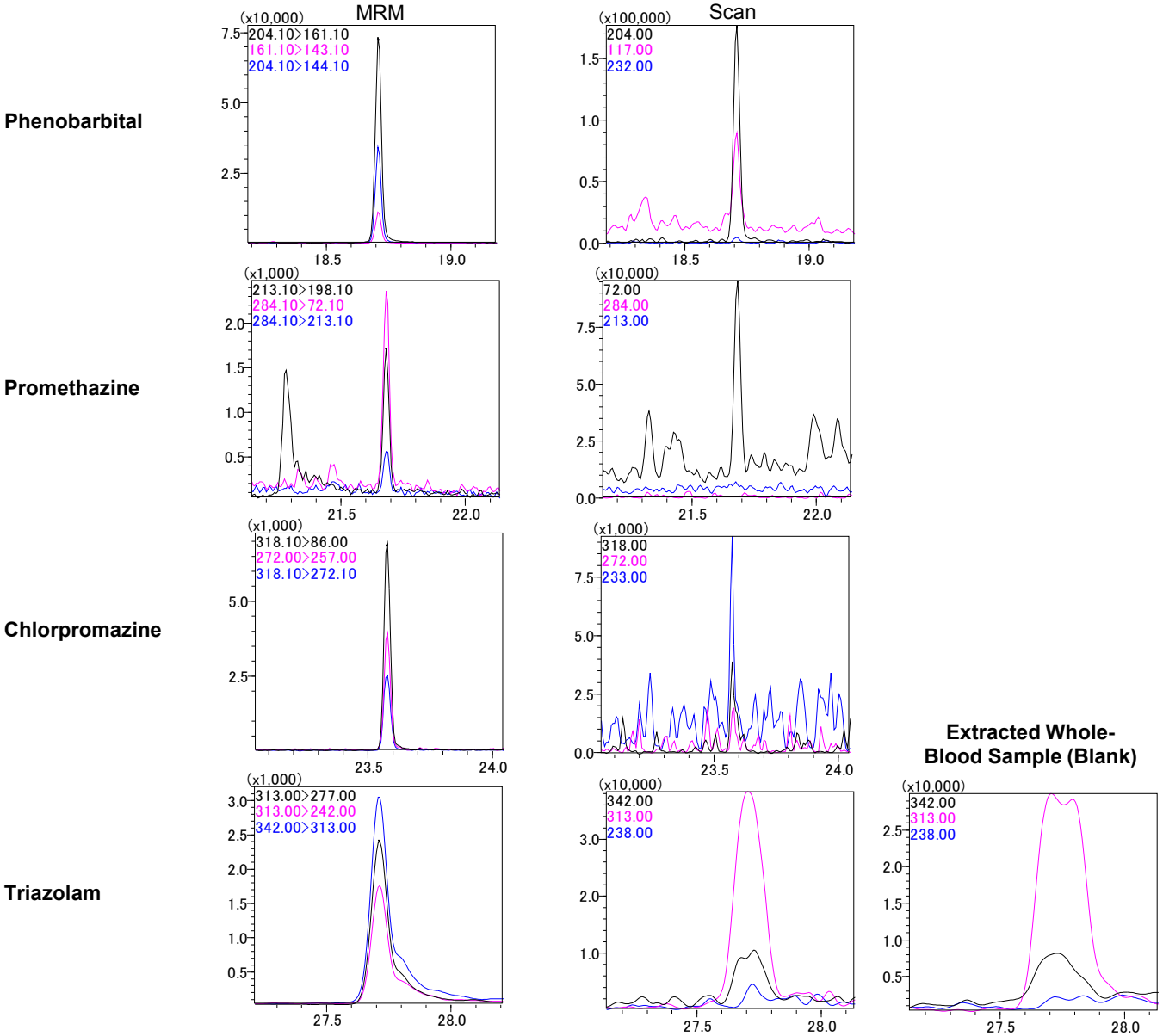

Fig. 1: Scan and MRM Mass Chromatograms for Four Toxicological Substances in Whole-Blood Samples (L: MRM; R: Scan)

Table 1: Area Repeatability at Five Replicates (Concentration in Whole Blood: 50 ng/mL)

	Data 1	Data 2	Data 3	Data 4	Data 5	Average	SD	%RSD
Phenobarbital	131,876	133,119	137,359	136,480	133,656	134,498	2323.7	1.73
Promethazine	2,756	2,873	2,742	2,885	2,829	2,817	65.7	2.33
Chlorpromazine	12,832	12,899	12,657	13,484	14,024	13,179	565.3	4.29
Triazolam	10,909	10,315	10,704	10,838	10,701	10,693	229.5	2.15

The data evaluated in this article was obtained from a sample that was spiked with the substances after extraction. There is no guarantee that a favorable recovery ratio will be obtained with the pretreatment method described above.

This data was provided by Associate Professor Kei Zaitsu in the Department of Legal Medicine & Bioethics, Nagoya University Graduate School of Medicine.

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Application Data Sheet

No.139

GC-MS
Gas Chromatograph Mass Spectrometer

Analysis of Volatile Toxic Substances Using Headspace GC/MS Part.1 - Paint Thinner and Alcohol -

Forensic toxicologists in the police laboratories and forensic medicine departments of university measure a variety of volatile substances in the course of investigating accidents, crimes, and other incidents.
Blood alcohol (ethanol) is measured to provide evidence in cases of traffic accidents caused by drinking, incidents involving alcohol such as physical assault and injury, and acute alcohol poisoning.
Paint thinner is a solvent with toluene, methanol, and ethyl acetate as its main constituents. Paint thinner also has anesthetic and stimulant properties. Paint thinner abuse by inhalation has become prevalent and laws have been enacted to prevent the harmful effects of its abuse.
Used in industrial applications, cyanide and azide are compounds that are relatively easy to procure. This has resulted in incidents of contamination by these toxic substances. After these incidents, testing regimes for poisons were enhanced in order to determine the cause of such incidents, i.e. crime or suicide.
Blood alcohol and paint thinner can be measured relatively simply using headspace sampler, with measurements performed on a routine basis at police laboratories and in university forensic departments.
There have been reports of the headspace method being used to measure cyanide and azide. These compounds are normally measured by performing PFB derivatization, solvent extraction, and then liquid injection for GC/MS analysis. However, the derivatization and extraction steps of this method are labor-intensive.

This two-part application presents details on the investigation of using headspace GC/MS to measure cyanide and azide, as well as information on optimizing column conditions to allow simultaneous measurement of alcohol and paint thinner. Of these two investigations, results obtained from measuring blood alcohol and paint thinner are presented below. Please see Application Data Sheet No. 140 for part two.

Sample Preparation

Blood Ethanol Analysis

An aqueous solution of 1-propanol (0.5 mg/mL), which was to be added to blood samples as the internal standard, was prepared by dissolving 1-propanol in distilled water. Standards for calibration curve of blood ethanol were prepared by making up samples of hemolyzed equine blood with ethanol concentrations of 0.03, 0.1, 0.3, 1.0, and 2.0 mg/mL, then adding 0.5 mL of each of these samples and 0.5 mL of 1-propanol (0.5 mg/mL) internal standard to a 20 mL headspace vial. Each vial was quickly sealed with a headspace cap and then agitated. To confirm that 2-propanol, which is used for sterilization during blood sampling, is separated from ethanol and 1-propanol on a chromatogram, an aqueous solution of ethanol, 2-propanol, and 1-propanol was also prepared.

Paint Thinner Analysis

Paint thinner (5 μL) was added to a 20 mL headspace vial, which was quickly sealed with a headspace cap.

HS-20 Headspace Sampler + GCMS-QP™2020

Analytical Conditions

Table 1 shows the headspace and GC/MS analytical conditions. Alcohol, cyanide, and azide were all measured using the same headspace and GC/MS conditions, and only paint thinner was measured with different conditions after changing the split ratio and detector voltage. With the HS-20 headspace sampler, even when GC/MS analysis conditions are changed, measurements can be performed within the same batch file by switching methods, as long as the same headspace conditions are used. Changing the split ratio is an effective way of analyzing both cyanides and azides that must be measured at trace quantities, and undiluted thinner solution that contains high-concentration constituents.

For this application, results were collected using the GCMS-TQ™8040 GC-MS/MS device, though the same results can be obtained using the GCMS-QP™2020 single-GC/MS device.

Table 1: Analytical Conditions

HS: HS-20
GC-MS: GCMS-TQ™8040

[HS]		[GC]	
Headspace mode: Loop		Column:	Rtx™-BAC2 (length: 30 m, 0.32 mm I.D., df = 1.2 μm, Restek Corporation)
Oven temp.:	60 °C		
Sample line temp.:	100 °C	Column oven temp.:	40 °C (5 min) → (40 °C /min) → 200 °C (1 min)
Transfer line temp.:	150 °C	Carrier gas:	Helium
Vial pressurization gas pressure:	70 kPa	Carrier gas control:	Linear velocity (62.5 cm/sec)
Vial warming time:	10 min	Injection mode:	Split
Vial pressurization time:	0.5 min	Split ratio:	10:1 (alcohol, cyanide, azide) 30:1 (paint thinner)
Loading time:	0.5 min		
Loading equalization time:	0 min	[MS]	
Injection time:	0.5 min	Interface temp.:	230 °C
Needle flush time:	5 min	Solvent elution time:	0.7 min
GC cycle time:	18 min	Measurement mode:	Scan
		Event time:	0.2 sec

Ion source temp.:	200 °C		
Data acquisition time:			1 - 10 min
Mass range:	*m/z* 10 - 300		
Emission current:	60 μA (standard)		

*Note: The detection voltage and other conditions must be optimized since they can differ depending on equipment status.

Analytical Results of Blood Ethanol

Fig. 1 shows the total ion current (TIC) chromatogram obtained when analyzing a standard of 0.3 mg/mL ethanol to which 2-propanol had been added—a compound that is used for sterilization during blood collection. The chromatogram shows complete separation of 2-propanol and that quantitative values were not affected by 2-propanol.

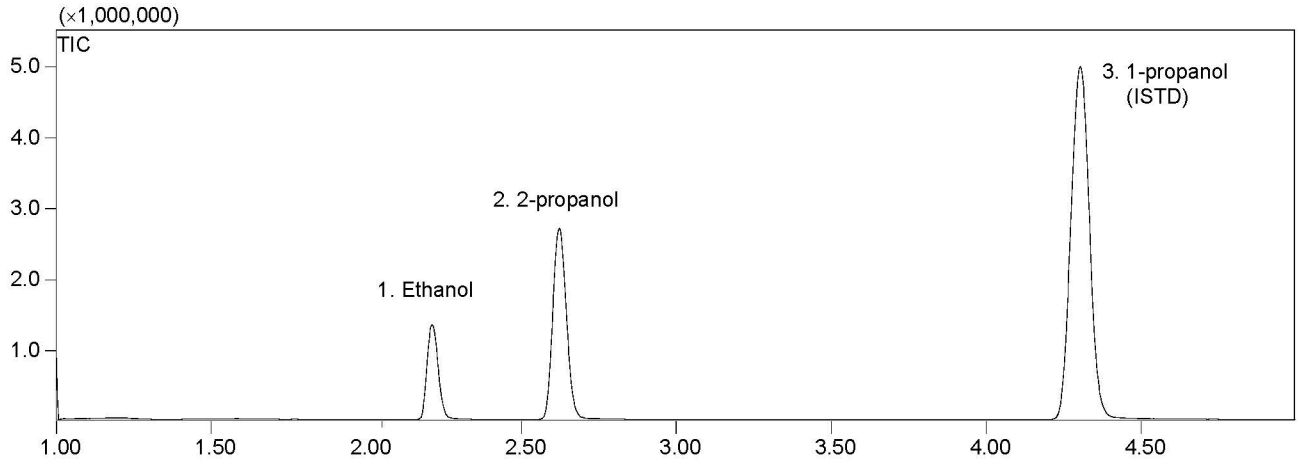

Fig. 1: Total Ion Current Chromatogram of 2-Propanol Added to 0.3 mg/mL Ethanol Standard

The chromatograms obtained when analyzing a blank and a 0.03 mg/mL standard are shown in Fig. 2. The calibration curve (0.03, 0.1, 0.3, 1.0, 2.0 mg/mL) obtained after internal standard correction is shown in Fig. 3. The calibration curve correlation coefficient (R) was 0.9999 or above, showing that linearity was good. Table 2 shows the reproducibility (n = 8) of measuring 0.3 mg/mL, which is the reference concentration used as the basis of the breath test in Japan's Road Traffic Act and Order for Enforcement of the Road Traffic Act. Table 3 shows the reproducibility (n = 8) of measuring 0.03 mg/mL, which is 1/10 the reference concentration. The accuracy of quantitative measurements of the 0.3 mg/mL concentration sample was 100.2 - 100.9 % (average 100.5 %) with a reproducibility relative standard deviation (%RSD) of about 0.2 %. The accuracy of quantitative measurements of the 0.03 mg/mL concentration sample was 89.4 - 97.1 % (average 95.2 %) with a reproducibility relative standard deviation (%RSD) of about 2.8 %.

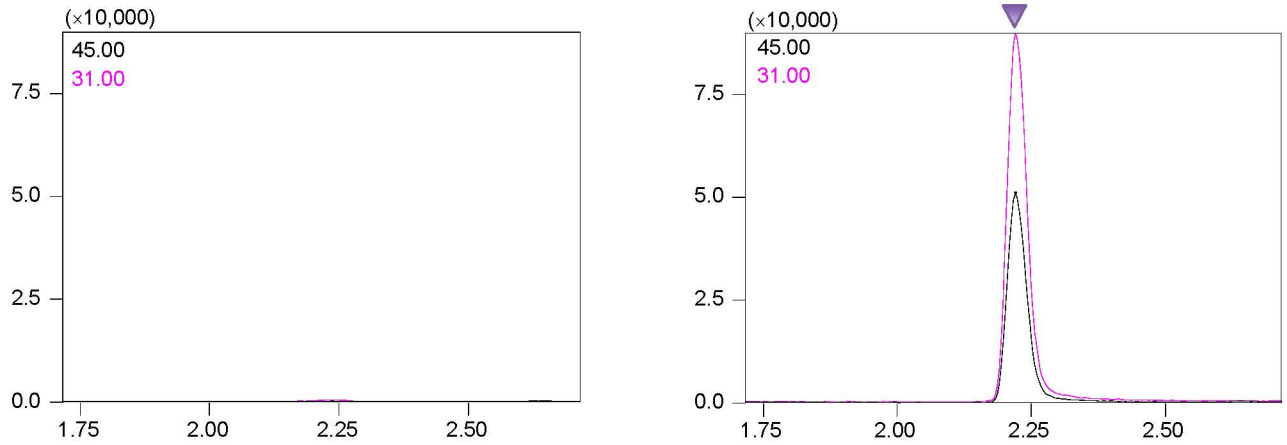

Fig. 2: Mass Chromatograms of Ethanol when Analyzing a Blank Sample and a 0.03 mg/mL Standard Sample
Left: Blank, Right: 0.03 mg/mL Standard

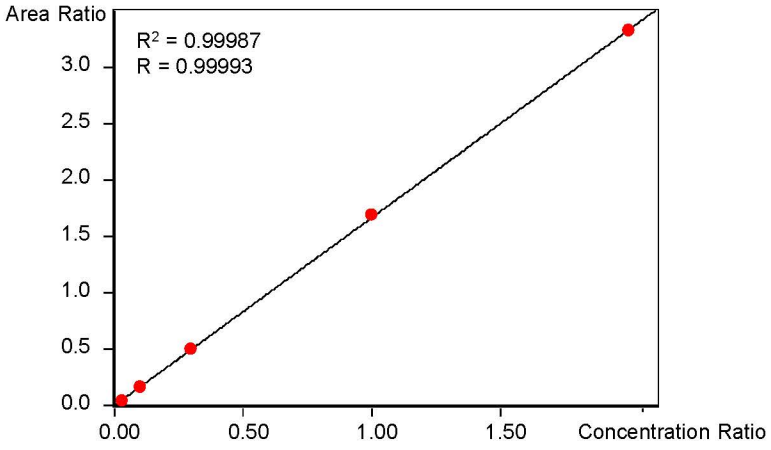

Fig. 3: Ethanol Calibration Curve (Concentration: 0.03 - 2.0 mg/mL)

Table 2: Reproducibility for 0.3 mg/mL (n = 8)

	Area Ratio	Concentration (mg/mL)	Accuracy (%)
1st	0.502	0.302	100.5
2nd	0.501	0.301	100.4
3rd	0.500	0.301	100.2
4th	0.504	0.302	100.8
5th	0.504	0.303	100.9
6th	0.503	0.302	100.7
7th	0.502	0.301	100.4
8th	0.502	0.301	100.5
Average	0.502	0.302	100.5
Standard Deviation (SD)	0.001	0.001	0.227
%RSD	0.226	0.226	0.226

Table 3: Reproducibility for 0.03 mg/mL (n = 8)

	Area Ratio	Concentration (mg/mL)	Accuracy (%)
1st	0.130	0.029	96.1
2nd	0.131	0.029	96.8
3rd	0.131	0.029	96.8
4th	0.129	0.029	95.5
5th	0.131	0.029	97.1
6th	0.131	0.029	97.1
7th	0.121	0.027	89.4
8th	0.126	0.028	92.9
Average	0.129	0.029	95.2
Standard Deviation (SD)	0.004	0.001	2.716
%RSD	2.849	2.853	2.853

Analytical Results of Paint Thinner

The total ion current chromatogram obtained after analyzing commercially available paint thinner is shown in Fig. 4. The mass spectra of each constituent of paint thinner (methanol, ethyl acetate, and toluene) are shown in Fig. 5. The analysis conditions used in this investigation allowed for separation of the three main constituents of paint thinner (methanol, ethyl acetate, and toluene) in 10 minutes.

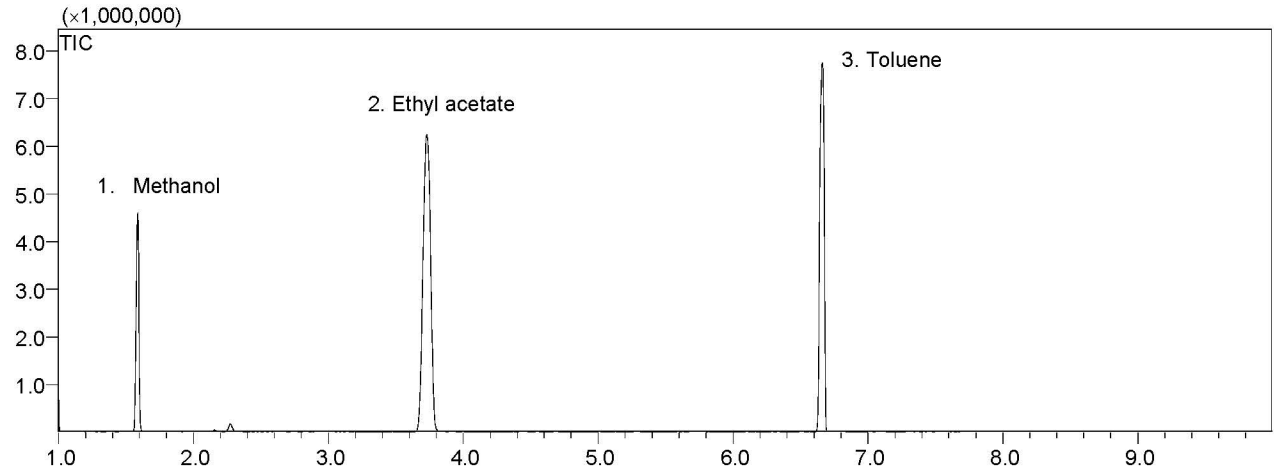

Fig. 4: Total Ion Current Chromatogram Obtained after Analysis of Undiluted Paint Thinner

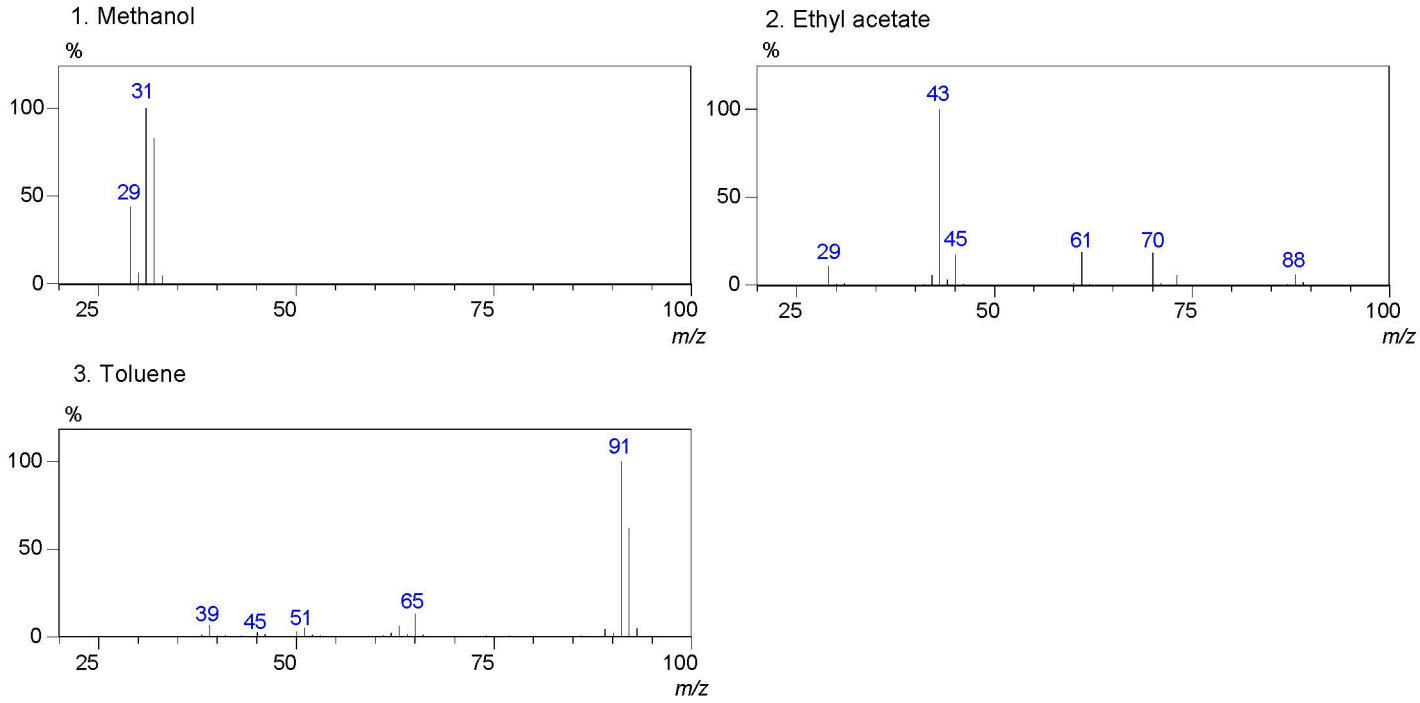

Fig. 5: Mass Spectra of Main Constituents Obtained after Analysis of Paint Thinner

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Application Data Sheet

No.140

GC-MS
Gas Chromatograph Mass Spectrometer

Analysis of Volatile Toxic Substances Using Headspace GC/MS Part.2 - Cyanide and Azide -

Forensic toxicologists in the police laboratories and forensic medicine departments of university measure a variety of volatile substances in the course of investigating accidents, crimes, and other incidents.
Used in industrial applications, cyanide and azide are compounds that are relatively easy to procure. This has resulted in incidents of contamination by these toxic substances. After these incidents, testing regimes for poisons were enhanced in order to determine the cause of such incidents, i.e. crime or suicide.
Blood alcohol and paint thinner can be measured relatively simply using headspace sampler, with measurements performed on a routine basis at police laboratories and in university forensic departments.
There have been reports of the headspace method being used to measure cyanide and azide. These compounds are normally measured by performing PFB derivatization, solvent extraction, and then liquid injection for GC/MS analysis. However, the derivatization and extraction steps of this method are labor-intensive.

This two-part application presents details on the investigation of using headspace GC/MS to measure cyanide and azide, as well as information on optimizing column conditions to allow simultaneous measurement of alcohol and paint thinner. Of these two investigations, results obtained from measuring cyanide and azide are presented below. Please see Application Data Sheet No. 139 for part one.

Sample Preparation

Aiming to carry out the same procedures as during blood alcohol analysis whenever possible, 1-propanol was used as the internal standard.

Standard Solutions

The standard solutions below were prepared as required.

- Aqueous solution of 1-propanol (0.5 mg/mL)
  5 mg of 1-propanol was weighed out, diluted in ultrapure water and made up to 10 mL in a measuring cylinder.
- Aqueous solution of ascorbic acid (0.1 M)
  1.76 g of L-ascorbic acid was weighed out, diluted in ultrapure water and made up to 10 mL in a measuring cylinder.
- Aqueous solution of phosphoric acid (50 %)
  Concentrated phsophoric acid (85 %) was diluted 1.7-fold in ultrapure water.
- Cyanide ion (CN-) standard solution (1 mg/mL)
  250 mg of potassium cyanide (KCN) was mixed with an aqueous solution of 0.1 M NaOH to make up 100 mL in a measuring cylinder.
- Azide ion (N3-) standard solution (1 mg/mL)
  155 mg of sodium azide (NaN_3) was diluted in ultrapure water and made up to 100 mL in a measuring cylinder.

Sample Preparation

Standards for calibration curve of cyanide and azide were prepared by adding each compound to hemolyzed equine blood to concentrations of 0.15, 0.5, 1.5, 5.0, and 10.0 μg/mL. 0.5 mL of each mixture of hemolyzed equine blood was then added to a 20 mL headspace vial, after which 0.5 mL of internal standard (aqueous solution of 1-propanol [0.5 mg/mL]) was added to each vial. 0.2 mL of 0.1 M ascorboc acid solution (aqueous) was then added, 0.2 mL of 50 % phosphoric acid was added to the inner wall, and the vial was quickly sealed with a headspace cap and agitated.

Analytical Conditions

Table 1 shows the headspace and GC/MS analytical conditions. Alcohol, cyanide, and azide were all measured using the same headspace and GC/MS conditions, and only paint thinner was measured with different conditions after changing the split ratio and detector voltage. With the HS-20 headspace sampler, even when GC/MS analysis conditions are changed, measurements can be performed within the same batch file by switching methods, as long as the same headspace conditions are used.

For this application, results were collected using the GCMS-TQ™8040 GC-MS/MS device, though the same results can be obtained using the GCMS-QP™2020 single-GC/MS device.

Table 1: Analytical Conditions

HS: HS-20
GC-MS: GCMS-TQ™8040

[HS]		[GC]			
Headspace mode:	Loop	Column:	Rtx™-BAC2 (length: 30 m, 0.32 mm I.D., df = 1.2 µm, Leistec Corporation)		
Oven temp.:	60 °C	Column oven temp.:	40 °C (5 min) → (40 °C/min) → 200 °C (1 min)		
Sample line temp.:	100 °C	Carrier gas:	Helium		
Transfer line temp.:	150 °C	Carrier gas control:	Linear velocity (62.5 cm/sec)		
Vial pressurization gas pressure:	70 kPa	Injection mode:	Split		
Vial warming time:	10 min	Split ratio:	10:1 (alcohol, cyanide, azide)		
Vial pressurization time:	0.5 min		30:1 (paint thinner)		
Loading time:	0.5 min	[MS]			
Loading equalization time:	0 min	Interface temp.:	230 °C	Ion source temp.:	200 °C
Injection time:	0.5 min	Solvent elution time:	0.7 min	Data acquisition time:	1 - 10 min
Needle flush time:	5 min	Measurement mode:	Scan	Mass range:	*m/z* 10 - 300
GC cycle time:	18 min	Event time:	0.2 sec	Emission current:	60 µA (standard)

*Note: The detection voltage and other conditions must be optimized since they can differ depending on equipment status.

Analytical Results of Blood Cyanide

Fig. 1 shows the mass chromatograms obtained when analyzing a blank and 0.15 µg/mL standard. The mass spectrum is shown in Fig. 2. The ion that appears at m/z 27, which is the HCN ion, was used for quantification. The calibration curve obtained by internal standard calibration (0.15, 0.5, 1.5, 5 and 10 µg/mL) is shown in Fig. 3. The calibration curve correlation coefficient (R) was 0.9999 or above, showing that linearity was good.

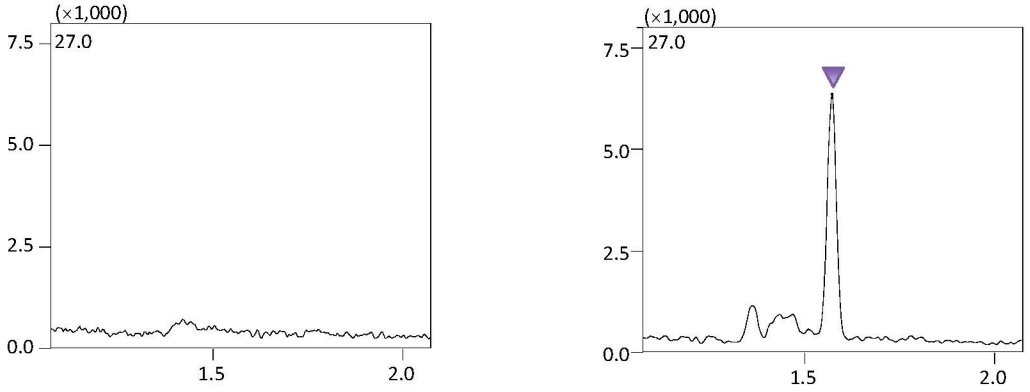

Fig. 1: Mass Chromatogram of Cyanide when Analyzing a Blank and 0.15 µg/mL Standard
Left: Blank, Right: 0.15 µg/mL Standard Sample

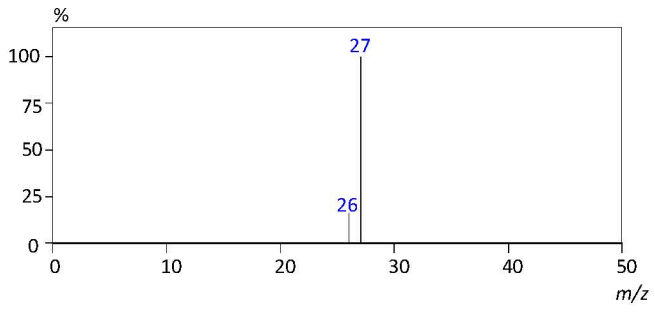

Fig. 2: Mass Spectrum of Cyanide

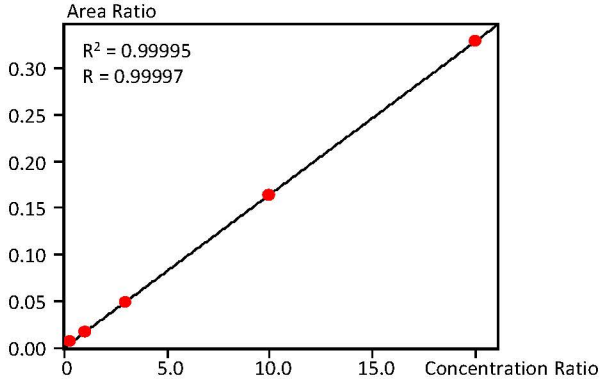

Fig. 3: Cyanide Calibration Curve
(Concentration: 0.15 - 10 µg/mL)

Intra-day reproducibility and Inter-day reproducibility were evaluated by repeating analysis of a 1.5 μg/mL standard (in hemolyzed equine blood) eight times each day for five days. Intra-day reproducibility (n = 8) on the first day is shown in Table 2, and Inter-day reproducibility over five days is shown in Table 3. The accuracy of intra-day repeatability of eight repeated measurements was 97.2 - 103.6 % (average 100.5 %), with a reproducibility relative standard deviation (%RSD) of about 2 %. The inter-day reproducibility of quantitative measurements of concentration performed over five days was within 5 %, which shows good quantitative performance.

Table 2: Intra-Day Reproducibility of Area Ratio and Concentration (n = 8, First Day)

	Area Ratio	Concentration (μg/mL)	Accuracy (%)
1st	0.053	1.546	103.0
2nd	0.054	1.555	103.6
3rd	0.053	1.519	101.2
4th	0.052	1.503	100.2
5th	0.051	1.476	98.4
6th	0.051	1.457	97.2
7th	0.053	1.520	101.3
8th	0.052	1.486	99.1
Average	0.052	1.508	100.5
Standard Deviation (SD)	0.001	0.034	2.251
%RSD	2.101	2.240	2.240

Table 3: Inter-Day Reproducibility of Qualitative Results Over Five Days

	Average Area Ratio	Average Concentration (μg/mL)	Concentration %RSD (Intra-Day Reproducibility)
1st Day	0.052	1.508	2.240
2nd Day	0.051	1.463	1.577
3rd Day	0.056	1.610	1.126
4th Day	0.055	1.603	2.287
5th Day	0.057	1.645	1.359
Inter-day Reproducibility (%RSD)	4.614	4.907	

Analytical Results of Blood Azide

Fig. 4 shows the mass chromatograms obtained after analyzing a blank and a 1.5 μg/mL standard. The mass spectrum is shown in Fig. 5. The ion that appears at *m/z* 43, which is the HN_3 ion, was used for quantification. The azide peak has a broad leading edge that reduces sensitivity compared to other volatile toxic substances, but sensitivity is deemed sufficient based on a reported blood concentration of about 5 μg/mL or above in cases of death. The calibration curve obtained by internal standard calibration (1.5, 5 and 10 μg/mL) is shown in Fig. 6. The calibration curve does not pass through the origin, but has a correlation coefficient (R) of 0.999 or above that shows linearity is good.

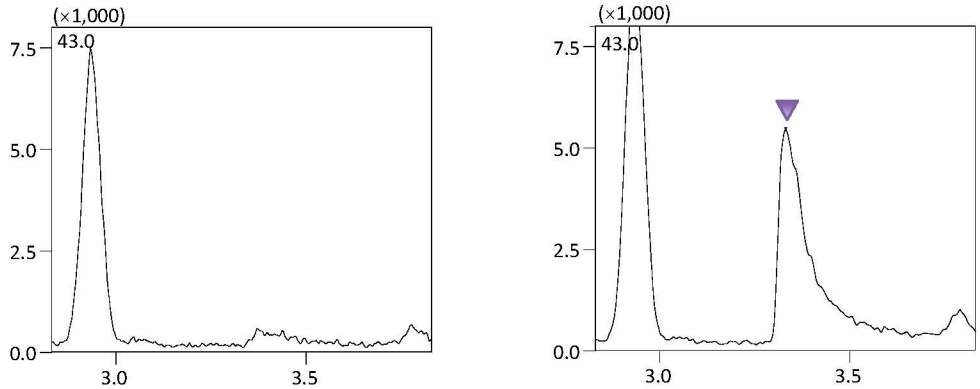

Fig. 4: Mass Chromatogram of Azide when Analyzing a Blank and a 1.5 μg/mL Standard
Left: Blank, Right: 1.5 μg/mL Standard Sample

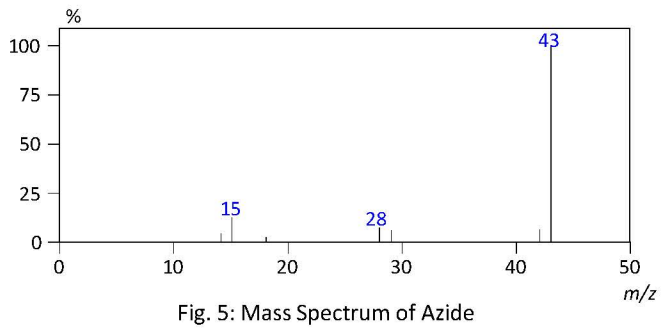

Fig. 5: Mass Spectrum of Azide

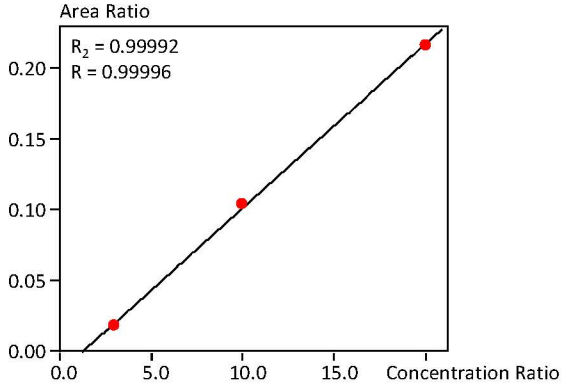

Fig. 6: Azide Calibration Curve
(Concentration: 1.5 - 10 μg/mL)

Intra-day reproducibility and Inter-day reproducibility were evaluated by repeating analysis of a 1.5 µg/mL standard (in hemolyzed equine blood) eight times each day for five days. Intra-day reproducibility (n = 8) on the first day is shown in Table 4, and Inter-day reproducibility over five days is shown in Table 5. The accuracy of intra-day repeatability of eight repeated measurements was 94.3 - 114.5 % (average 101.6 %), with a reproducibility relative standard deviation (%RSD) of about 7 %. The inter-day reproducibility of quantitative measurements of concentration performed over five days was within 2 %, which shows good quantitative performance.

Table 4: Intra-Day Reproducibility of Area Ratio and Concentration (n = 8, First Day)

	Area Ratio	Concentration (mg/mL)	Accuracy (%)
1st	0.018	1.420	94.7
2nd	0.018	1.449	96.6
3rd	0.020	1.513	100.9
4th	0.022	1.593	106.2
5th	0.023	1.623	108.2
6th	0.025	1.718	114.5
7th	0.019	1.464	97.6
8th	0.018	1.415	94.3
Average	0.020	1.524	101.6
Standard Deviation (SD)	0.003	0.110	7.307
%RSD	12.594	7.190	7.190

Table 5: Inter-Day Reproducibility of Qualitative Results Over Five Days

	Average Area Ratio	Average Concentration (mg/mL)	Concentration %RSD (Intra-Day Reproducibility)
1st Day	0.020	1.524	7.190
2nd Day	0.019	1.489	6.258
3rd Day	0.019	1.460	6.618
4th Day	0.019	1.463	7.077
5th Day	0.019	1.465	4.552
Inter-Day Reproducibility (%RSD)	3.295	1.839	

Summary

This application investigated whether the volatile toxic substances cyanide and azide could be measured using the same column and analytical conditions as used to analyze alcohol (ethanol) in blood. Cyanide and azide could be measured using the same analytical conditions as blood alcohol simply by adding ascorbic acid solution (aqueous) and phosphoric acid solution (aqueous) to the test sample.

The HS-20 headspace sampler provides high-performance vial heating and a sample loop of minimal length and inertness that reduces the carryover and allows for easy switching between analysis of high-concentration ethanol, such as in blood alcohol testing, and cyanide and azide that are present at trace level.

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Application Data Sheet

No.28

GCMS

Gas Chromatograph Mass Spectrometer

Analysis of a Benzodiazepine-Based Drug Using GC-MS

Benzodiazepine drugs are commonly used in sleeping aids and tranquilizers, and sometimes in crimes or suicide. Therefore, these chemical substances are often analyzed by forensic laboratories for criminal or academic investigations. This datasheet shows the results from using GC-MS to measure 9 types of benzodiazepine drugs.

Analysis Conditions

Table 1: Analysis Conditions

GC-MS :GCMS-QP2010 Ultra
Column : Rxi®-5Sil MS（30 mL. X 0.25 mmI.D., df=0.25 µm, Shimadzu GLC P/N:13623)
Glass insert :Silanized splitless insert (P/N: 221-48876-03)

[GC]
Vaporization chamber temperature : 260℃
Column oven temperature : 60℃ (2min) -> (10℃/min) -> 320℃ (10min)
Injection mode : Splitless
Sampling time : 1 min
High pressure injection method: 250 kPa (1.5 min)
Carrier gas : Helium
Control mode :Linear velocity (45.6 cm/sec)
Purge flow rate :3.0 ml/min
Sample injection quantity :1.0 µL

[MS]
Interface temperature : 280℃
Ion source temperature : 200℃
Solvent elution time : 2.0 min
Measurement mode : Scan
Mass range : *m/z* 35-600
Event time : 0.3 sec
Emission current : 150 µA (high sensitivity)

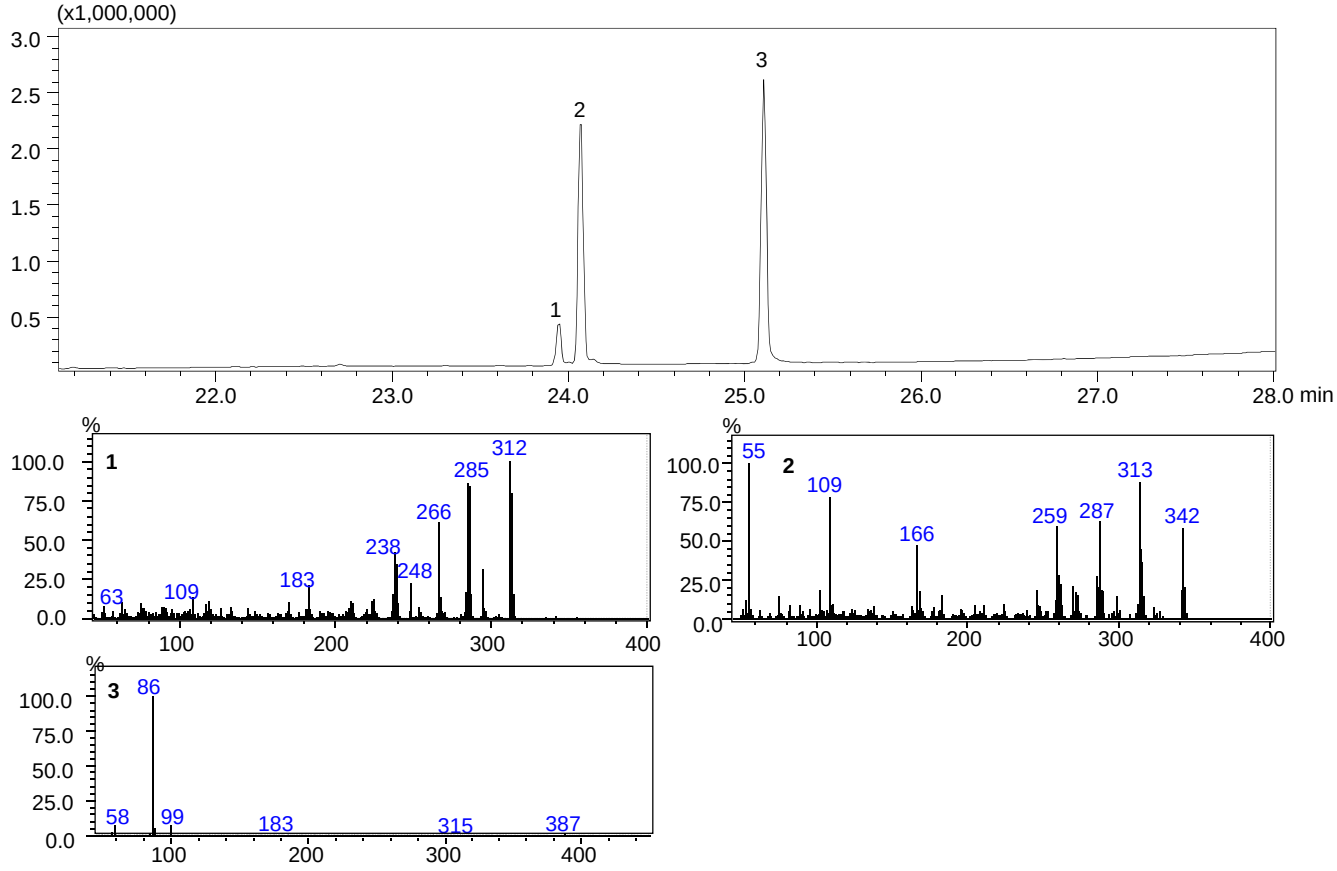

Fig. 1: Total Ion Current Chromatogram and Mass Spectra
1: Flunitrazepam, 2: Flutoprazepam, 3: Flurazepam

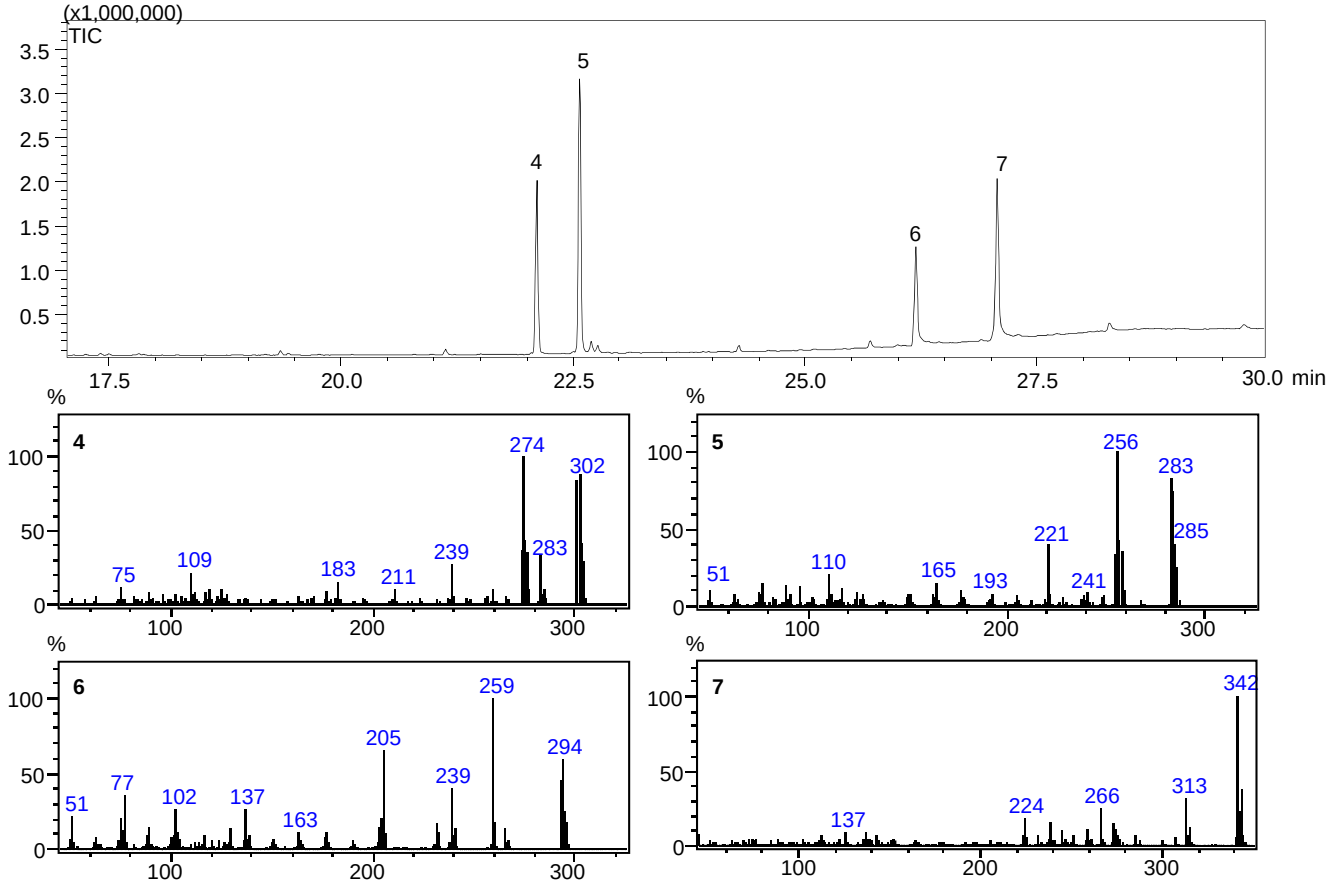

Fig. 2: Total Ion Current Chromatogram and Mass Spectra
4: Fludiazepam, 5: Diazepam, 6: Estazolam, 7: Etizolam

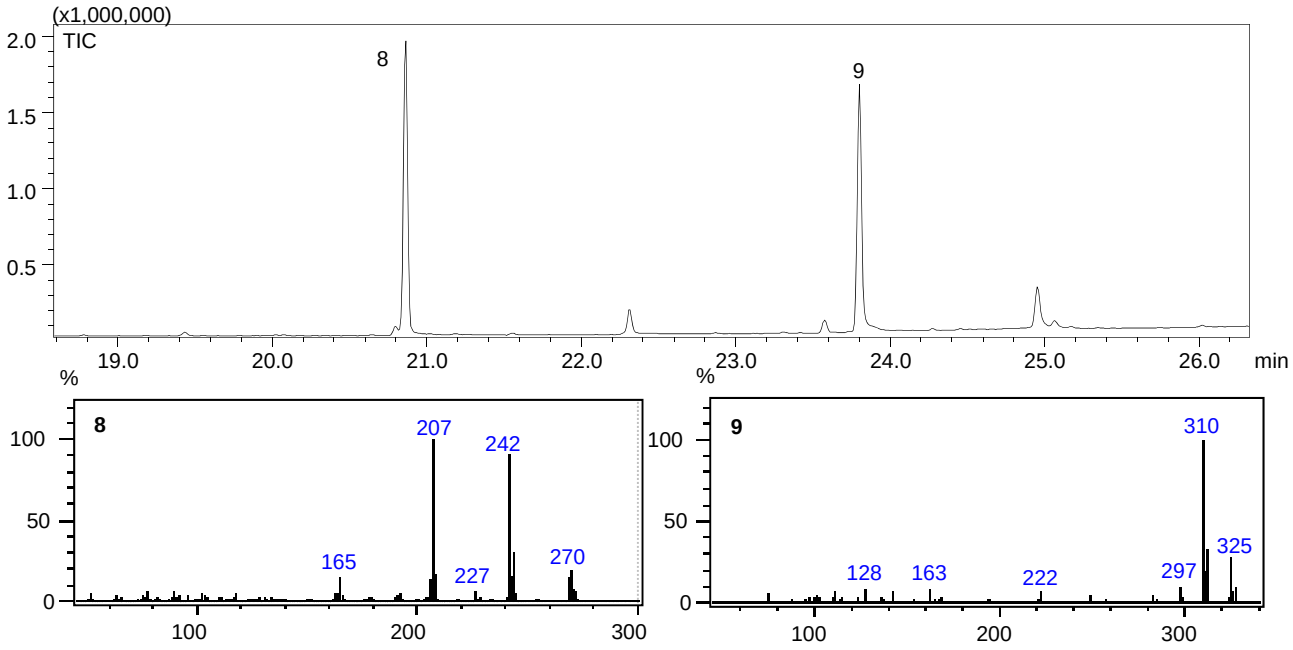

Fig. 3: Total Ion Current Chromatogram and Mass Spectra
8: Medazepam, 9: Midazolam

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Technical Report

Comprehensive Detection and Structural Elucidation of Synthetic Cathinones Using GC-MS/MS

Yuki Sakamoto[1], Haruhiko Miyagawa[1]

Abstract:

A rapid analytical method that allows comprehensive detection and structural elucidation of synthetic cathinones was developed using a gas chromatograph/tandem mass spectrometer (GC-MS/MS) capable of two-stage mass spectrometry. The proposed method consists of three simultaneous analytical procedures: 1) selective detection of the carbonyl group, characteristic of cathinones, using multiple reaction monitoring (MRM) and determination of both 2) iminium cations and 3) substituted benzoyl cations generated by α-cleavage of amine and benzoyl moieties, respectively, using product ion scan. For all cathinones examined, single peaks were detected at the same retention time on MRM chromatograms in procedure 1) and on total ion current chromatograms (TIC) in procedures 2) and 3). MRM in procedure 1) showed a transition of the substituted benzoyl cation > substituted phenyl cation due to CO elimination by collision-induced dissociation (CID), which demonstrated the existence of a carbonyl group in the structures. Each product ion mass spectrum for the substituted benzoyl cation allowed to not only determine the substituted group on the aromatic ring for all the cathinones, but also differentiate the corresponding positional isomers for ethyl, methoxy, and methylenedioxy substitutions, although identification of the substituted position for methyl, bromine, and fluorine groups on the benzene ring was difficult. On the other hand, the difference in product ion mass spectra between structural isomers of iminium cations was significantly clear, leading to easy discriminative identification of the isomers.

Keywords: synthetic cathinones, designer drugs, GC-MS/MS, structural elucidation

1. Introduction

In recent years, synthetic drugs, whose chemical structures have been partially modified from that of a controlled substance such as a narcotic or stimulant, in order to evade drug regulations, have become a major social problem globally. These include cathinones, which have become the principal drugs involved in synthetic drug abuse, along with indole synthetic cannabinoids. Cathinones (Fig. 1) have a β-keto-phenethyl-amine skeleton and resemble methamphetamine (MA) stimulants in their basic structure, thereby exhibiting a central nervous system-stimulant action similar to that of MA. Various new cathinones are in circulation as synthetic drugs modified with different types of substituents and regioisomers, including modifications to the benzene ring, different types of alkyl groups modifying the α-carbon (so-called side chains), and different types of alkyl groups modifying the nitrogen.

Many methods have been reported for the identification of these synthetic drugs, which mainly involve the use of nuclear magnetic resonance (NMR), gas chromatography/mass spectrometry (GC/MS), or liquid chromatography/tandem mass spectrometry (LC/MS/MS). NMR provides detailed structural information on the constituent hydrogen and carbon atoms of compounds, and is very useful for the structural elucidation. However, it requires several milligrams of isolated and purified objective compounds, making it difficult to apply this approach to highly sensitive analysis of biological samples. Although LC/MS and LC-MS/MS are useful for verifying molecular weight and quantifying drugs in biological samples, they are inferior to GC/MS in terms of retention time precision. Owing to the differences in analysis conditions, LC/MS and LC-MS/MS exhibit differences in the mass spectrum patterns obtained, and thus, are thought to be unsuitable for structural elucidation of unknown cathinones.

Moreover, GC/MS exhibits high retention time precision and allows for simultaneous observation of many ions, reflecting structural differences in the mass spectra obtained through electron ionization (EI). Furthermore, GC/MS shows similar EI mass spectra between instrument types and has many kinds of available spectral databases. Therefore, GC/MS is the most popular method in the field of forensic science.

For the identification of these synthetic drugs, comparison with reference standards is indispensable. However, synthetic drugs that have partially modified chemical structures are not provided as reference standards, and are not registered in mass spectral databases. Thus, there is a high demand for analytical techniques that are applicable, without a reference standard, to structural elucidation and comprehensive detection of cathinones modified with different types of substituents.

Structural elucidation of cathinones is particularly important for determining substituents modifying the benzene ring and hence yielding regioisomers, as well as for certain alkyl group types modifying side chains and the nitrogen and thereby yielding structural isomers. The EI-mass spectra of phenethylamines, obtained using GC/MS, are considered inferior for the detection of structure-reflecting ions, because only a fragment of the α-cleaved amine moiety has relatively high strength.

In this study, we used GC-MS/MS in an EI mode to obtain further cleaved characteristic fragment ions reflective of cathinone substructures. We performed comprehensive detection of cathinones and elucidated their structures, which in turn enabled easy differentiation of cathinone isomers and identification of substituents of various and diverse cathinones.

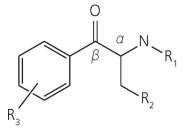

Fig. 1 Main Structure of Cathinones

1 Analytical & Measuring Instruments Division

2. Experimental

A GCMS-TQ8040 triple quadrupole gas chromatograph–mass spectrometer was used for the GC-MS/MS analysis. Table 1 shows detailed analytical conditions. For the evaluation, we selected 62 types of cathinones with different types of substituents located at different positions on the benzene ring and different types of alkyl groups modifying the side chains and nitrogen. Table 2 shows the examined combinations of substituents. As an analysis mode, we performed simultaneous scan/product ion scan/MRM measurements combining three MS/MS measurement types, 1) to 3), in addition to the EI-scan. Fig 2 shows a schematic of this method.

1) MRM measurements accounting for the modification of nine types of substituents to the benzene ring in benzoyl>phenyl transitions
2) Product ion scan measurements using 10 types of different *m/z* produced by α-cleavage of the amine as precursor ions
3) Product ion scan measurements using nine types of different *m/z* produced by α-cleavage of the benzoyl as precursor ions

In addition, Table 3 shows parameters of MRM and precursor ion scans for ions produced by α-cleavage of the benzoyl, and Table 4 shows parameters of precursor ion scans for iminium ions produced by α-cleavage of the amine.

Table 1 GC-MS/MS Analytical Conditions

GC/MS	: GCMS-TQ8040
GC conditions	
Column	: SH-Rxi-5Sil MS (length: 30 m, inner diameter: 0.25 mm, film thickness 0.25 µm)
Injection temperature	: 260°C
Column oven temperature	: 60°C (2 min) – (15°C/min) – 320°C (5 min)
Carrier gas	: Helium
Flow control	: Constant linear velocity (45.6 cm/sec)
Injection mode	: Splitless
MS conditions	
Ionization mode	: EI
Ion source temperature	: 200°C
Interface temperature	: 280°C
Analysis mode	: Scan/product ion scan/MRM simultaneous measurement
Collision gas	: Argon (200 kPa)

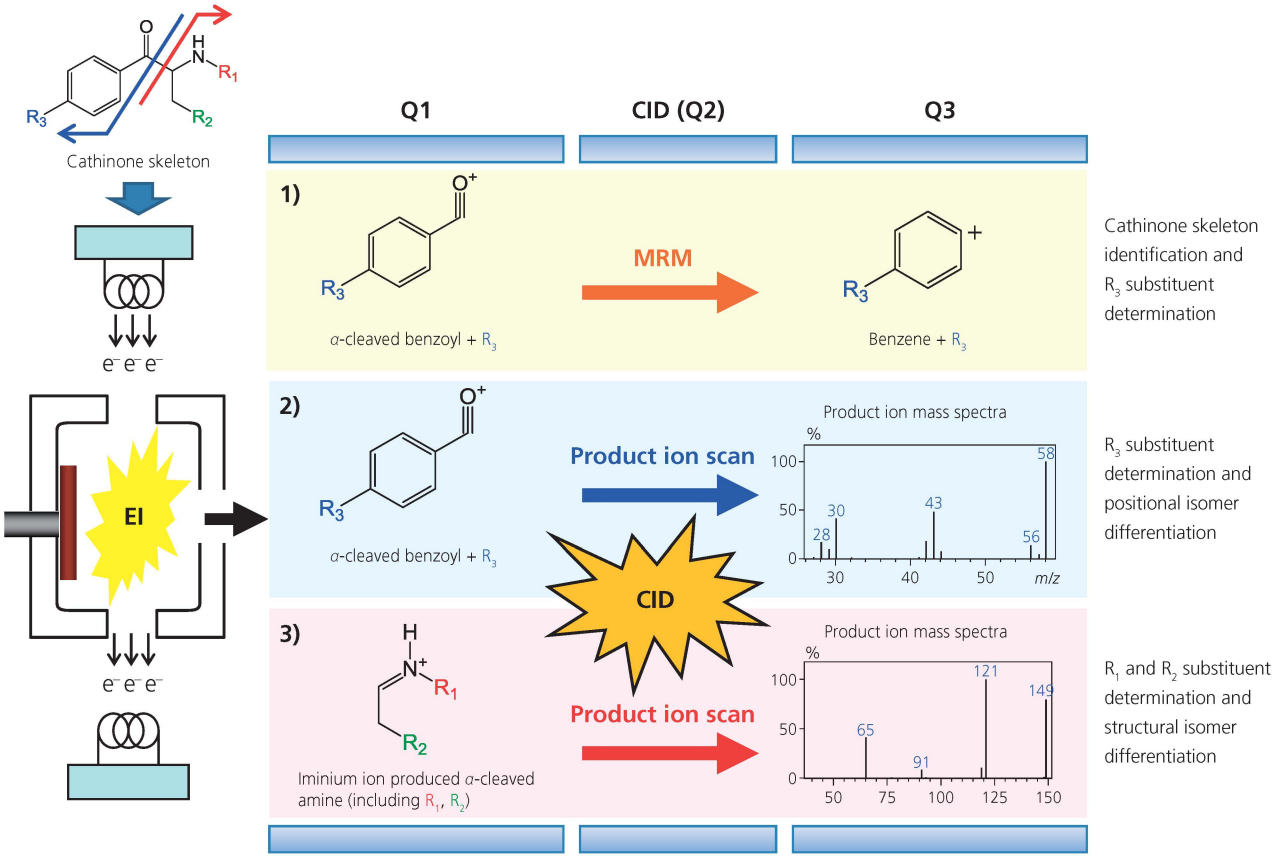

Fig. 2 Schematic of Three Types of MS/MS Measurements

Table 2 Evaluated Cathinones and Their Substituents

No.	Compound	R_1	R_2	R_3
1	Methcathinone	Methyl	H	H
2	*N*-Ethyl-*N*-methylcathinone	*N*-Ethyl-*N*-methyl	H	H
3	Ethcathinone	Ethyl	H	H
4	*N*,*N*-Diethylcathinone	*N*,*N*-Diethyl	H	H
5	α-PPP	Pyrrolidinyl	H	H
6	Buphedrone	Methyl	Methyl	H
7	*N*-Ethyl-*N*-methylbuphedone	*N*-Ethyl-*N*-methyl	Methyl	H
8	*N*-Ethylbuphedrone (NEB)	Ethyl	Methyl	H
9	*N*,*N*-Diethylbuphedrone	*N*,*N*-Diethyl	Methyl	H
10	α-PBP	Pyrrolidinyl	Methyl	H
11	Pentedrone	Methyl	Ethyl	H
12	*N*-Ethyl-*N*-methylpentedrone	*N*-Ethyl-*N*-methyl	Ethyl	H
13	*N*-Ethylpentedrone	Ethyl	Ethyl	H
14	*N*,*N*-Diethylpentedrone	*N*,*N*-Diethyl	Ethyl	H
15	α-PVP	Pyrrolidinyl	Ethyl	H
16	*N*-Desmethylhexedrone	H	*n*-Propyl	H
17	Hexedrone	Methyl	*n*-Propyl	H
18	*N*,*N*-Dimethylhexedrone	*N*,*N*-Dimethyl	*n*-Propyl	H
19	α-PHP	Pyrrolidinyl	*n*-Propyl	H
20	α-PHPP	Pyrrolidinyl	*n*-Butyl	H
21	α-POP	Pyrrolidinyl	*n*-Pentyl	H
22	α-PNP	Pyrrolidinyl	*n*-Hexyl	H
23	2-Methylmethcathinone	Methyl	H	Methyl (*ortho*)
24	3-Methylmethcathinone	Methyl	H	Methyl (*meta*)
25	4-Methylmethcathinone	Methyl	H	Methyl (*para*)
26	4-Methylethcathinone	Ethyl	H	Methyl (*para*)
27	MPPP (Desethylpyrovalerone)	Pyrrolidinyl	H	Methyl (*para*)
28	4-Methylbuphedrone	Methyl	Methyl	Methyl (*para*)
29	4-Methyl-*N*-ethylbuphedrone	Ethyl	Methyl	Methyl (*para*)
30	MPBP	Pyrrolidinyl	Methyl	Methyl (*para*)
31	4-Methylpentedrone	Methyl	Ethyl	Methyl (*para*)
32	4-Ethyl-*N*-ethylpentedrone	Ethyl	Ethyl	Ethyl (*para*)
33	MPVP	Pyrrolidinyl	Ethyl	Methyl (*para*)
34	4-Ethylcathinone	H	H	Ethyl (*para*)
35	2-Ethylmethcathinone	Methyl	H	Ethyl (*ortho*)
36	3-Ethylmethcathinone	Methyl	H	Ethyl (*meta*)
37	4-Ethylmethcathinone	Methyl	H	Ethyl (*para*)
38	4-Ethyl-*N*,*N*-dimethylcathinone	*N*,*N*-Dimethyl	H	Ethyl (*para*)
39	2-Bromomethcathinone	Methyl	H	Br (*ortho*)
40	3-Bromomethcathinone	Methyl	H	Br (*meta*)
41	4-Bromomethcathinone	Methyl	H	Br (*para*)
42	4-Chloromethcathinone	Methyl	H	Cl (*para*)
43	2-Fluoromethcathinone	Methyl	H	F (*ortho*)
44	3-Fluoromethcathinone	Methyl	H	F (*meta*)
45	4-Fluoromethcathinone	Methyl	H	F (*para*)
46	4-Fluorooctedrone (4F-Octedrone)	Methyl	*n*-Pentyl	F (*para*)
47	4-Iodomethcathinone	Methyl	H	I (*para*)
48	2-Methoxymethcathinone	Methyl	H	Methoxy (*ortho*)
49	3-Methoxymethcathinone	Methyl	H	Methoxy (*meta*)
50	4-Methoxymethcathinone	Methyl	H	Methoxy (*para*)
51	4-Methoxyethcathinone	Ethyl	H	Methoxy (*para*)
52	MOPPP	Pyrrolidinyl	H	Methoxy (*para*)
53	2,3-Methylenedioxymethcathinone	Methyl	H	Methylnedioxy (2,3-)
54	Methylone (bk-MDMA)	Methyl	H	Methylnedioxy (3,4-)
55	Ethylone (bk-MDEA)	Ethyl	H	Methylnedioxy (3,4-)
56	MDPPP	Pyrrolidinyl	H	Methylnedioxy (3,4-)
57	bk-BDB	H	Methyl	Methylnedioxy (3,4-)
58	bk-MBDB	Methyl	Methyl	Methylnedioxy (3,4-)
59	3,4-Methylenedioxy-*N*,*N*-dimethylbuphedrone	*N*,*N*-Dimethyl	Methyl	Methylnedioxy (3,4-)
60	Buthylone	H	Ethyl	Methylnedioxy (3,4-)
61	Penthylone	Methyl	Ethyl	Methylnedioxy (3,4-)
62	*N*,*N*-Dimethylpentylone	*N*,*N*-Dimethyl	Ethyl	Methylnedioxy (3,4-)

Table 3 MRM Transition and Precursor *m/z* of a Product Ion Scan for the α-Cleaved Benzoyl

Precursor *m/z*	MRM transition	R_3	CE (V)
105	105 > 77	H	10
119	119 > 91	Methyl	10
123	123 > 95	F	10
133	133 > 105	Ethyl or Dimethyl	10
135	135 > 107	Methoxy	10
139	139 > 111	Cl	10
149	149 > 121	Methylenedioxy	10
183	183 > 155	Br	10
231	231 > 203	I	10

Table 4 Precursor *m/z* of a Product Ion Scan for Iminium Ions Produced by α-Cleaved Amine

Precursor *m/z*	R_1	R_2	CE (V)
44	H	H	15
58	Methyl	H	15
	H	Methyl	
72	*N*,*N*-Dimethyl	H	15
	Ethyl	H	
	Methyl	Methyl	
	H	Ethyl	
86	*N*-Ethyl-*N*-methyl	H	15
	N,*N*-Dimethyl	Methyl	
	Ethyl	Methyl	
	Methyl	Ethyl	
	H	*n*-Propyl	
98	Pyrrolidinyl	H	15
100	*N*,*N*-Diethyl	H	15
	N-Ethyl-*N*-methyl	Methyl	
	N,*N*-Dimethyl	Ethyl	
	Ethyl	Ethyl	
	Methyl	*n*-Propyl	
	H	*n*-Butyl	
112	Pyrrolidinyl	CH_3	15
114	*N*,*N*-Diethyl	Methyl	15
	N-Ethyl-*N*-methyl	Ethyl	
	N,*N*-Dimethyl	*n*-Propyl	
	Ethyl	*n*-Propyl	
	Methyl	*n*-Butyl	
	H	*n*-Pentyl	
126	Pyrrolidinyl	Ethyl	15

Precursor *m/z*	R_1	R_2	CE (V)
128	*N*,*N*-Diethyl	Ethyl	15
	N-Ethyl-*N*-methyl	*n*-Propyl	
	N,*N*-Dimethyl	*n*-Butyl	
	Ethyl	*n*-Butyl	
	Methyl	*n*-Pentyl	
	H	*n*-Hexyl	
140	Pyrrolidinyl	*n*-Propyl	15
142	*N*,*N*-Diethyl	*n*-Propyl	15
	N-Ethyl-*N*-methyl	*n*-Butyl	
	N,*N*-Dimethyl	*n*-Pentyl	
	Ethyl	*n*-Pentyl	
	Methyl	*n*-Hexyl	
	H	*n*-Heptyl	
154	Pyrrolidinyl	*n*-Butyl	15
156	*N*,*N*-Diethyl	*n*-Butyl	15
	N-Ethyl-*N*-methyl	*n*-Pentyl	
	N,*N*-Dimethyl	*n*-Hexyl	
	Ethyl	*n*-Hexyl	
	Methyl	*n*-Heptyl	
168	Pyrrolidinyl	*n*-Pentyl	15
170	*N*,*N*-Diethyl	*n*-Pentyl	15
	N-Ethyl-*N*-methyl	*n*-Hexyl	
	N,*N*-Dimethyl	*n*-Heptyl	
	Ethyl	*n*-Heptyl	
182	Pyrrolidinyl	*n*-Hexyl	15
184	*N*,*N*-Diethyl	*n*-Hexyl	15
	N-Ethyl-*N*-methyl	*n*-Heptyl	
196	Pyrrolidinyl	*n*-Heptyl	15
198	*N*,*N*-Diethyl	*n*-Heptyl	15

3. Results and Discussion

3-1. Detection of Benzoyl Skeletons by MRM Measurement

It is known that benzoyl cations produced by α-cleavage of the benzoyl and ions eliminated with a CO molecule from benzoyl cations are observed in EI-mass spectra of cathinones. These ions have a weak relative intensity compared to the iminium ion produced by α-cleavage of the amine. However, the presence of β-carbonyl groups is very important for the differentiation of cathinones and phenethylamines. Therefore, we attempted to selectively detect cathinones with the carbonyl group using the MRM transition of the benzyl cation as a precursor ion and benzene as a product ion, with carbonyl eliminated by collision-induced dissociation (CID). We set MRM transitions corresponding to combinations of "benzoyl>phenyl" accounting for the masses of nine types of substituents modifying the benzene ring.

We evaluated the optimal CID collision energy in as many established MRM measurements as possible and set the collision energy for all MRM measurements at 10 V. In addition, MRM transitions corresponding to combinations of "benzoyl>phenyl" were verified in all 62 types of cathinones examined.

Identification of cathinones requires the verification of two types of ions present in EI-mass spectra, benzoyl cations produced by α-cleavage of the benzoyl and ions eliminated with the CO molecule from benzoyl cations. However, relative intensities of these ions are very weak with EI-scans, making data processing of mass spectra time-consuming. In addition, sometimes these ions are buried in the background noise of biological samples or low-concentration samples, which increases the risk of misidentification.

Since CO elimination from benzoyl cations was selectively monitored in MRM measurements, it was possible to detect the presence of two types of ions produced from this reaction as peaks on MRM chromatograms. Therefore, it was easy to detect the benzoyl skeleton, characteristic of cathinones. In addition, when a peak of set MRM transitions corresponding to substituents modifying benzene rings was detected, the types of substituents modifying the benzene ring could be identified.

3-2. Product Ion Scan Measurement of Ions Produced by *α*-Cleavage of Benzoyl

If a MRM measurement in section 3-1 suggested the possibility of a cathinone, further specification, such as a modified position of substituents to the benzene ring, was important for structure elucidation. Therefore, we utilized a product ion scan, which accounts for the substituents shown in Table 3, to determine both positions and types of substituents attached to the benzene ring. Regarding the compounds examined, in addition to the basic methcathinone skeleton, methyl groups, ethyl groups, methoxy groups, methylenedioxy groups, bromine, and fluorine as substituents modifying the benzene ring and their *o*-, *m*-, and *p*-position substituents for regioisomers were selected.

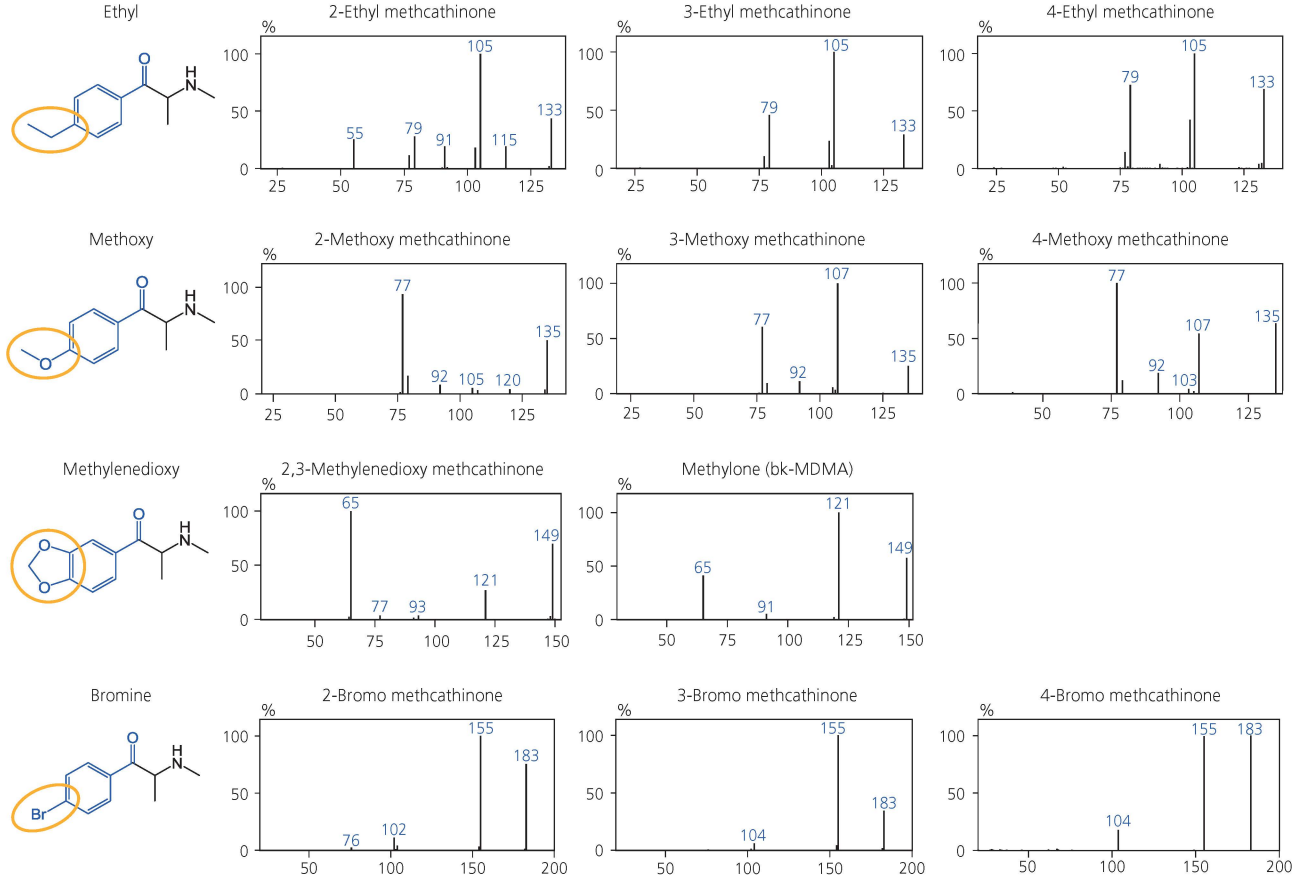

Fig. 3 Product Ion Mass Spectra of *α*-Cleaved Benzoyl Cations

For each compound, we set benzoyl cations produced by α-cleavage of benzoyl as precursor *m/z* and optimized the CID collision energy to obtain product ion mass spectra changing the collision energy. In the case when a methyl or ethyl group was substituted to the benzene ring, if the collision energy was set higher, elimination of methyl or ethyl group substituted to the benzene ring occurred, and the ion intensity reflecting the structure of the aromatic ring side chain was relatively weak. Therefore, the optimal collision energy was set at 10 V. Fig. 3 shows representative product ion mass spectra.

If an ethyl, methoxy, or methylenedioxy group was substituted to the benzene ring, three or more characteristic ions with a high relative intensity were observed in each product ion spectrum.

In addition, their regioisomers could be differentiated by the relative intensity and respective characteristic ions. However, if a methyl, bromine, or fluorine group was substituted to the benzene ring, only two types of ions were observed, a precursor ion and an ion eliminated with the CO molecule from each precursor ion. In the case of these substituents, it was possible to identify the type of the substituent, but regioisomers were difficult to differentiate from the product ion in a mass spectrum.

3-3. Product Ion Scan Measurement of Ions Produced by α-Cleavage of the Amine

It is known that iminium ions derived from α-cleavage of the amine are detected with a high intensity in EI-mass spectra of cathinones. Amine skeletons forming iminium ions produced by α-cleavage of the amine have many structural isomers. However, identifying structural isomers of the amine moiety is difficult using only EI-mass spectra. Therefore, we attempted to elucidate amine moieties using the mass spectrum of a product ion scan, with iminium ions produced by the α-cleaved amine set as precursor *m/z*.

Product ion mass spectra are largely dependent on the collision energy. Therefore, we examined the optimum collision energy allowing for structural isomer differentiation of amine moieties. We determined that 15 eV was the optimum collision energy, at which product ions reflecting differences in the amine moiety were observed with a relatively strong intensity in the product ion mass spectra.

Representative product ion mass spectra obtained using iminium ions produced by α-cleavage of the amine as precursor ions are shown in Fig. 4 (precursor *m/z* 86) and Fig. 5 (precursor *m/z* 100). The mass spectrum patterns were clearly different even if precursor ions of the same *m/z* were selected. These differences were reflective of the amine grade and the type of the alkyl group substituted to the nitrogen atom, and were evidenced by different base peak *m/z*, allowing for easy differentiation of structural isomers.

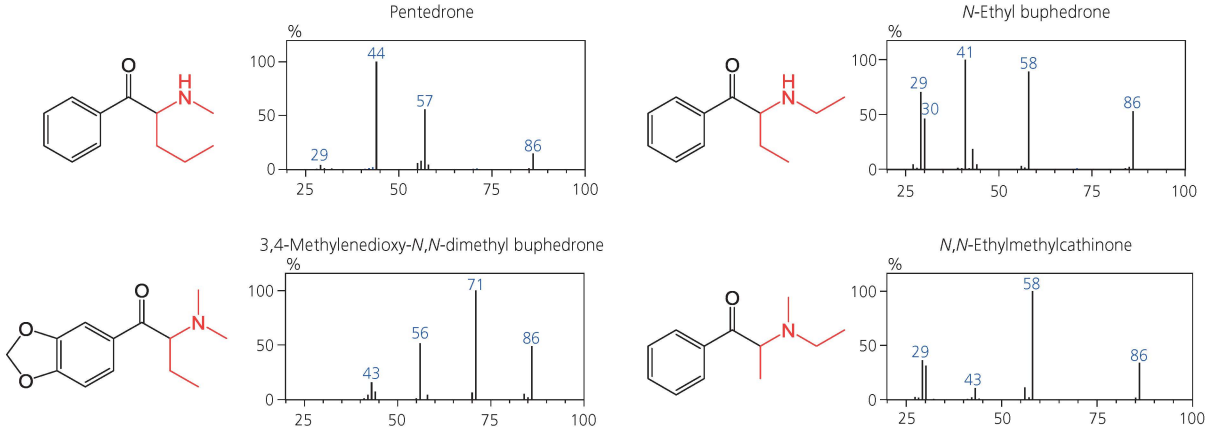

Fig. 4 Product Ion Mass Spectra Using Iminium Ion *m/z* 86 Produced by α-Cleavage of the Amine

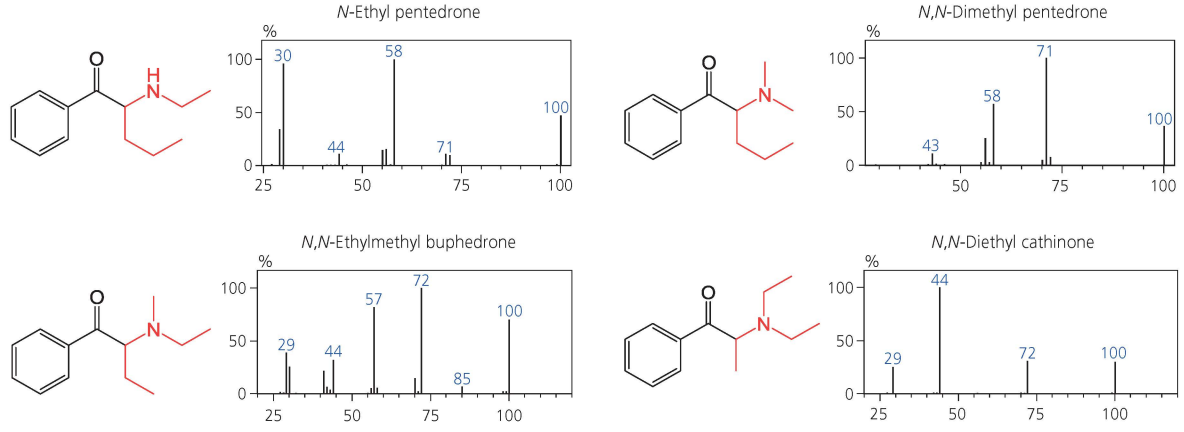

Fig. 5 Product Ion Mass Spectra Using Iminium Ion *m/z* 100 Produced by α-Cleavage of the Amine

3-4. Example of Comprehensive Detection and Structural Elucidation Using This Technique

Combining the three types of MS/MS measurements in theory enables the comprehensive detection and structural elucidation of cathinones. If a sample containing cathinone is analyzed, peaks on chromatograms obtained from the three respective types of MS/MS measurements are detected at the same retention times. By confirming the transitions observed in the MRM chromatogram peak, it is possible to evaluate whether the detected peak has the characteristic cathinone structure and which of the nine substituents modifies the benzene ring.

In addition, based on the results from product ion scan measurements of ions produced by α-cleavage of the benzoyl group, it is possible to determine substituents modifying the benzene ring by automatically searching pre-registered product ion spectrum databases for product ion spectra. Moreover, the positional isomer can be specified according to the type of substituents. In addition, based on the results from the product ion scan, in which iminium ions produced by α-cleavage of the amine are used as precursors, amine moieties can be identified.

Fig. 6 shows an example of the analysis performed using this method. A cathinone was detected, and three chromatograms from each MS/MS measurement type were obtained with the same retention times in each respective measurement. The MRM transition (*m/z* 133>105) of the obtained MRM chromatogram (1) confirmed that the peak had a characteristic cathinone structure, and the substituent modifying the benzene ring was a dimethyl or ethyl group.

In addition, the product ion spectra of precursor *m/z* 133 produced by α-cleavage of the benzoyl as precursors indicated that an ethyl group modified the *m*-position (2). Furthermore, the result of the product ion scan of *m/z* 58 produced by α-cleavage of the amine indicated that the amine moiety consisted of $R_1 = CH_3$, R_2 :H (3). Based on these results, the detected peak was estimated to be 3-ethylmethcathinone. Measurement of a reference standard showed an identical retention time, confirming the detected peak as 3-ethylmethcathinone.

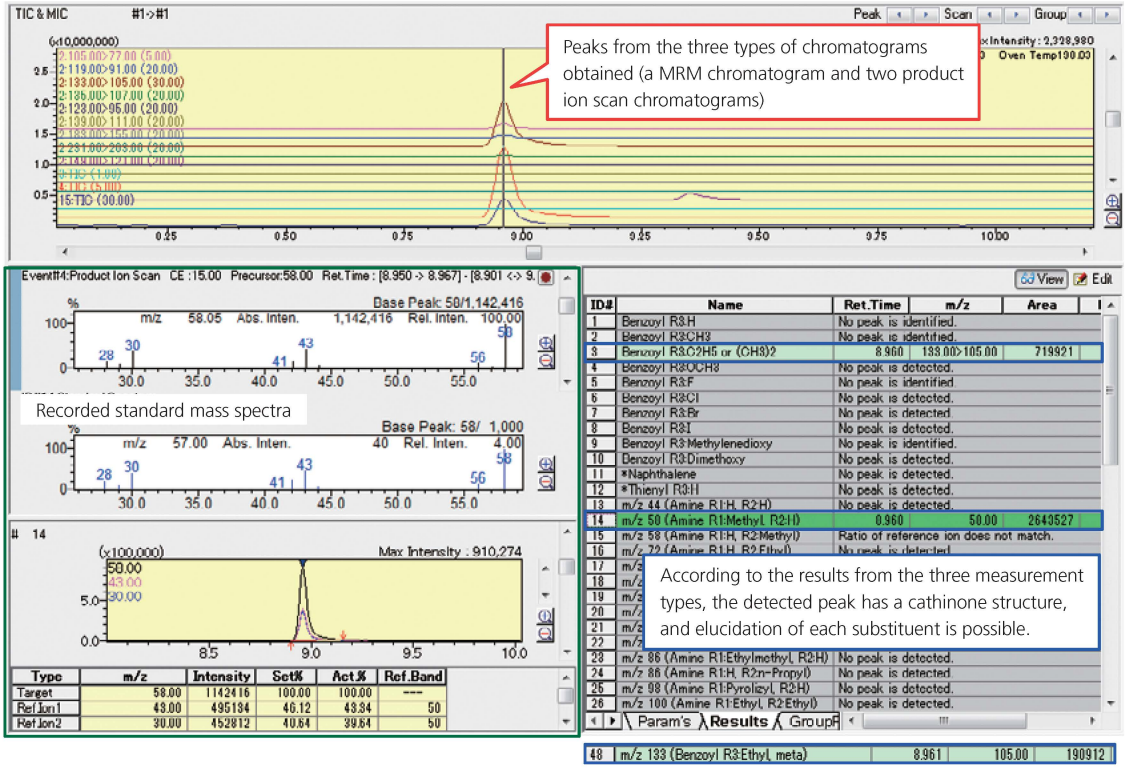

Fig. 6 Data Processing Procedures Using This Proposed Method

4. Conclusions

We performed comprehensive detection and structural elucidation of cathinones using MRM and product ion scans with GC-MS/MS. By combining measurements from product ion scans, which use precursor ions reflective of the structure of the α-cleaved amine and benzoyl produced by EI, and MRM, which selectively detects the benzoyl moiety, all examined cathinones could be detected on individual MRM chromatograms.

In product ion scans using iminium ions produced by α-cleavage of the amine as precursors, clear differences were observed for base peaks in the product ion spectra, as well as in the mass spectral pattern. The product ion scan allows elucidation of the substructure of the amine moiety, which is difficult to achieve using an EI-scan with GC/MS. In product ion measurements using ions produced by α-cleavage of the benzoyl as precursors, it was difficult to distinguish the regioisomers of some substituents, such as methyl group and halogen atoms. However, the regioisomers of ethyl, methoxy, and methylenedioxy groups could be differentiated by the mass spectral pattern.

GC-MS/MS allows ions fragmented by EI to be cleaved by CID. The main structure of cathinones is divided into two substructures, amine and benzoyl moieties, which are analyzed independently for the structural elucidation. A collection of product ion mass spectra of amine and benzoyl substructures can greatly reduce the effort required to obtain reference standards for comparison with the collection of EI-scan mass spectra of each individual cathinone. In recent years, new synthetic cathinones have been circulated, such as α-PVT modified with a thienyl group instead of the benzoyl moiety. Cathinones with partial structural modifications are expected to widely circulate in the future. A collection of additional information on MRM transitions and product ion scan mass spectra reflecting these substructures is expected to be very useful for the structural elucidation of newly derived cathinones.

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Nevertheless, GCMS-TQ8040 can dramatically increase the productivity by automating tedious method creation processes and simultaneously analyzing multiple components with high sensitivity.

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- Includes a new firmware protocol.
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- Twin Line MS system minimizes the replacement of columns.

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- Smart MRM automatically creates optimized methods.
- Automatically searches for optimal transitions.
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- Patented high-sensitivity ion source technology offers even higher sensitivity.
- OFF-AXIS Ion Optics reduces noise.
- Capable of performing high-sensitivity analysis even as a single GC-MS system.

Simultaneous measurement in a multiple analysis mode

GCMS-TQ8040 achieved a maximum scanning speed of 20,000 u/sec using a high-efficiency collision cell UF sweeper, which is capable of achieving a high CID efficiency and high-speed ion transport with Advanced Scanning Speed Protocol (ASSP). Using this application, a three-mode simultaneous measurement (scan/MRM/product ion scan) is performed. It is possible to use product ion scanning with a maximum scanning speed of 20,000 u/sec, allowing for 10 or more precursor *m/z* types per single run.

	Compound Name	Start Time (min)	End Time (min)	Acq. Mode	Event Time(sec)	Scan Speed	Start m/z	End m/z	Precursor m/z	CE
1-1		2.30	43.00	Q3 Scan	0.050	10000	43.00	500.00		
1-2		2.30	43.00	MRM	0.050					
1-3	Amine m/z44	2.30	43.00	Product Ion Scan	0.005	20000	20.00	45.00	44.00	15.00
1-4	Amine m/z58	2.30	43.00	Product Ion Scan	0.005	20000	20.00	59.00	58.00	15.00
1-5	Amine m/z72	2.30	43.00	Product Ion Scan	0.008	20000	20.00	73.00	72.00	15.00
1-6	Amine m/z86	2.30	43.00	Product Ion Scan	0.008	20000	20.00	87.00	86.00	15.00
1-7	Amine m/z98	2.30	43.00	Product Ion Scan	0.010	20000	20.00	99.00	98.00	15.00
1-8	Amine m/z100	2.30	43.00	Product Ion Scan	0.010	20000	20.00	101.00	100.00	15.00
1-9	Amine m/z112	2.30	43.00	Product Ion Scan	0.010	20000	20.00	113.00	112.00	15.00
1-10	Amine m/z114	2.30	43.00	Product Ion Scan	0.010	20000	20.00	115.00	114.00	15.00
1-11	Amine m/z126	2.30	43.00	Product Ion Scan	0.010	20000	20.00	127.00	126.00	15.00
1-12	Amine m/z128	2.30	43.00	Product Ion Scan	0.010	20000	20.00	129.00	128.00	15.00
1-13	Amine m/z140	2.30	43.00	Product Ion Scan	0.010	20000	20.00	141.00	140.00	15.00
1-14	Amine m/z142	2.30	43.00	Product Ion Scan	0.015	20000	20.00	143.00	142.00	15.00
1-15	Amine m/z154	2.30	43.00	Product Ion Scan	0.015	20000	20.00	155.00	154.00	15.00
1-16	Amine m/z156	2.30	43.00	Product Ion Scan	0.015	20000	20.00	157.00	156.00	15.00
1-17	Amine m/z168	2.30	43.00	Product Ion Scan	0.015	20000	20.00	169.00	168.00	15.00
1-18	Amine m/z170	2.30	43.00	Product Ion Scan	0.015	20000	20.00	171.00	170.00	15.00
1-19	Amine m/z182	2.30	43.00	Product Ion Scan	0.015	20000	20.00	183.00	182.00	15.00
1-20	Amine m/z184	2.30	43.00	Product Ion Scan	0.015	20000	20.00	185.00	184.00	15.00
1-21	Amine m/z196	2.30	43.00	Product Ion Scan	0.015	20000	20.00	197.00	196.00	15.00
1-22	Amine m/z198	2.30	43.00	Product Ion Scan	0.015	20000	20.00	199.00	198.00	15.00
1-23	Benzoyl R3:H	2.30	43.00	Product Ion Scan	0.012	10000	20.00	106.00	105.00	10.00
1-24	Benzoyl R3:Methyl	2.30	43.00	Product Ion Scan	0.015	10000	20.00	120.00	119.00	10.00
1-25	Benzoyl R3:Ethyl or Dimet	2.30	43.00	Product Ion Scan	0.015	10000	20.00	134.00	133.00	10.00
1-26	Benzoyl R3:Methoxy	2.30	43.00	Product Ion Scan	0.015	10000	20.00	136.00	135.00	10.00
1-27	Benzoyl R3:F	2.30	43.00	Product Ion Scan	0.015	10000	20.00	124.00	123.00	10.00
1-28	Benzoyl R3:Cl	2.30	43.00	Product Ion Scan	0.015	10000	20.00	140.00	139.00	10.00
1-29	Benzoyl R3:Br	2.30	43.00	Product Ion Scan	0.020	10000	20.00	184.00	183.00	10.00
1-30	Benzoyl R3:I	2.30	43.00	Product Ion Scan	0.025	10000	20.00	232.00	231.00	10.00
1-31	Benzoyl R3:Methylenediox	2.30	43.00	Product Ion Scan	0.020	10000	20.00	150.00	149.00	10.00
1-32	Thienyl R3:H	2.30	43.00	Product Ion Scan	0.012	10000	20.00	112.00	111.00	10.00
1-33	Benzoyl R3:Dimethoxy	2.30	43.00	Product Ion Scan	0.020	10000	20.00	166.00	165.00	10.00
1-34	Naphthalene R3:H	2.30	43.00	Product Ion Scan	0.020	10000	20.00	156.00	155.00	10.00

MS/MS analysis conditions in this application

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A Novel Platform of On-line Sample Pre-treatment and LC/MS/MS Analysis for Screening and Quantitation of Illicit Drugs in Urine

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A Novel Platform of On-line Sample Pre-treatment and LC/MS/MS Analysis for Screening and Quantitation of Illicit Drugs in Urine

Introduction

In recent years, LC/MS/MS methods are adopted in analyses of illicit and prescription drugs in toxicological samples such as urine and serum. Sample pre-treatment is always a critical step in the whole analysis procedure and on-line sample pre-treatment is desired not only for improving analysis throughput, but also minimizing human errors. The CLAM-2000 module is designed for on-line sample pre-treatment in high throughput LC/MS/MS analysis of drugs and metabolites in biological samples such as plasma/serum and urine. Many sample preparation process can be performed automatically such as dispensing solvents, sample-reagent mixing by vortexing, sample filtering by vacuum filtration, and sample derivatisation with heating. Internal standard and reagent for derivatization or other purposes can be added to a sample before or after protein crash. We describe development of an automated sample pre-treatment using a Shimadzu CLAM-2000 module coupled with Shimadzu LCMS-8040 TQ system. It involves IS addition, protein precipitation, filtration and transferring the final solution to LC/MS/MS for analysis. This new platform was applied and evaluated for quantitation of 18 illicit drugs with 14 isotope-labelled internal standards (IS).

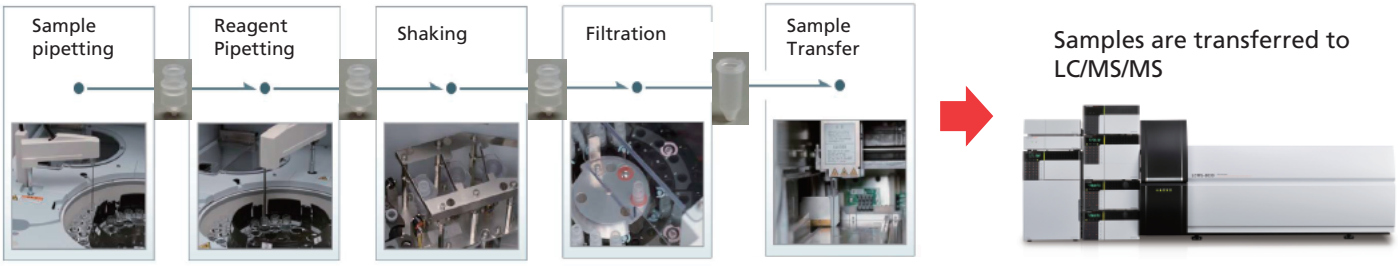

Figure 1: Procedure of protein crash and spiked-sample preparation

Experimental

Sample preparation and analytical conditions

A total of 18 illicit drugs and 14 isotope-labeled internal standards (except for phencyclidine, methaqualone, methadone and propoxyphene) were used for setting up the MRM quantitation method. The urine samples, internal standards mixed solution and organic solvents were pre-loaded onto the CLAM-2000. An automated batch-run program allows sample pre-treatment and analysis to perform concurrently on the CLAM-LC-MS/MS platform. Table 1 shows the analytical conditions on LCMS-8040. Figure 2 illustrates the automated workflow on the CLAM-2000 module. An aliquot of 20 uL of urine sample was dispensed into a filtration vial. Then, 20 µL of mixed internal standard (IS) stock solution was added to the sample, followed by addition of 40 µL of organic solvent (MeOH : ACN = 1 : 1 in volume). The sample mixture was vortexed and filtered into a collection vial before injecting to LCMS-8040. A Phenomenex Biphenyl column (100 x 2.1 mm I.D., 2.6µm) was used for the analysis of 18 analytes and 14 IS with a gradient elution program of 11 minutes. A calibration series of spiked standard samples in urines were prepared in concentrations of 20, 50 and 200 ng/mL. The concentration of each IS was 100 ng/mL. A LCMS-8040 with ESI was employed in this work.

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Table 1: Analytical conditions on LCMS-8040

Column	: Biphenyl 2.6µ, 100A (100 mmL x 2.10mm I.D.)
Mobile Phase	: A: Water with 0.1% FA
	B: Methanol with 0.1% FA
Elution Program	: Gradient elution (11.0 minutes)
	B: 3% (0 to 0.5 min) → 90% (5.5 to 7.0 min) → 3% (7.5 to 11.0 min)
Flow Rate	: 0.4 mL/min
Oven Temp.	: 40°C
Injection	: 5 µL
Interface	: ESI
MS Mode	: MRM, Positive
Block Temp.	: 400°C
DL Temp.	: 250°C
CID Gas	: Ar, 270 kPa
Nebulizing Gas	: N_2, 2.0 L/min
Drying Gas	: N_2, 15.0 L/min

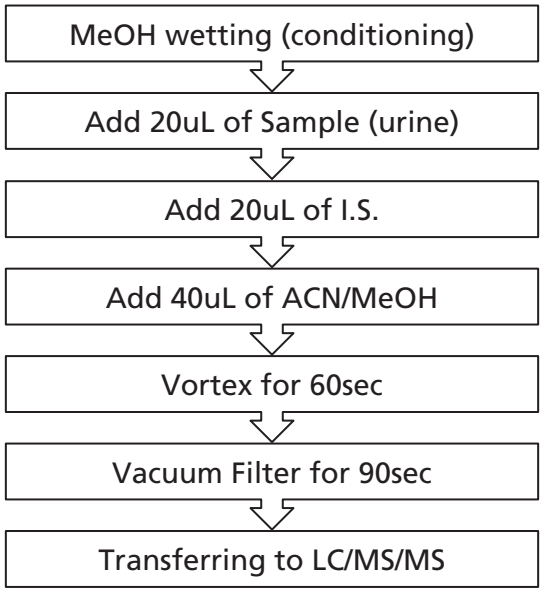

Figure 2: Typical auto-workflow of urine sample via protein-crash and adding IS for LC/MS/MS by CLAM-2000

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Results and Discussion

MRM-based method for eighteen illicit drugs

Table 2 shows the summarized results of optimized MRM transitions and parameters of the eighteen analytes and fourteen isotope-labelled internal standards (IS). However, four isotope-labelled ISs were not available. Three MRM transitions were selected for each compound except PROP with one as the quantitation ion and the other two for confirmation. A gradient elution program was optimized with a total runtime of eleven minutes. The MRM chromatograms of a mixed standard sample in urine are shown in Figure 3.

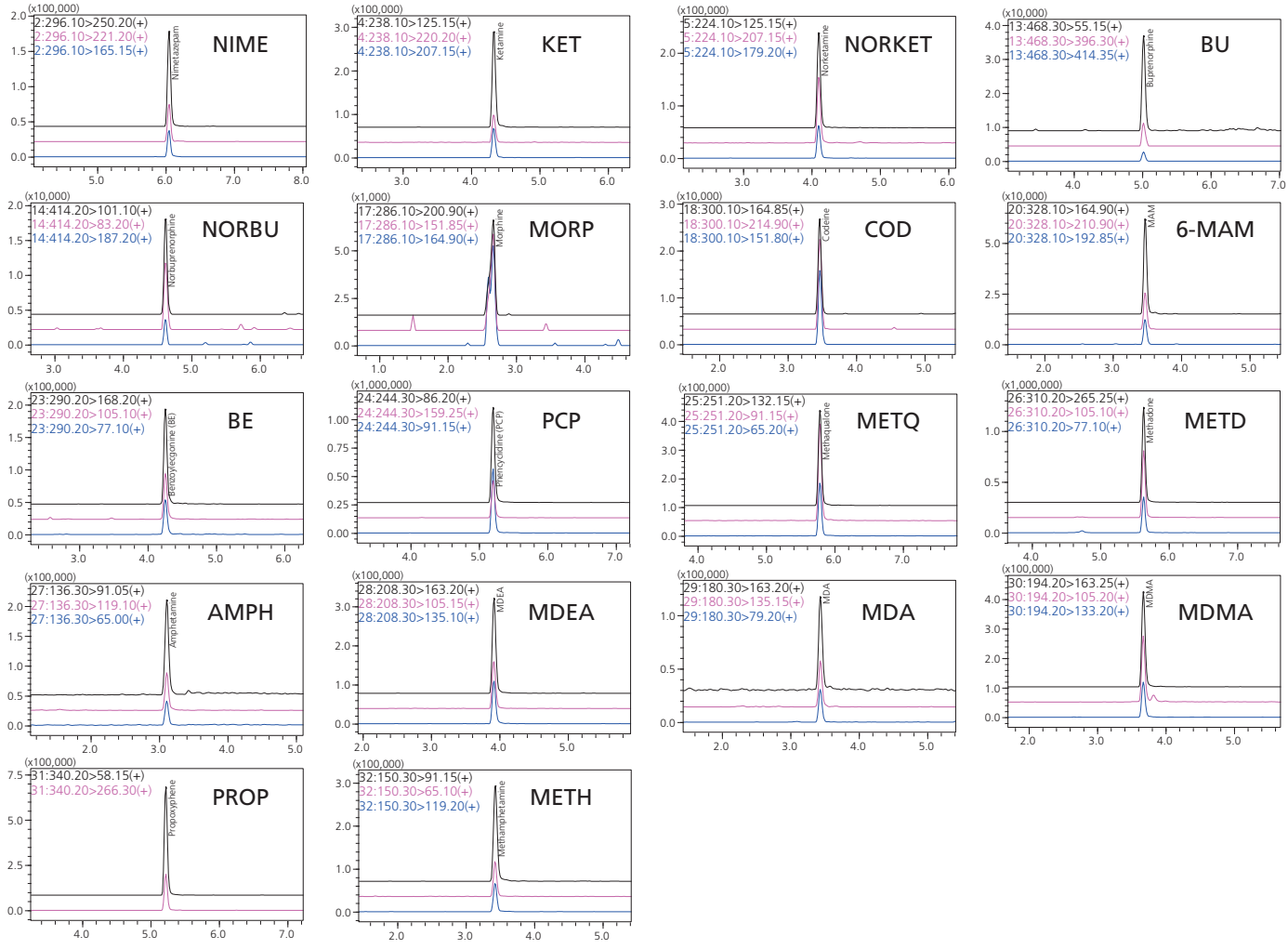

Figure 3: Individual MRM chromatograms of eighteen illicit drugs each (200 ng/mL) and fourteen ISs (100 ng/mL) spiked in urine obtained on CLAM-LC/MS/MS platform.

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Table 2: MRM transitions and parameters of the illicit drugs on LCMS-8060

Standard				Internal Standard			
Compd.	R.T (min)	MRM (m/z)	CE (V)	Compd.	R.T (min)	MRM (m/z)	CE (V)
Nimetazepam (NIME)	6.053	296.1>250.2	-26	D5-Nitrazepam (D5-NITRA)	5.63	287.2>241.2	-26
		296.1>221.2	-34			287.2>185.2	-36
		296.1>165.2	-57			287.2>212.2	-34
Ketamine (KET)	4.334	238.1>125.2	-28	D4-KET	4.32	242.2>129.1	-27
		238.1>220.2	-16			242.2>224.2	-15
		238.1>207.2	-14			242.2>211.2	-15
Norketamine (NORKET)	4.105	224.1>125.2	-24	D4-NORKET	4.09	228.1>129.1	-25
		224.1>207.2	-13			228.1>211.2	-12
		224.1>179.2	-15			228.1>183.2	-16
Buprenorphine (BU)	5.019	468.3>55.2	-60	D4-BU	5.01	472.3>59.2	-54
		468.3>414.4	-36			472.3>400.3	-40
		468.3>396.3	-41			472.3>101.1	-43
Norbuprenorphine (NORBU)	4.622	414.2>83.2	-52	D3-NORBU	4.61	417.3>83.2	-50
		414.2>101.1	-44			417.3>101.2	-41
		414.2>187.2	-38			417.3>187.2	-41
Morphine (MORP)	2.663	286.1>200.9	-26	D3-MORP	2.66	289.1>157.1	-43
		286.1>151.9	-61			289.1>165.1	-41
		286.1>164.9	-41			289.1>153.1	-41
Codeine (COD)	3.474	300.1>164.9	-45	D3-COD	3.46	303.1>151.8	-67
		300.1>214.9	-27			303.1>164.9	-45
		300.1>151.8	-65			303.1>214.9	-27
6-MAM	3.475	328.1>164.9	-39	D6-MAM	3.46	334.1>164.9	-40
		328.1>210.9	-26			334.1>210.9	-27
		328.1>192.9	-29			334.1>192.9	-30
Benzoylecgonine (BE)	4.260	290.2>168.2	-20	D3-BE	4.25	293.2>171.3	-20
		290.2>105.1	-31			293.2>105.1	-29
		290.2>77.1	-53			293.2>77.1	-55
Phencyclidine (PCP)	5.204	244.3>86.2	-12	N.A.			
		244.3>159.3	-14				
		244.3>91.2	-30				
Methaqualone (METQ)	5.786	251.2>132.2	-27	N.A.			
		251.2>91.2	-45				
		251.2>65.2	-61				
Methadone (METD)	5.632	310.2>265.3	-17	N.A.			
		310.2>105.1	-27				
		310.2>77.1	-53				
Propoxyphene (PROP)	5.220	340.2>58.2	-23	N.A.			
		340.2>266.3	-10				
Amphetamine (AMPH)	3.111	136.3>91.5	-21	D5-AMPH	3.066	141.3>124.3	-19
		136.3>119.1	-14			141.3>92.2	-14
		136.3>65.0	-37			141.3>93.2	-17
MDEA	3.919	208.3>163.2	-14	D6-MDEA	3.906	214.3>166.2	-13
		208.3>105.2	-27			214.3>136.2	-20
		208.3>135.1	-22			214.3>108.2	-26
MDA	3.440	180.3>163.2	-12	D5-MDA	3.424	185.3>168.3	-12
		180.3>135.2	-19			185.3>110.3	-23
		180.3>79.2	-32			185.3>138.3	-20
MDMA	3.678	194.2>163.3	-14	D5-MDMA	3.664	199.3>165.3	-13
		194.2>105.2	-24			199.3>107.2	-26
		194.2>133.2	-21			199.3>135.2	-20
Methamphetamine (METH)	3.430	150.3>91.2	-21	D8-METH	3.403	158.3>93.2	-21
		150.3>119.2	-15			158.3>124.3	-15
		150.3>65.1	-43			158.3>92.2	-19

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Performance of MRM-based Quantitative Method

Linearity of the calibration curves with both IS method (14 analytes) and external standard method (4 analytes) were constructed using the standard samples prepared by pre-spiked in urine matrix are shown in Figure 4. The method parameters are summarized in Table 3. It can be seen that good linearity with R^2 greater than 0.995 was obtained for the eighteen illicit drugs in the range from 20 ng/mL to 200 ng/mL in urine.

Accuracy of the quantitation method was evaluated with pre-spiked standard samples at all concentrations. The results are shown in Table 3, which indicate that reliable quantitation accuracy was obtained, except Methadone at 20 ng/mL with an accuracy of 130%.

Process Efficiency (P.E) was evaluated based on the peak area (external standard) or peak ratios (IS method) of pre-spiked samples and neat-spiked sample at all concentrations. The results shown in Table 3 indicate the P.E obtained for the 18 analytes are between 62~122% except four analytes with higher values, Norbuprenorphine, Morphine, MAM, and Methadone. This could be due to interference from urine, which causes ion enhancement.

Specificity of the method for detection and confirmation of the eighteen illicit drugs was evaluated (Figure 5). The confirmation criteria for each target include quantifier MRM peak, its ratios with reference MRM transitions as well as retention time.

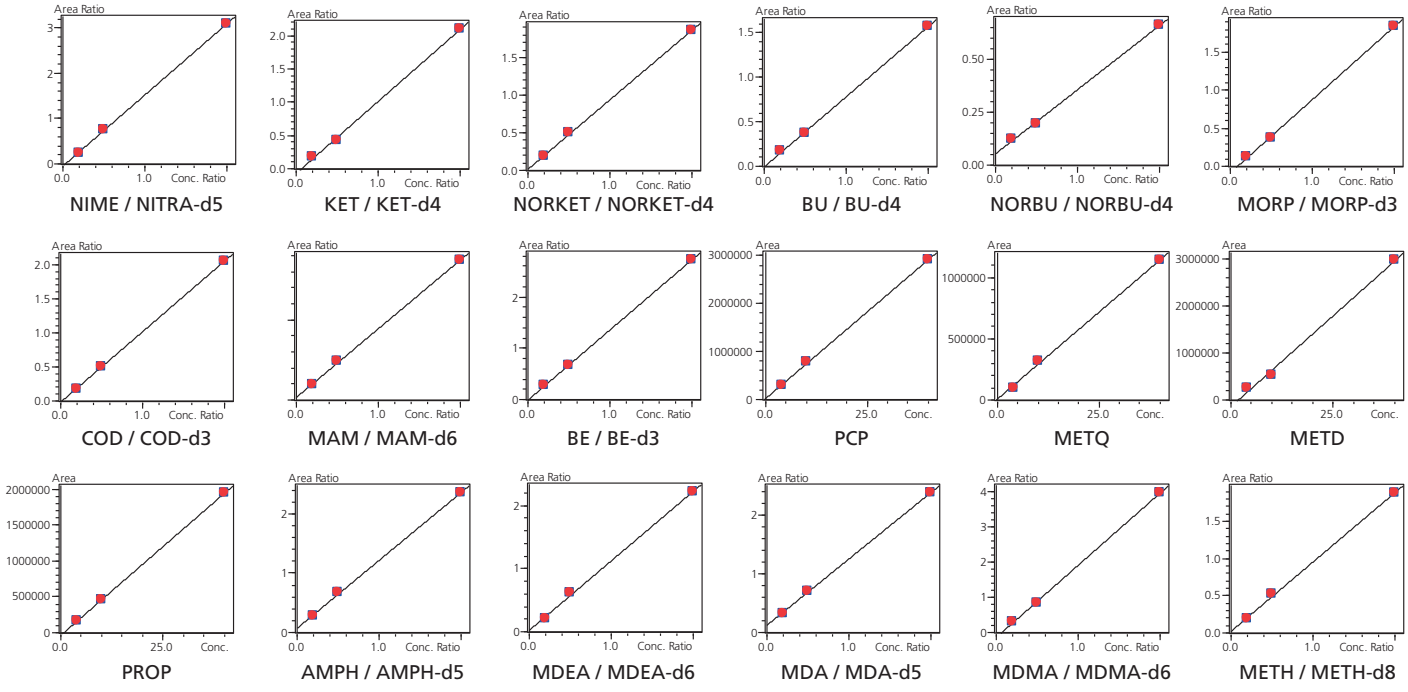

Figure 4: Calibration curves of 14 illicit drugs with isotope-labelled internal standards and 4 illicit drugs with external standard in human urine on LCMS-8040. Details are shown in Table 3.

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Table 3: MRM quantitation method of eighteen illicit drugs

Compd.	Accuracy (%)			R2	*Cut Off ng/mL	Avg. P.E (%)
	20 ng/mL	50 ng/mL	200 ng/mL			
NIME	93.7	103.0	99.9	0.9997	5	99.1
KET	117.5	91.6	100.3	0.9983	100	107.9
NORKET	91.6	104.0	99.8	0.9996	100	106.5
BU	94.5	102.7	99.9	0.9998	2	118.1
NORBU	112.8	93.9	100.3	0.9991	2	183.0
MORP	108.7	95.8	100.2	0.9995	300	62.2
COD	96.0	101.9	99.9	0.9999	300	88.8
MAM	89.6	105.0	99..8	0.9994	10	139.5
BE	102.5	98.8	100.1	0.9999	150	111.3
PCP	92.0	103.8	99.8	0.9996	25	88.3
METQ	80.8	109.2	99.6	0.9980	250	101.9
METD	130.2	85.5	100.6	0.9951	250	161.6
AMPH	91.7	104.0	99.8	0.9996	200	92.3
MDEA	84.0	107.7	99.7	0.9986	200	84.5
MDA	92.1	103.8	99.8	0.9996	200	80.8
MDMA	110.4	95.0	100.2	0.9994	200	98.5
PROP	102.2	98.9	100.0	0.9999	300	122.1
METH	83.7	107.8	99.7	0.9985	200	84.0

*The Cut Off is based on European Guidelines for Workplace Drug Testing in Urine

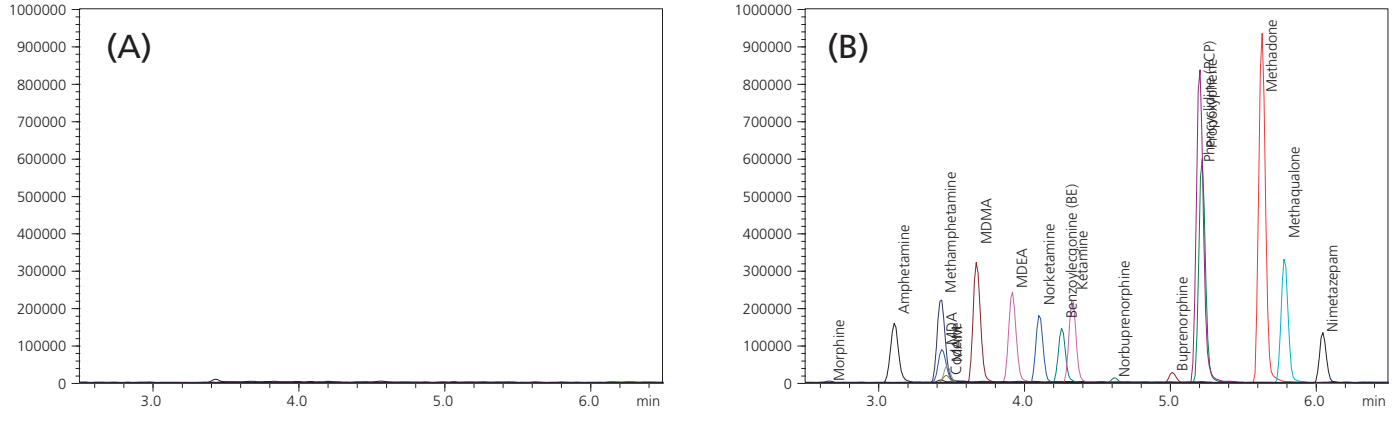

Figure 5: Total MRM chromatograms of (A) blank urine and (B) spiked urine with eighteen illicit drugs (200 ng/mL).

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Conclusions

A fully automated method of sample pretreatment and quantitation for eighteen illicit drugs in human urine was developed on a novel platform of CLAM-LC/MS/MS. The method performance was evaluated on the linearity, accuracy, specificity and process efficiency.

Acknowledgement

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Disclaimer: The data and instruments presented in this Application News are intended for Research Use Only (RUO). Not for use in diagnostic procedures.

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Application News

SSI-BioTech-001

Liquid Chromatography Mass Spectrometry

Fully Automated Sample Preparation and LCMS Analysis of Drugs in Oral Fluid

Summary: The Clinical Laboratory Automation Module (CLAM-2000) is a fully automated sample preparation module that is integrated with a Shimadzu LC/MS analyzer. This system has been used for analysis of 122 drugs and deuterated internal standards in oral fluid matrix with detection limits of 2 ng/mL.

Background: The CLAM-2000 is the first sample preparation module that is fully integrated with LC separation and MS detection of small molecules. Additionally, the CLAM-2000 is capable of parallel processing up to four samples simultaneously, which enables it to keep up with current sub-five-minute LCMS methods. One key advantage of the CLAM-2000 is its great reproducibility as it commonly achieves %RSDs of 10%. This system offers high sample throughput and increased safety for laboratory personnel.

Method: Oral fluid analysis was performed using the CLAM-2000 integrated with a Shimadzu Nexera LC system and a Shimadzu 8050 triple quadrupole mass spectrometer. A gradient of 10% to 60% methanol was implemented over seven minutes. All samples, calibrators and quality controls were made in Quantisol oral fluid diluent (Immunalysis, Pomona, CA).

Analytical Method Summary	
# Drug Compounds	122
Time for first result	12 mins
Sample to sample time	7 mins
LOQ	2 ng/mL
Average %RSD	7.5%
R^2 Values	≥ 99

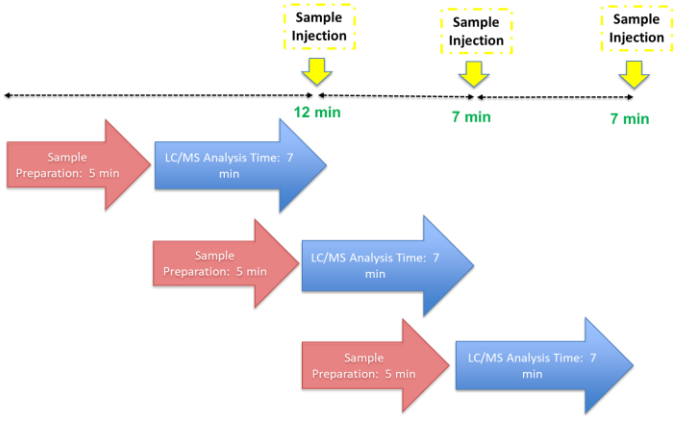
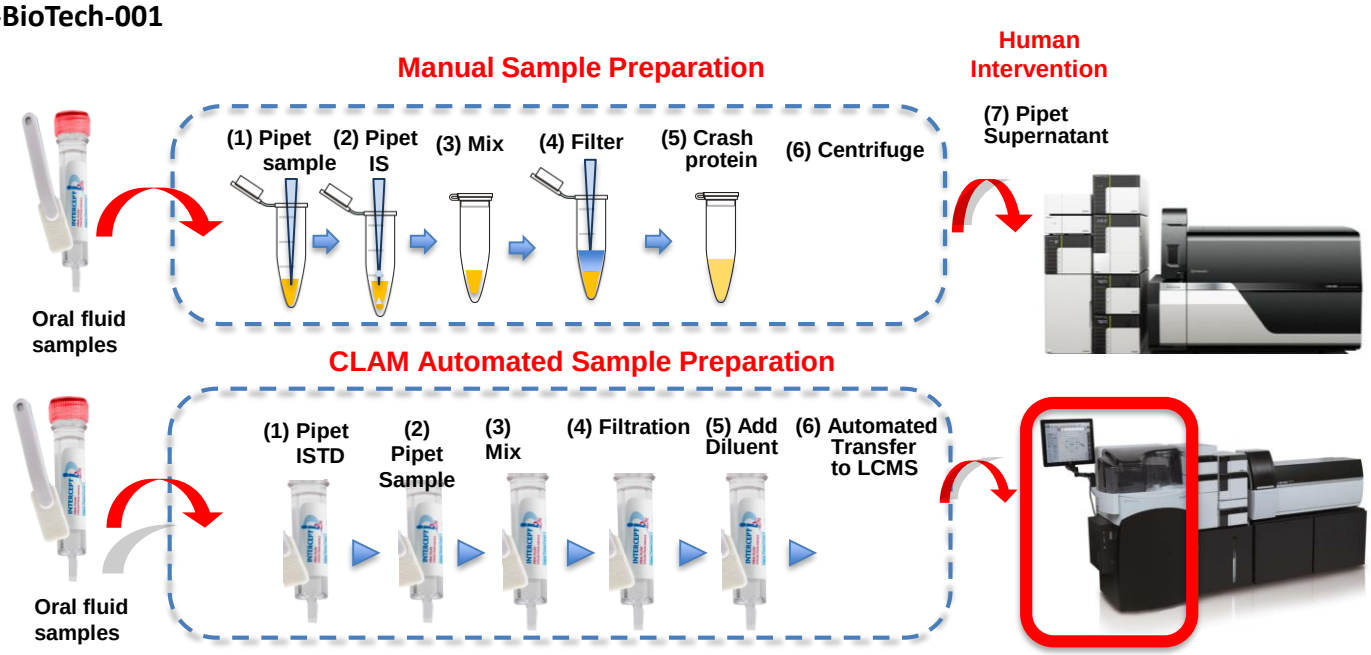


Figure 1: CLAM-2000 workflow with parallel sample processing



Graph 1: Comparison of manual sample preparation and LCMS analysis and CLAM-2000 fully automated sample preparation and LCMS analysis. The many steps of human intervention required for manual preparation not only introduce human error but costs more because employees are being paid to do routine tasks that are more easily accomplished by automation.

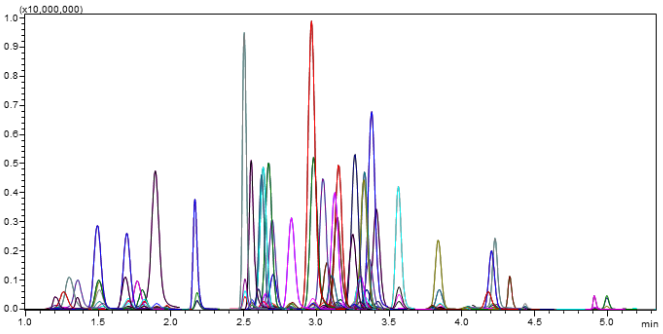
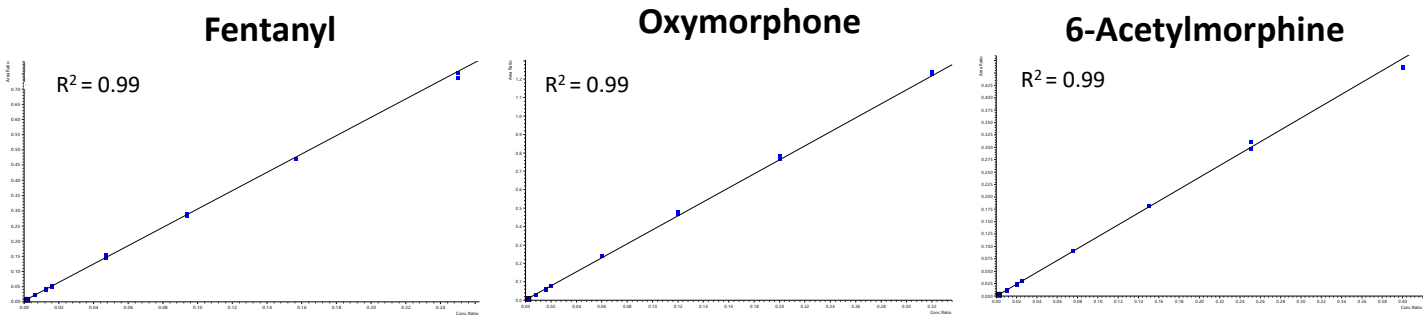
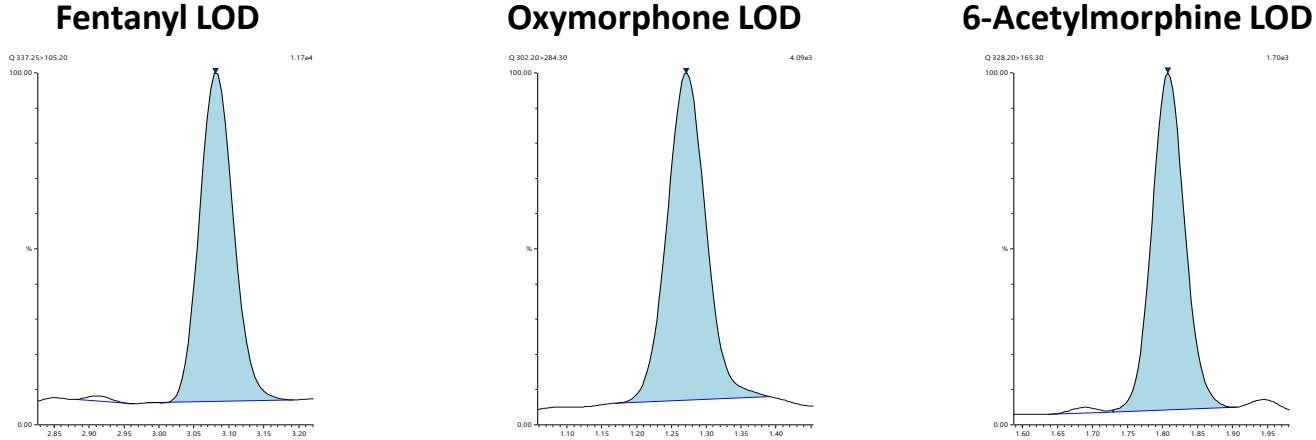


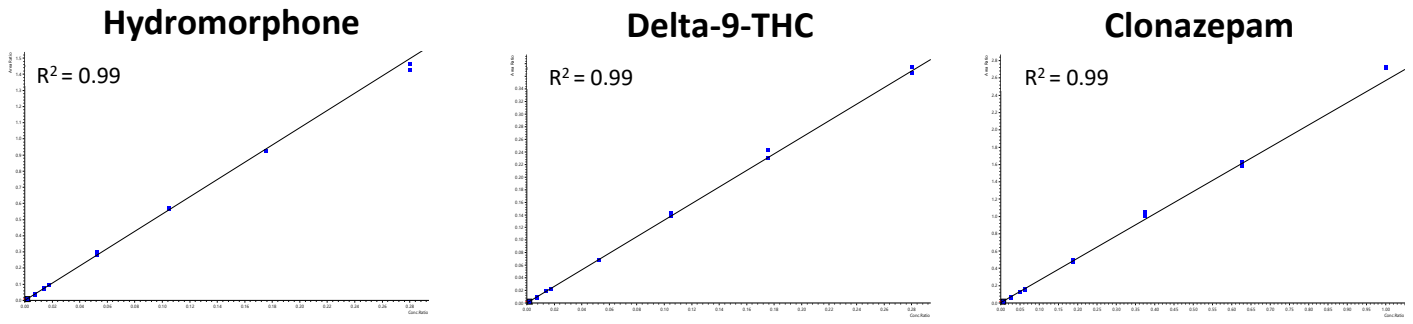
Figure 2: Simultaneous acquisition of 61 compounds in five minutes



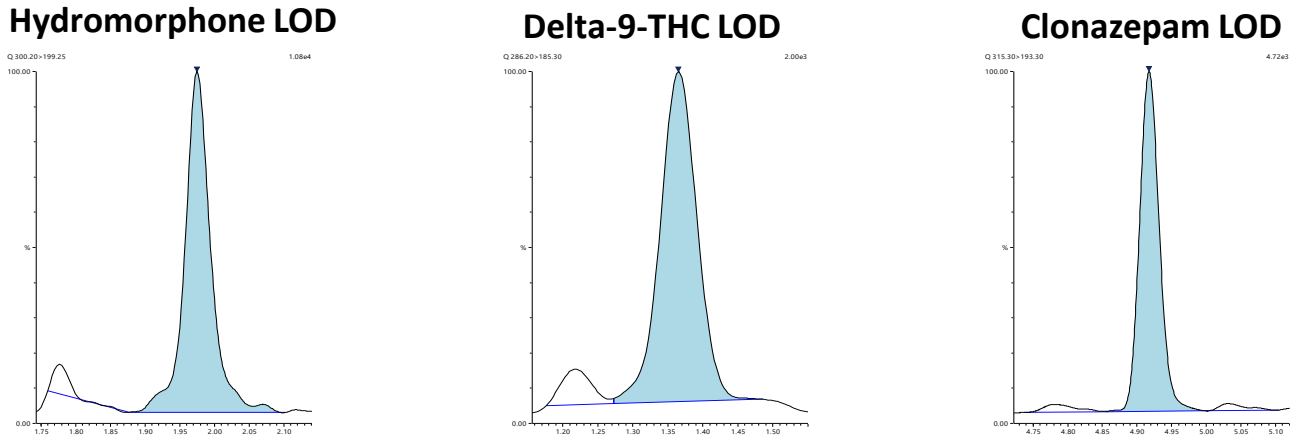
Figures 3-5: Calibration curves for Fentanyl, Oxymorphone, 6-Acetylmorphine. All have R^2 values of greater than 0.99



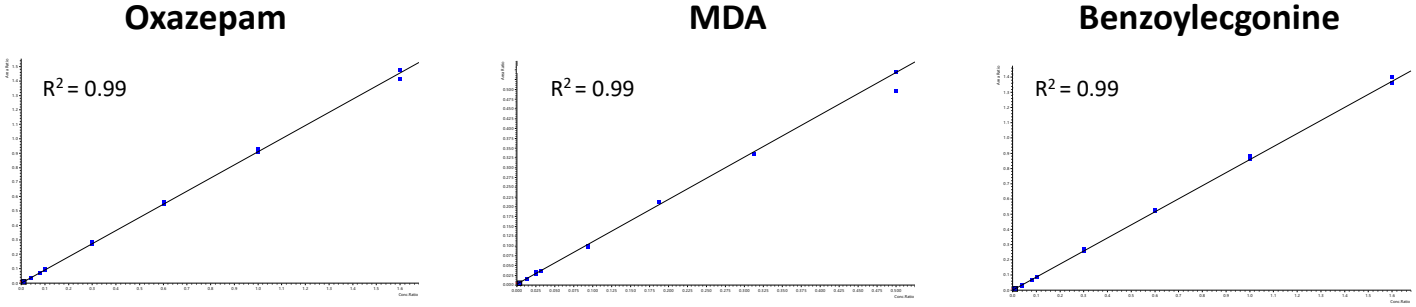
Figures 6-8: Extracted ion chromatograms at limits of quantification for Fentanyl, Oxymorphone, 6-Acetylmorphine.



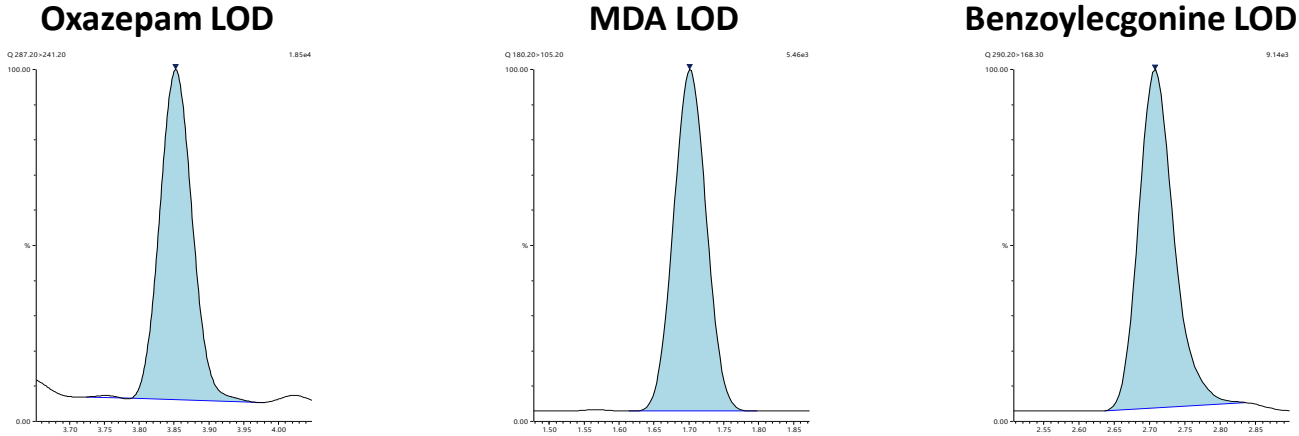
Figures 9-11: Calibration curves for Hydromorphone, Delta-9-THC, and Clonazepam. All have R^2 values ≥ 0.99



Figures 12-14: Extracted ion chromatograms at limits of quantification for Hydromorphone, Delta-9-THC, and Clonazepam.



Figures 15-17: Calibration curves for Oxazepam, MDA, and Benzoylecgonine. All have R^2 values of greater than 0.99



Figures 18-20: Extracted ion chromatograms at limits of quantification for Oxazepam, MDA, and Benzoylecgonine

Oral Fluid Laboratory Cutoff Concentrations		
	SAMSHA	Laboratory
THC	2	2
Cocaine	8	1.25
Benzoylecgonine	8	1.25
Codeine	15	2
Morphine	15	2
Hydrocodone	15	1.25
Hydromorphone	15	1.25
Oxycodone	15	2
Oxymorphone	15	2
6-Acetylmorphine	2	1
Phencylcidine	2	1.25
Amphetamine	15	7.5
Methamphetamine	15	1.25
MDMA	15	1.25
MDA	15	1.25
MDEA	15	1.25

Table 1: Laboratory SAMSHA cutoff concentrations for oral fluid analysis of drugs of abuse and cutoff concentrations from laboratory validated oral fluid analysis method

Precision Results: n = 7			
Interday			
	Conc (ng/mL)	SD	CV
Fentanyl	0.125	0.008	6.296
	1.25	0.101	8.16
	12.5	0.458	3.623
	Conc (ng/mL)	SD	CV
Oxymorphone	2	0.112	5.826
	20	0.815	4.197
	200	3.025	1.526
	Conc (ng/mL)	SD	CV
6-Acetylmorphine	1	0.082	8.296
	10	0.283	2.842
	100	3.263	3.255
Intraday			
	Conc (ng/mL)	SD	CV
Fentanyl	0.125	0.011	8.963
	1.25	0.059	5.221
	12.5	0.628	5.347
	Conc (ng/mL)	SD	CV
Oxymorphone	2	0.142	6.982
	20	1.076	5.424
	200	6.097	3.066
	Conc (ng/mL)	SD	CV
6-Acetylmorphine	1	0.064	6.553
	10	0.309	3.198
	100	4.325	4.496

Table 2: Standard deviation and coefficient of variation for three QC levels from Fentanyl, Oxymorphone and 6-Acetylmorphine. Both Interday and Intraday results show CV's ≤ 10

Results and Discussion: Fully automated sample preparation, LC separation and MS analysis of 122 drugs and deuterated internal standards were performed using the CLAM-2000 LC/MS system. No sample preparation was performed by lab personnel aside from loading the oral fluid collection devices into the instrument carousel.

After the first sample preparation and LCMS analysis, which takes eleven minutes, the sample to sample analysis time is equal to the LCMS method time (seven minutes). Calibration curves for the drugs of abuse exhibited R^2 values ≥ 0.99 and limits of quantification ranged from 2 ng/mL for most compounds to 7.5 ng/mL for Amphetamine. This novel sample preparation method achieved percent relative standard deviations of ~10% or less for all compounds

Conclusion: The fully automated sample preparation and analysis of over one hundred drugs and internal standards was performed with the CLAM-2000 LCMS system. A high degree of reproducibility from sample to sample was exhibited using the CLAM-2000. Additionally, this fully automated sample preparation and LC/MS analysis system reduces sample preparation time by humans, increases laboratory efficiency, improves safety while providing high accuracy and reproducibility. A complete sample preparation and analysis solution for drug analysis is possible when the CLAM-2000 is coupled with an LCMS system and Insight software which allows importation of data files into modern LIMS systems for an efficient and productive laboratory workflow.

ULTRA FAST MASS SPECTROMETRY

LCMS-8030 LCMS-8040 LCMS-8050 LCMS-8060 LCMS-2020 LCMS-IT-TOF

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