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Solutions for Pharmaceutical Impurities

Application Notebook

Solutions for

Pharmaceutical Impurities

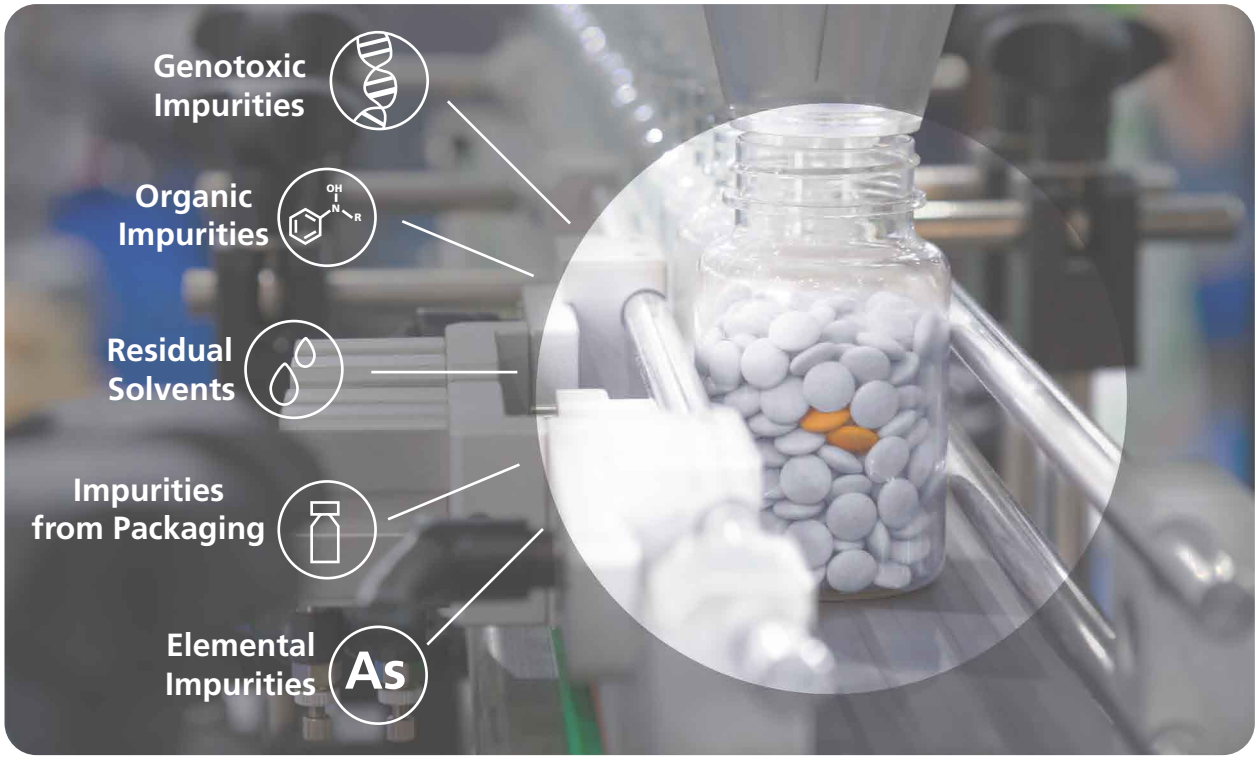
Application Notebook

Introduction

With an ever-increasing consumption of pharmaceutical products by the world population, there are considerable concerns about the unknowns and impurities which are ingested along with these medicinal drugs. The nature of these impurities and their consequent effect may pose a potential hazard to human health. This makes identification, estimation, quantification and control of these impurities matter of great concern and challenge. Regulatory agencies are laying increasingly defined guidelines for different types of impurities both for drug substances and drug products.

Chemical properties of these impurities is diverse. Accordingly, laboratories need to use variety of analytical equipment like LC-MS/MS, UHPLC, GC-MS, GC-MS/MS, ICP-MS. Shimadzu has been a trusted scientific partner for these analytical equipment, with workflows suitable for impurity analysis and compliance platforms tailor-made for pharmaceutical business environment.

In this handbook, we share a variety of methodologies for different classes of impurities like elemental, organic, residual solvent and more. Methods described are in synchronization with relevant ICH guidelines and create a sound platform for scientists to initiate their quest for answers with confidence.



Genotoxic Impurity Analysis

Analysis of Potential Genotoxic Impurities in Active Pharmaceutical Ingredients
This article introduces the analysis of sulfonic acid esters utilizing the GCMS-QP2010 Ultra.

Analysis of Potential Genotoxic Impurities in Active Pharmaceutical Ingredients (2)
This data sheet details the quantitative analysis of sulfonate esters in active pharmaceutical ingredients (API) using GC-MS.

Analysis of Potential Genotoxic Impurities in Active Pharmaceutical Ingredients using Headspace-GC/MS
This article describes the analysis of sulfonic acid esters in pharmaceuticals using a headspace sampler coupled to GC-MS.

Simultaneous Determination of Potential Genotoxic Impurities in Active Pharmaceutical
Quantitation of trace levels of sulfonate esters using a direct injection gas chromatography method coupled with selective ion monitoring mass spectrometry.

Simultaneous Analysis of 8 Sulfonate Esters Genotoxic Impurities in Drugs
A method for the simultaneous analysis of 8 sulfonate esters genotoxic impurities in drugs by GC-MS/MS.

Analysis of Potential Genotoxic Impurities in Active Pharmaceutical Ingredients (3)
-Analysis of Haloalcohols and Glycidol Part 1-
This application data sheet introduces analysis of haloalcohols and glycidol in an active pharmaceutical ingredient (API) using the GC-MS.

Analysis of Potential Genotoxic Impurities in Active Pharmaceutical Ingredients (4)
- Analysis of Haloalcohols and Glycidol Part 2-
Using the GCMS system to conduct quantitative analysis of haloalcohols and glycidol in an active pharmaceutical ingredient.

Analysis of Potential Genotoxic Impurities in Active Pharmaceutical Ingredients (5)
- Analysis of Alkyl Halides-
This data sheet describes the analysis of 18 alkyl halides using headspace-GC/MS.

Detection of 8 Volatile Nitrosoamines in Drugs by GC-MS/MS Method
A method for the simultaneous analysis of 8 volatile nitrosoamines in drugs by GC-MS/MS.

Organic Impurity Analysis

High Speed Analysis of Pharmaceutical Impurities in Compliance with European Pharmacopoeia Using Nexera-i MT
This article introduces a method for high speed analysis of pharmaceuticals and related substances incompliance with the EP using the Nexera-i MT integrated high performance liquid chromatograph.

Isolation and identification of Atorvastatin degradation impurities by UFPLC
Traditional Prep LC with novel fraction trapping for up to five compounds of interest using Prominence UFPLC.

Analysis of Impurities in Pharmaceutical Ingredients Using Trap-Free Two-Dimensional HPLC and Triple Quadrupole LC-MS/MS (LCMS-8040)
Detection of impurities using trap-free two-dimensional HPLC allows online conversion of non-volatile mobile phase to volatile mobile phase.

Residual Solvent Analysis

Analysis of Residual Solvents in drug products using Nexis GC-2030 combined with HS-20 head space sampler
- USP <467> Residual Solvents Procedure A -
Analysis of residual solvents using the Shimadzu HS-20 Headspace Sampler and Nexis GC-2030 Gas Chromatograph.

Analysis of Residual Solvents in Pharmaceuticals Using Headspace GC-FID/MS Detector Splitting System
In a single measurement, FID and MS data was simultaneously obtained using a detector splitting system for residual solvent test in pharmaceuticals.

Impurities from Packaging

Analysis of styrene leached from polystyrene cups using GC-MS coupled with Headspace (HS) sampler

A sensitive, selective, accurate and reliable method for styrene determination using low carryover headspace sampler coupled with GC-MS-QP2010 Ultra.

Simultaneous determination of residual solvents in pharmaceutical packaging materials using headspace-GC-MS

A highly sensitive and precise method utilizing Headspace-GC/MS-QP2010 Ultra has been developed for the analysis of residual solvents in pharmaceutical packaging materials.

Simultaneous Determination of Phthalate Esters in Pharmaceuticals Using GC-MS

A GCMS method was developed for the analysis phthalate esters in pharmaceuticals and drug products using Shimadzu GC-MS-QP2010 Ultra.

Elemental Impurity Analysis

Analysis of ICH Q3D Guideline for Elemental Impurities in Drug Products Using ICPMS-2030

Analysis of 24 elements included in the ICH Q3D guideline using inductively coupled plasma mass spectrometry.

Analysis of Toxic Elements in Supplements as per USP 2232 Using the ICPMS-2030

Quantitative analysis performed on arsenic, cadmium, mercury, and lead in supplements using the Shimadzu ICPMS-2030 ICP mass spectrometer.

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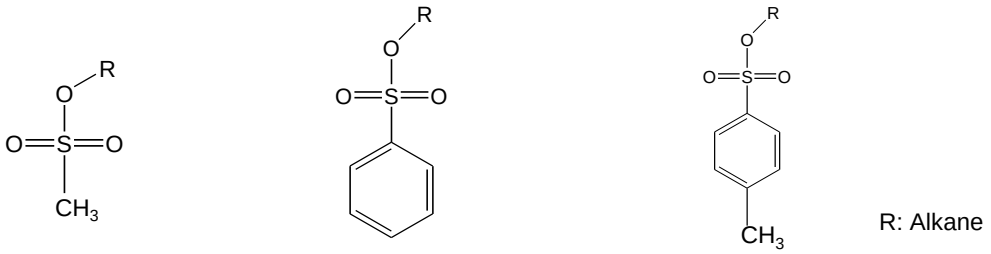
Application Data Sheet

No.18B

GCMS
Gas Chromatograph Mass Spectrometer

Analysis of Potential Genotoxic Impurities in Active Pharmaceutical Ingredients

Chemicals such as methanesulfonic acid (mesylate), benzenesulfonic acid (besilate), and *p*-toluenesulfonic acid (tosylate) are used in the process of synthesizing active pharmaceutical ingredients. These compounds are likely to generate sulfonic acid ester (Fig. 1) as a reaction byproduct. These compounds are known as potential genotoxic impurities (PGI) and are a significant cause for concern among pharmaceutical manufacturers. This article introduces the analysis of sulfonic acid esters utilizing the GCMS-QP2010 Ultra.



Methanesulfonic acid ester　　Benzenesulfonic acid ester　　*p*-toluenesulfonic acid ester

Fig. 1: Structural Formulas for Sulfonic Acid Esters

Analysis Conditions

FASST (Fast Automated Scan/SIM Type), which is capable of simultaneous Scan and SIM measurements, was used as the measurement mode. The analysis conditions are shown in Table 1, while the SIM measurement monitoring *m/z* values are shown in Table 2.

Table 1: Analysis Conditions

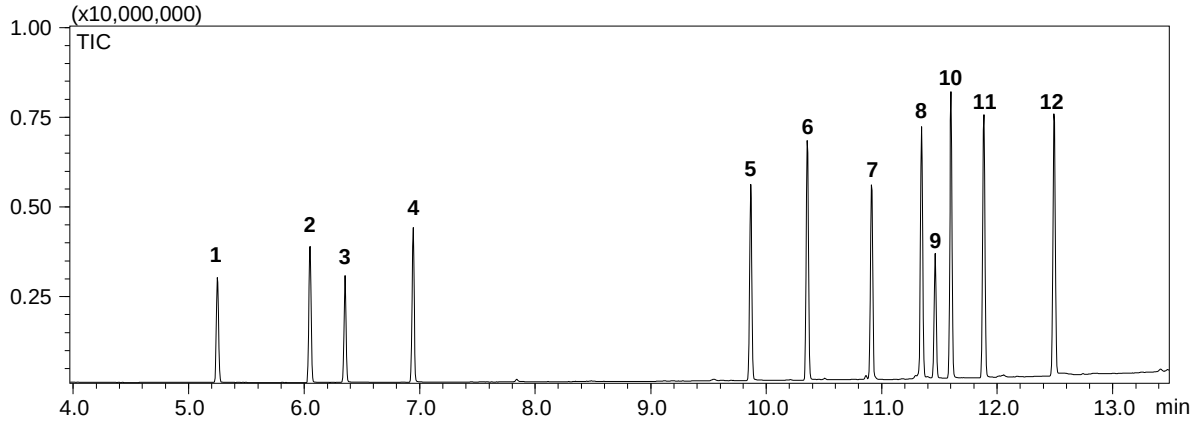
GC-MS	: GCMS-QP2010 Ultra
Column	: Rtx-200 (30 mL. X 0.25 mm I.D., df=0.25 µm, Restek P/N: 15023)
Glass insert	: Deactivated split liner with wool (P/N: 225-20803-01)

[GC]		[MS]	
Injection temp.	: 280°C	Interface temp.	: 280°C
Column oven temp.	: 70°C (2 min) →(15°C/min)→320°C (3min)	Ion source temp.	: 230°C
Injection mode	: Split	Solvent cut time	: 1.5 min
Carrier gas	: He	Tuning mode	: High sensitivity
Flow control mode	: Linear velocity (40 cm/sec)	Acquisition mode	: FASST(Scan/SIM measurements)
Purge flow rate	: 3.0 mL/min	Scan mass range	: *m/z* 40 - 330
Split ratio	: 10	Scan event time	: 0.1 sec
Injection volume	: 1.0 µL	SIM monitoring *m/z*	: See Table 2
		SIM event time	: 0.3 sec

Table 2: Monitoring *m/z* for Target Compounds

	Monitoring *m/z*		Monitoring *m/z*
Methyl methanesulfonate	80, 95	Methyl *p*-toluenesulfonate	155, 186
Ethyl methanesulfonate	109, 97	Ethyl *p*-toluenesulfonate	155, 200
Isopropyl methanesulfonate	123, 79	Isopropyl *p*-toluenesulfonate	172, 155
n-propyl methanesulfonate	109, 97	*n*-propyl *p*-toluenesulfonate	155, 172
Methyl benzenesulfonate	172, 141	Butyl *p*-toluenesulfonate	173, 91
Ethyl benzenesulfonate	141, 186		
Butyl benzenesulfonate	141, 159		

Analysis Results



ID	Compound Name	Retention Time	ID	Compound Name	Retention Time
1	Methyl methanesulfonate	5.252	7	Methyl *p*-toluenesulfonate	10.911
2	Ethyl methanesulfonate	6.052	8	Ethyl *p*-toluenesulfonate	11.345
3	Isopropyl methanesulfonate	6.355	9	Isopropyl *p*-toluenesulfonate	11.462
4	*n*-propyl methanesulfonate	6.944	10	Butyl benzenesulfonate	11.598
5	Methyl benzenesulfonate	9.865	11	*n*-propyl *p*-toluenesulfonate	11.883
6	Ethyl benzenesulfonate	10.356	12	Butyl *p*-toluenesulfonate	12.491

Fig. 2: Total Ion Current Chromatogram

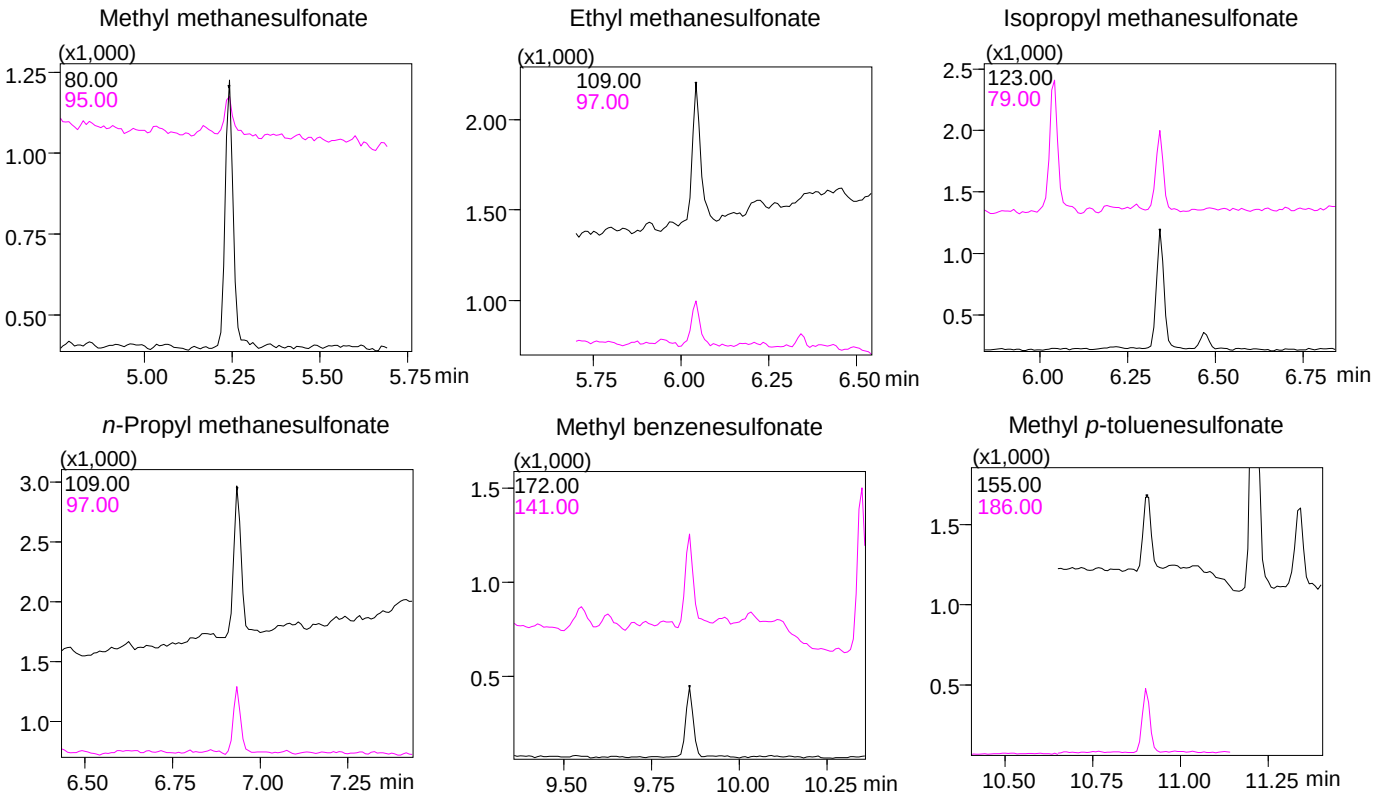


Fig. 3: Mass Chromatograms for Sulfonic Acid Esters

The standard concentration is 10 ng/mL. It is equivalent to 1 ng/mg in active pharmaceutical ingredients when diluted 100 times in pretreatment with a recovery ratio of 100 %.

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Application Data Sheet

No.40

GCMS
Gas Chromatograph Mass Spectrometer

Analysis of Potential Genotoxic Impurities in Active Pharmaceutical Ingredients (2)

This Application Datasheet introduces evaluation results with respect to quantification in the GC/MS analysis of sulfonate esters, so-called PGI (potential genotoxic impurities), in active pharmaceutical ingredients (API). Please refer to GC-MS Application Datasheet No. 18, “Analysis of Potential Genotoxic Impurities in Active Pharmaceutical Ingredients,” for the analysis conditions and the total ion current chromatogram for the sulfonate esters.

Experiment

Gabexate mesylate and amlodipine besilate, commercially-available research reagents, were used as the API. Solvent extraction was utilized as the extraction method, and the other pretreatment procedures are shown in Fig. 1. The API were dissolved in Milli-Q water to a concentration of 10 mg/mL, and 1 mL of the solution was extracted. 2 mL of ethyl acetate was added. After agitation, the sample was centrifuged for 5 minutes at 2,000 rpm, and the organic phase was isolated. This extraction procedure was repeated a total of 3 times, and all of the organic phase extracts were mixed together. Afterwards, 1 g of anhydrous sodium sulfate was added to the organic phase to dehydrate it. After concentration under a stream of nitrogen gas, it was adjusted to 1 mL using acetic ether to arrive at the experimental solution

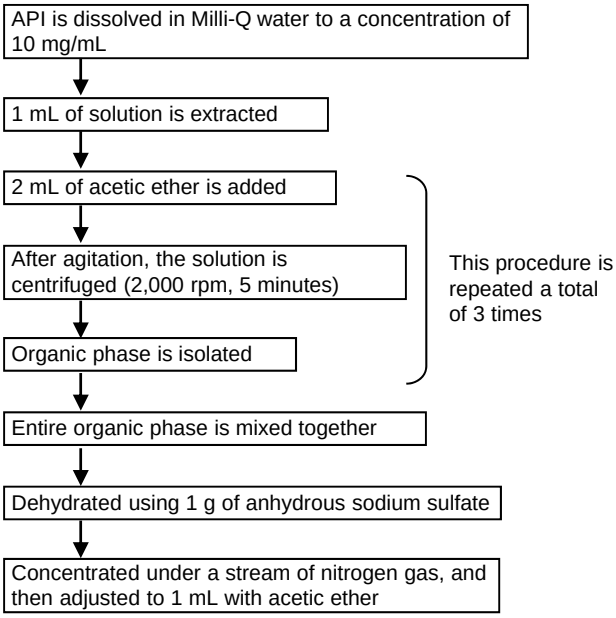

Fig. 1: Pretreatment Procedure

Linearity of the Calibration Curve

Utilizing the analysis conditions shown in GC-MS Application Datasheet 18, a calibration curve was created with the standard sulfonate ester solutions at a concentration of 0.01 µg/mL to 10 µg/mL. If pretreatment is performed as shown above, this concentration range is equivalent to 1 ng/mg to 1,000 ng/mg of API. The calibration curve correlation coefficient (R) was at least 0.9996, indicating that favorable linearity was obtained.

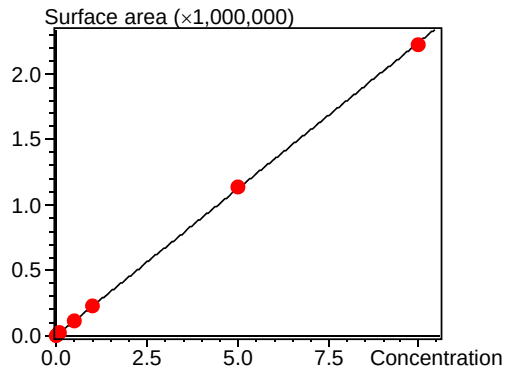

Fig. 2. Methyl Methanesulfonate Ester Calibration Curve

Table 1: Calibration Curve Correlation Coefficients (Concentration: 0.01 µg/mL to 10 µg/mL)

Name of Compound	R (Correlation Coefficient)
Methyl methanesulfonate ester	0.9999
Ethyl methanesulfonate ester	0.9999
Methanesulfonic acid isopropyl ester	0.9999
Methanesulfonic acid n-propyl ester	0.9999
Benzenesulfonic acid methyl ester	0.9999
Benzenesulfonic acid ethyl ester	0.9999
p - toluenesulfonic acid methyl ester	0.9998
p - toluenesulfonic acid ethyl ester	0.9998
Benzenesulfonic acid isopropyl ester	0.9996
Benzenesulfonic acid butyl ester	0.9998
p - toluenesulfonic acid n-propyl ester	0.9998
p - toluenesulfonic acid butyl ester	0.9996

Calibration Curve Weighting: 1/C (Concentration)

Spiked Recovery Test

To perform the spiked recovery test, 1 mL of gabexate mesylate solution (concentration: 10 mg/mL), which was confirmed to not contain the target compound, was extracted, and pretreatment was performed by adding 100 ng of the sulfonic esters. In this case, the concentration of the sulfonic esters in the API was 10 ng/mg. The spiked recovery test was repeated 5 times, and the recovery ratio and recovery ratio repeatability were calculated (Table 2). Favorable results were obtained, with an average recovery ratio in the range of 90.8% to 116.6%, and a recovery ratio repeatability (%RSD) of 3.4% max. for 5 repetitions.

Table 2: Recovery Ratio and Repeatability Results for the Spiked Recovery Test

Name of Compound	Recovery Ratio (%)					Average Recovery Ratio	Recovery Ratio Repeatability %RSD
	NO.1	NO.2	NO.3	NO.4	NO.5		
Methyl methanesulfonate ester	96.4	98.6	93.7	97.3	98.7	96.9	2.1
Ethyl methanesulfonate ester	89.5	89.6	84.4	89.4	89.5	88.5	2.6
Methanesulfonic acid isopropyl ester	92.1	93.4	89.0	94.0	94.1	92.5	2.3
Methanesulfonic acid n-propyl ester	101.7	100.3	99.9	101.8	102.0	101.1	1.0
Benzenesulfonic acid methyl ester	116.3	115.6	112.3	111.7	111.1	113.4	2.1
Benzenesulfonic acid ethyl ester	119.6	116.2	116.2	117.4	113.5	116.6	1.9
p - toluenesulfonic acid methyl ester	107.1	104.0	101.7	105.2	103.9	104.4	1.9
p - toluenesulfonic acid ethyl ester	99.4	96.0	93.2	90.9	95.7	95.0	3.4
Benzenesulfonic acid isopropyl ester	93.8	91.9	88.5	92.0	88.0	90.8	2.7
Benzenesulfonic acid butyl ester	104.8	102.6	100.2	99.1	99.3	101.2	2.4
p - toluenesulfonic acid n-propyl ester	108.1	107.1	104.6	100.5	102.9	104.6	3.0
p - toluenesulfonic acid butyl ester	103.6	105.2	102.4	97.7	100.2	101.8	2.9

Measurement of the API Sample

Pretreatment was performed using commercially-available amlodipine besilate as the research reagent. Fig. 3 shows the total ion current chromatogram and mass chromatograms for the sulfonic esters detected. Four sulfonic esters were detected.

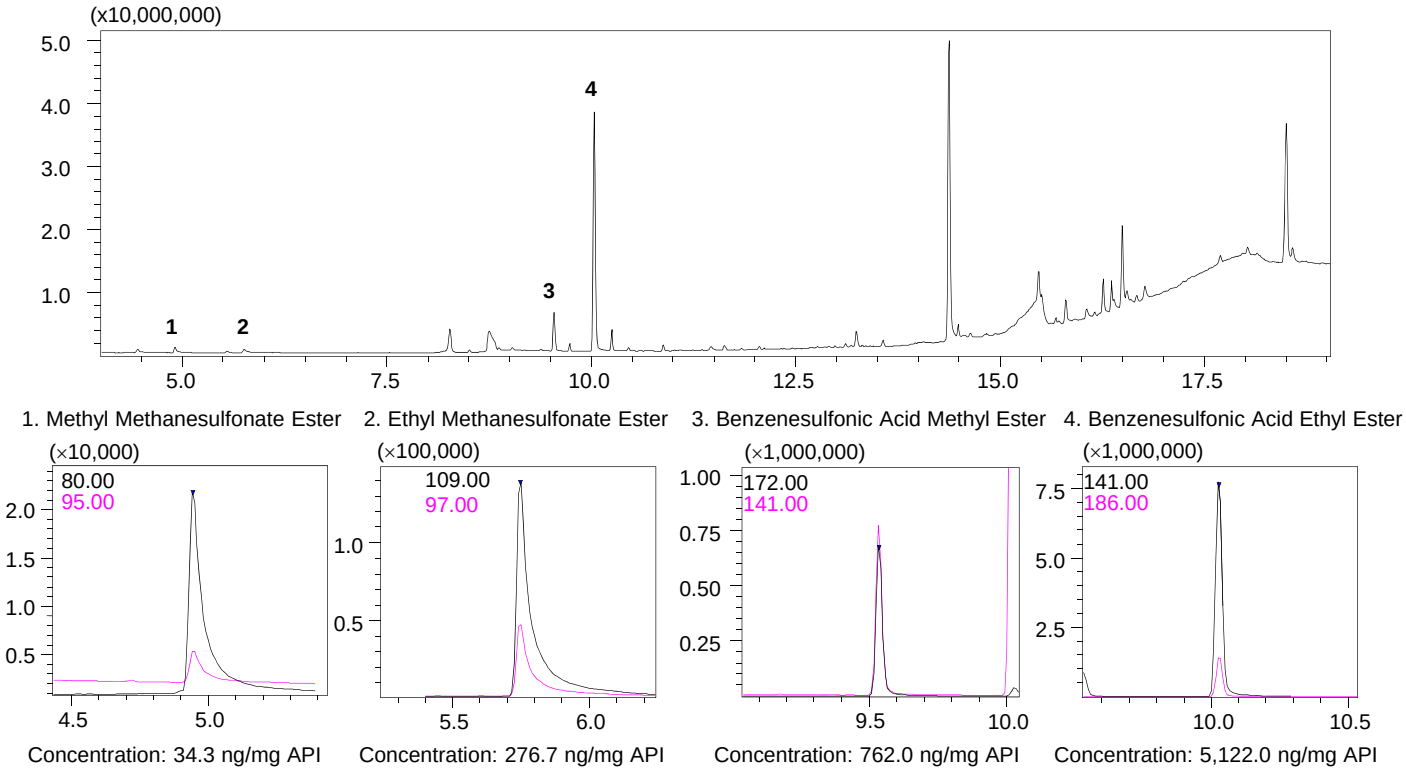

Fig. 3: Total Ion Current Chromatogram and Mass Chromatograms for the Sulfonic Esters Detected

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Application News

No. SSL-CA14-320

Gas Chromatography Mass Spectrometry

Analysis of Potential Genotoxic Impurities in Active Pharmaceutical Ingredients using Headspace-GC/MS

Abstract

Analysis of sulfonic acid esters in pharmaceuticals has been developed using a headspace sampler coupled to GC/MS. The calibration curves showed excellent linearity (R>0.999) over the range of 1~100 µg/L. Good recovery of 90.84~109.23% was obtained at spike-and-recovery test at 0.15µg/g level. The established method demonstrates high sensitivity and reliability for routine analysis.

Introduction

Recently, genotoxic impurities have received considerable attention from regulatory bodies and pharmaceutical manufacturers. In almost every literature on genotoxic impurities, it has been reported that sulfonate esters play an important role and may be produced during drug synthesis. Furthermore, traces of low molecular alcohol impurities (e.g. methanol) in these processes may lead to the production of chemicals like methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), and isopropyl methanesulfonate (IMS). As a result, these compounds can lead to mutations or cause cancer.

According to the guidelines from European Medicine Agency, a Threshold of Toxicological concern (TTC) based acceptable intake of a mutagenic impurity of 1.5 µg per person per day is considered to be associated with a negligible risk and can, in general, be used for most pharmaceuticals as a default value, to derive an acceptable limit for control. A derivatization procedure on the determination of MMS, EMS and IMS in active substances has been recently published as a compendial method in the European Pharmacopoeia, supplement 7.3.

This article introduces the analysis of sulfonic acid esters in pharmaceuticals utilizing Shimadzu Headspace sampler coupled to GCMS-QP2020.

Experimental

Instrumentation and Analytical Conditions

Shimadzu GCMS-QP2020 and HS-20 sampler using the analytical conditions described in Table 1.

Table 1 Analytical Conditions of HS-20 sample and GCMS-QP2020

HS-20 Sampler Conditions		
Injection mode	:	Trap Mode
Vial Warming	:	80 °C
Sample Line Temperature	:	120 °C
Transfer Line Temperature	:	130 °C
Trap Cooling Temperature	:	-20 °C
Trap Heating Temperature	:	300 °C
Trap standy Temperature	:	80 °C
Vial Warming Time	:	20 min
Injection Time	:	0.5 min
Injection Volume	:	1 mL
GC-MS Conditions:		
Column	:	SH-Rtx-Wax (60mx0.32mmx0.50 µm)
Column Temp. Program	:	40 °C (5 min) 20 °C/min 200 °C (3min)
Control Mode	:	Constant liner velocity
Linear Velocity	:	40.2 cm/sec
Injection Mode	:	Split
Split Ratio	:	1:30
Ion Source Temperature	:	230 °C (EI)
Interface Temperature	:	250 °C
Acquisition Mode	:	SIM Mode

Table 2 GC/MS conditions for analysis of derivatized analytes

No.	Compounds	Derivatized compounds	Quantitation Ions	Qualification Ions
1	Methyl methanesulfonate (MMS)	Iodomethane	142	127, 141
2	Ethyl methanesulfonate (EMS)	Iodoethane	156	127, 128
3	Isopropyl methanesulfonate (IMS)	2-Iodoethane	170	43, 127

Preparation of standard solutions

The diluent was prepared by mixing acetonitrile and water in 80:20 (v/v) ratio. The stock solution A was prepared by dissolving MMS, EMS and IMS at 30 mg each in 10 mL of toluene solution. The prepared toluene solution was transferred to a 25 mL volumetric flask and diluted to volume with diluent. The derivatization agent B was prepared by dissolving 50 g of sodium iodide and 30 mg of sodium thiosulfate into a 50 mL volumetric flask, and diluting to volume with water.

For linearity validation, stock solution A was diluted with diluent to give concentrations 2, 5, 10, 20, 50, 100 and 200 µg/L. 0.5 mL of derivatization agent B was added to 0.5 mL of each of these concentrations in a 20 mL headspace vial. It was subsequently filled up to volume with diluent. These dilutions should be prepared fresh before use.

Results and Discussion

Standard chromatograms

The standard chromatogram and the peak identities are shown in Fig.1 and Table 3 respectively.

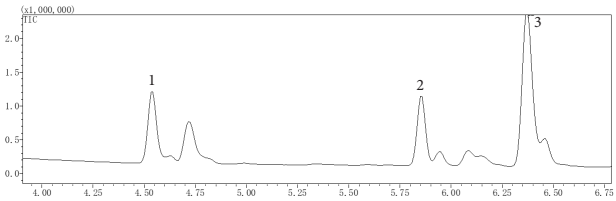

Fig. 1 TIC Chromatograms of 3 derivatized standard analytes at 5 mg/L

Table 3 Peak Identities

No.	Analytes	CAS	Retention time (min)
1	Iodomethane	74-88-4	4.538
2	Iodoethane	75-03-6	5.854
3	2-Iodoethane	75-30-9	6.372

Calibration curves

Linearity of analytes was determined at concentrations of 1, 2.5, 5, 10, 25, 50 and 100 µg/L. All compounds showed excellent linearity with coefficients of determination R>0.9995 (Figure 2). The Instrument Detection Limit (IDL) of each compound is determined at S/N ratio of 3, by injecting 1 µg/L standard mixture. The linearity of each compound is shown in Table 4.

Table 4 Method validation data

No.	Compound name	R	S/N (1 µg/L)	IDL (ng/g)
1	Iodomethane	0.9999	357.71	0.16
2	Iodoethane	0.9998	315.00	0.20
3	2-Iodoethane	0.9995	163.08	0.36

Measurement of the Active Pharmaceutical Ingredient (API) sample

The commercially-available API sample was prepared by the procedure described in the previous section. Analysis was carried out by headspace injection prior to GC/MS and the sample results are shown in Table 5.

Table 5 Sample results

No.	Compound name	Result (µg/g)
1	Iodomethane	N.D*
2	Iodoethane	N.D*
3	2-Iodoethane	N.D*

*N.D: Not detected.

Recoveries and relative standard deviations (RSDs)

For method evaluation, blank samples (n=6) spiked with 0.15 µg/g stock solution A were carried out as per the procedure described in Section 1.4. The spiked recovery test was repeated 5 times and demonstrated good recovery and repeatability in matrix, as shown in Table 6.

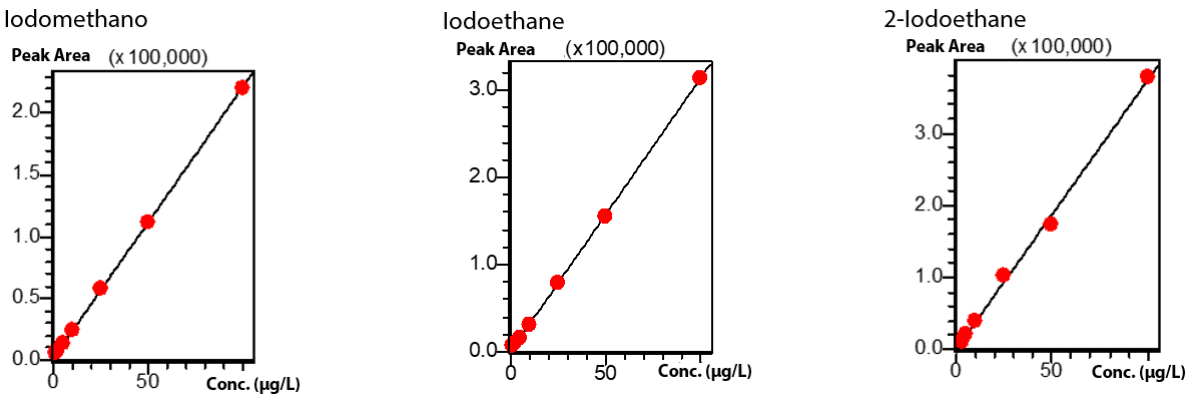

Fig. 2 Calibration Curves of target compounds

Table 6 Results of the testing samples spiked at 0.15 μg/g

No.	Analytes	Recoveries (%)						Mean recoveries (%)	RSD% (n=6)
		1	2	3	4	5	6		
1	Iodomethane	98.67	93.56	89.33	89.71	97.25	84.512	92.17	5.79
2	Iodoethane	103.94	110.37	106.99	109.79	114.42	109.88	109.23	3.22
3	2-Iodoethane	93.00	89.55	86.58	91.70	92.22	91.97	90.84	2.63

Conclusion

Analytical method of 8 volatile nitrosoamines in drug substances has been established by using Shimadzu's triple quadrupole gas chromatography mass spectrometer (GCMS-TQ8040). This method requires simple pretreatment and gives good repeatability. The recoveries of eight volatile nitrosamines were between 67.0 and 99.7%. This method can serve as a reference for the detection of volatile nitrosoamines in drugs.

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SHIMADZU
Excellence in Science

Application News

No. SSL-CA14-335

Gas Chromatography Mass Spectrometry

Simultaneous determination of potential genotoxic impurities in Active Pharmaceutical Ingredients by direct injection GC/MS

Abstract

A direct injection gas chromatography method coupled with selective ion monitoring mass spectrometry (GCMS-QP2020/SIM Mode) was developed for the quantitation of trace levels of sulfonate esters, also known as Potential Genotoxic Impurities (PGI), in active pharmaceutical ingredients (APIs). As a result, all target compounds show good linear calibrations of R > 0.9995 over the range 5~200 µg/L and instrument detection limit (IDL) less than 2.459 µg/L were estimated based on the S/N ratio of more than 3. Acceptable recoveries of 77.03~112.79% were obtained at spiked levels of 10 µg/g (n=3). Good repeatability (relative standard deviations) of the mixed standard solution at 20 µg/L (n=6) were achieved in the range of 1.94%~4.44%. In summary, this method demonstrates high precision and repeatability for the analysis of PGIs utilizing GCMS-QP2020.

Introduction

Recently, the issue of potential genotoxic impurities (PGIs) in active pharmaceutical ingredients (APIs) and drug products continues to receive considerable attention. Some impurities like sulfonic acid esters are identified as genotoxic impurities (GTI) which pose a significant safety risk because they induce damage to the genetic material in the cells through interactions with the DNA sequence and structure. As a result, GTI can lead to mutations or cause cancer. Therefore, exposure to trace levels of GTI in API may be of significant toxicological concern. EMEA and FDA guidelines have established a threshold of toxicological concern (TTC) of 1.5 µg/day (1.5 ppm, assuming a daily dose of 1 g) for each GTI as an acceptable threshold for any marketing authorization application.

A derivatization procedure on the determination of methyl, ethyl and isopropyl methanesulfonates in active substances has been recently published as a compendial method in the European Pharmacopoeia, supplement 7.3. The use of GC-MS after headspace-derivatization has found wide application in rapid analysis, but the derivatization step can cause matrix interference in the presence of chemicals like methyl, ethyl or isopropyl substances in APIs. For this study, the aim was to establish a simple, rapid and sensitive GC/MS method by direct injection, which enable achieving high throughput analysis and minimizing sample preparation for the routine analysis.

Experimental

Instrumentation and Analytical Conditions

Shimadzu GCMS-QP2020 and the analytical conditions detailed in Table 1.

Table 1 Analytical Conditions of GCMS-QP2020

GC-MS Conditions		
Column	:	Intercap 35 (30mx0.25mmx0.25 µm)
Column Temp. Program	:	50 °C (1 min) 15 °C/min 260 °C (5min)
Control Mode	:	Constant linear velocity
Linear Velocity	:	47.2 cm/sec
Injection Mode	:	Splitless
Injection Time	:	1 min
Ion Source Temperature	:	230 °C (EI)
Interface Temperature	:	280 °C
Acquisition Mode	:	SIM Mode (See Table 2)

Table 2 Mass spectrometry parameters for the GC /MS determination of target compounds

No.	Compound Name	CAS	Retention Time (min)	Quantitation Ion	Qualification Ion 1	Qualification Ion 2
1	Methyl methanesulfonate	66-27-3	5.050	80	79	65
2	Ethyl methanesulfonate	62-50-0	5.770	79	109	97
3	Isopropyl methanesulfonate	926-06-7	6.004	123	59	79
4	Methyl benzenesulfonate	80-18-2	10.494	77	141	172
5	Ethyl benzenesulfonate	515-46-8	10.996	77	186	141
6	Methyl p-toluenesulfonate	80-48-8	11.490	91	155	186
7	Ethyl p-toluenesulfonate	80-40-0	11.952	155	200	91
8	Isopropyl p-toluenesulfonate	2307-69-9	12.044	172	91	214

Sample preparation

0.05 g of the API sample was weighed into a centrifuge tube and 2 mL of ethyl acetate was subsequently added. The sample mixture was vortexed for 10 min. The extract was subjected to a 0.22 µm nylon filter, before injection to the GCMS using Selected Ion Monitoring (SIM) mode.

Results and Discussion

Standard chromatograms

The TIC chromatograms and the standard mass chromatograms are shown in Fig 1 and Fig 2, respectively

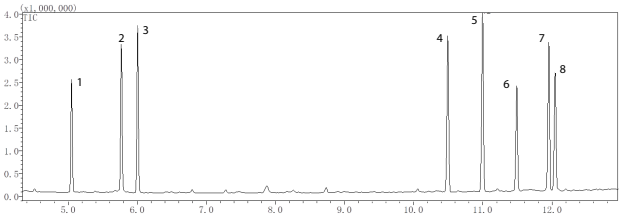

Fig. 1 TIC chromatograms of sulfonic acid (5 µg/ml)

Calibration curves

The standard stock solution was prepared by dissolving sulfonic acid esters at 50 mg each into a 50 mL volumetric flask. Stock solutions were further diluted volumetric flask to 1000 µg/mL with diluent (ethyl acetate). Calibration levels for each of the eight compounds at concentrations 5, 10, 20, 50, 100, and 200 µg/L, are shown in Figure 3. The Instrument Detection Limit (IDL) of each compound was determined at S/N ratio of 3, by injecting 5 µg/L standard mixture. The result of each compound is listed in Table 3.

Table 3 Method validation data

No.	Compound name	R	IDL (µg/L)	RSD%
1	Methyl methanesulfonate	0.9999	0.135	3.04
2	Ethyl methanesulfonate	0.9999	0.142	1.94
3	Isopropyl methanesulfonate	0.9999	0.059	2.40
4	Methyl benzenesulfonate	0.9999	0.112	2.63
5	Methyl benzenesulfonate	0.9999	0.415	2.70
6	Methyl p-toluenesulfonate	0.9997	2.459	2.75
7	Ethyl p-toluenesulfonate	0.9999	0.458	3.01
8	Isopropyl p-toluenesulfonate	0.9995	0.006	4.44

Repeatability results

Repeatability was determined by injecting standard mixture (n=6) spiked at the 20 µg/L level. The results are shown in Table 3.

Spiked recovery test

To perform the spiked recovery test, API samples were extracted, and pretreatment was performed as described in the previous section. After sample preparation, 50 µL of standard stock solution of concentration 10 µg/mL was spiked into the sample. The final concentration of the sulfonic esters in the API sample was 10.0 µg/g (Figure 4). The spiked recovery test was repeated thrice, and the recovery ratio and repeatability were calculated as shown in Table 4.

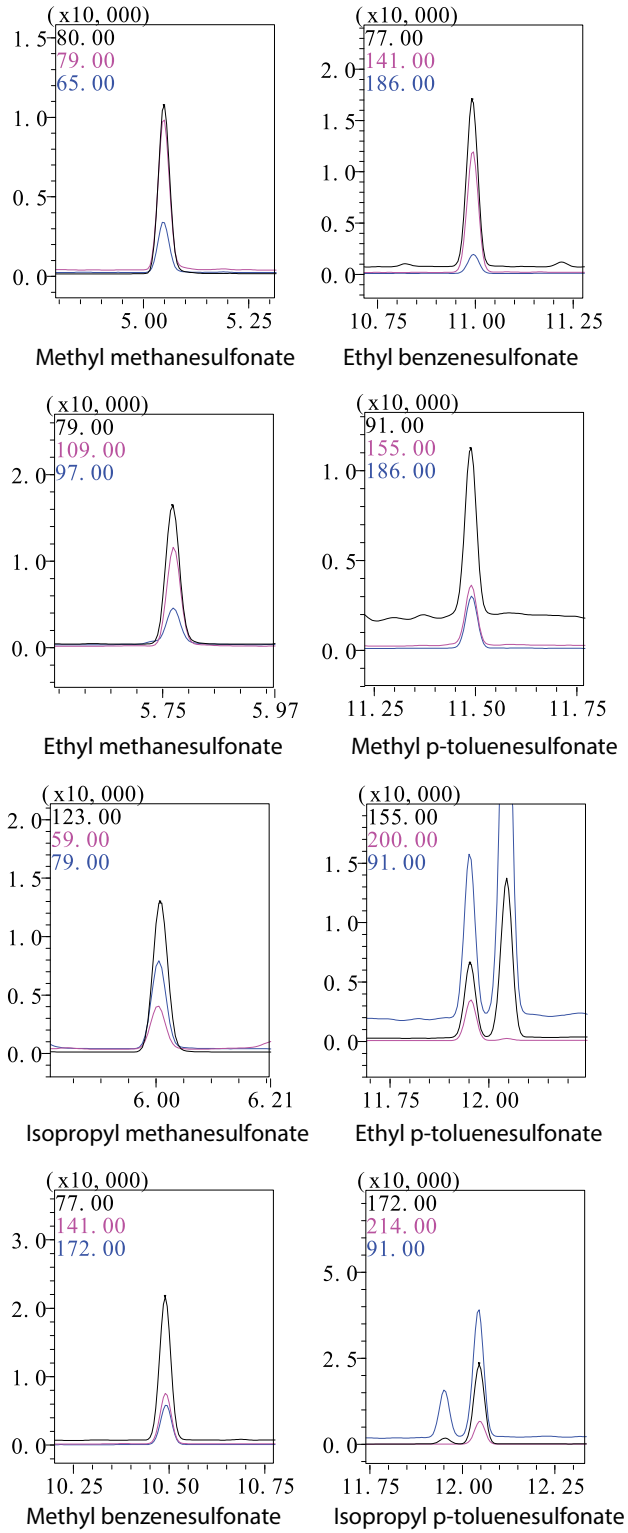

Fig.2 Mass Chromatograms for Sulfonic Acid Esters (5 µg/ml)

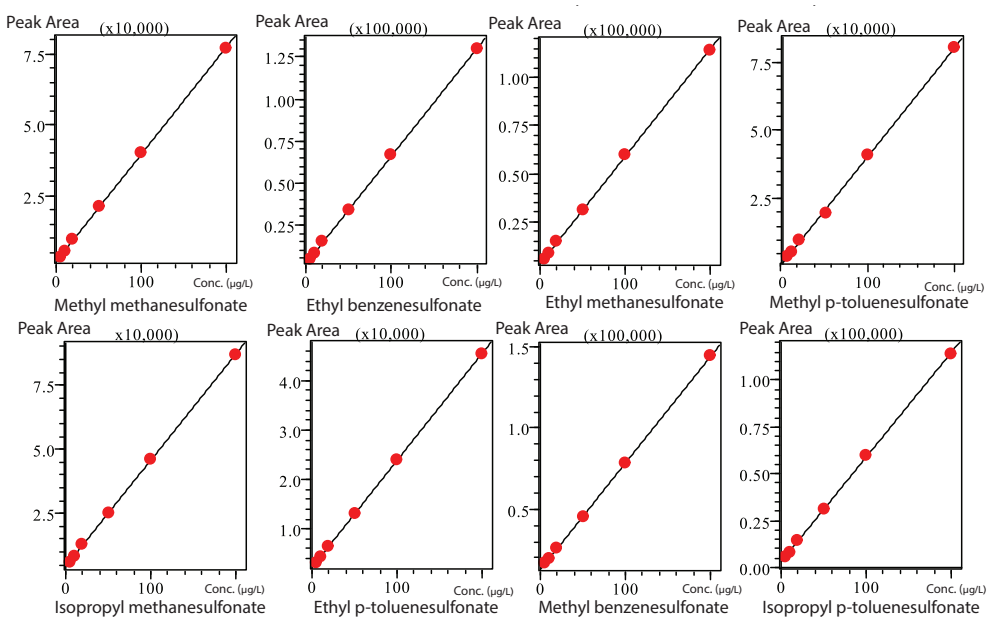

Figure 3. Calibration curves

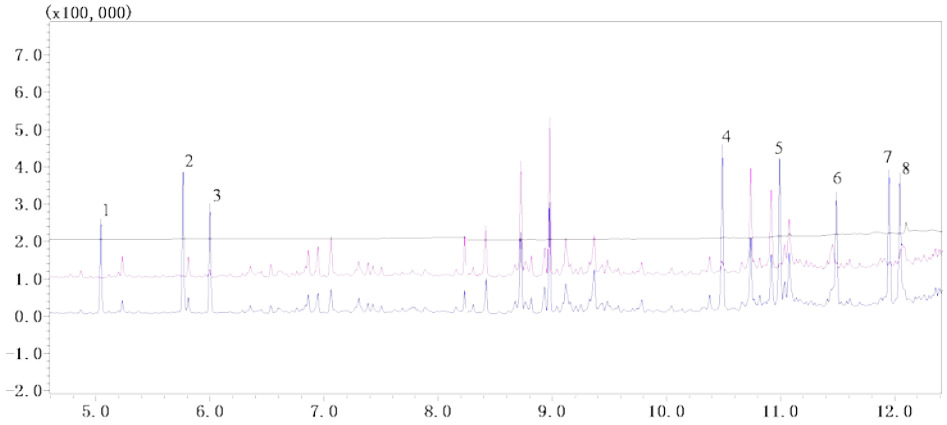

Figure 4. Chromatogram comparison at 10.0 μg/g
(Black - blank, Pink - API sample, Blue - Spiked API sample)

Conclusion

This article introduces the analysis of sulfonic acid esters utilizing GCMS-QP2020 by direct injection. All target compounds showed good linearity over the range 5~200 μg/L, calculated IDLs are lower than 2.459 μg/L and acceptable recoveries of 77.03~112.79%. This study demonstrates a derivatization-free, simple, rapid and sensitive GC-MS method for routine analysis.

Table 4 Recovery ratio and repeatability results for the spiked recovery test

No	Compound name	Conc. (μg/g)	Spiked concentrations 10.0 μg/g	
			Recovery ratio (%)	RSD%
1	Methyl methanesulfonate	N.D*	110.24	1.82
2	Ethyl methanesulfonate	N.D*	112.79	0.86
3	Isopropyl methanesulfonate	N.D*	98.59	2.69
4	Methyl benzenesulfonate	N.D*	94.24	2.15
5	Methyl benzenesulfonate	N.D*	77.03	0.80
6	Methyl p-toluenesulfonate	N.D*	98.55	1.36
7	Ethyl p-toluenesulfonate	N.D*	86.82	1.15
8	Isopropyl p-toluenesulfonate	N.D*	95.11	2.32

*N.D.: Not detected

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Application News

No. SSL-CA14-332

Gas Chromatography Mass Spectrometry

Simultaneous Analysis of 8 Sulfonate Esters Genotoxic Impurities in Drugs

Abstract

A method for the simultaneous analysis of 8 sulfonate esters genotoxic impurities in drugs by GC-MS/MS was developed. The results showed linear correlation coefficients R > 0.9993 for all the components over the range of 1 – 200 µg/L. Repeatability experiments were performed on 10 µg/L sulfonate esters standard solutions. The relative standard deviations (RSD%) of the peak areas of each component were below 5%. The recoveries of each component at 1.0 µg/g and 10.0 µg/g were 74.21% - 81.72% and 88.33% - 101.60% respectively. The established method can serve as a reference method for the detection of sulfonate esters in drugs.

Introduction

In recent years, genotoxic impurities have become the focus of attention. Methanesulfonic acid, benzenesulfonic acid and other sulfonic acids and traces of lower alcohols produce alkyl sulfonates such as methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), as well as some aryl sulfonates during the synthesis of drugs. These substances can be alkylated with DNA, which may cause cancer. Therefore, it is particularly important to detect and control these genotoxic impurities in drugs.

The traditional analytical methods are headspace derivatization and direct injection. The headspace derivatization method is simple in operation and can well exclude interference from complex matrixes, but false-positive derivatives may also be generated in the process of headspace derivation, which may interfere with the determination. The traditional direct injection (GC-FID) method is not stable, and may be susceptible to contamination. The reproducibility is poor, and interference may be observed. In contrast, the application of GC-MS is relatively common and can be applied to the detection of sulfonate ester impurities. However, drugs with a complex matrix tend to interfere with the sample analysis. In this application, a Shimadzu GCMS-TQ8040 triple quadrupole mass spectrometer was used to establish a method for the simultaneous determination of eight sulfonate esters in drugs. The method is simple to handle, and has low chemical interference and high sensitivity.

Experimental

Instrumentation and Analytical Conditions

Triple Quadrupole Mass Spectrometer GCMS-TQ8040 using the analytical conditions described in Table 1.

Table 1 Analytical Conditions of GCMS-TQ8040

GC-MS Conditions:		
Column	:	Intercap 35 (30 m x 0.25 mm x 0.25 µm)
Injection Temp.	:	260 °C
Column Temp. Program	:	50 °C (1 min) 15 °C/min 260 °C (1min)
Flow Control Mode	:	Linear Velocity 47.2 cm/sec
Injection Mode	:	Splitless (1 min)
Ion Source Temperature	:	230 °C
Interface Temperature	:	280 °C
Detector voltage	:	Tuning Voltage +0.4 kV
Acquisition Mode	:	MRM (Acquisition parameters are shown in Table 2)

Table 2 GC/MS conditions for analysis of derivatized analytes

No	Compound Name	CAS No.	Quantitation Ion	CE	Qualification Ion 1	CE	Qualification Ion 2	CE
1	Methyl methanesulfonate	66-27-3	80.00>65.00	9	109.00>79.00	6	80.00>63.00	21
2	Ethyl methanesulfonate	62-50-0	109.00>79.00	6	109.00>79.00	3	97.00>79.000	12
3	Isopropyl methanesulfonate	926-06-7	123.00>79.00	9	123.00>59.00	3	97.00>79.00	9
4	Methyl benzenesulfonate	80-18-2	77.00>51.00	15	141.00>77.00	9	172.00>77.00	27
5	Ethyl benzenesulphonate	515-46-8	141.00>77.00	9	77.00>51.00	15	141.00>95.00	6
6	Methyl p-toluenesulfonate	80-48-8	91.00>65.00	12	155.00>91.00	15	186.00>91.00	18
7	Ethyl p-toluenesulfonate	80-40-0	155.00>91.00	15	91.00>65.00	12	200.00>91.00	27
8	Isopropyl p-toluenesulfonate	2307-69-9	91.00>65.00	15	155.00>91.00	155	172.00>107.00	15

Sample Preparation

For drugs that are soluble in organic solvent

0.05 g of drug powder was weighed in a test tube and 2 ml of ethyl acetate was added. The mixture was vortex and subsequently extracted for 20 minutes by ultrasonication.

For water-soluble drugs:

0.05 g of drug powder was weighed in a test tube and 2 ml of methanol/water (v/v, 5:1) was added. The mixture was vortex and subsequently extracted for 20 minutes by ultrasonication. The mixture was further extracted with 2 ml of ethyl acetate followed by dehydration by sodium sulfate, then filtered and analyzed.

Results and Discussion

Standard chromatograms

The MRM chromatogram and the MRM mass chromatograms of the mixed standards are shown in Fig.1 and Fig. 2, respectively.

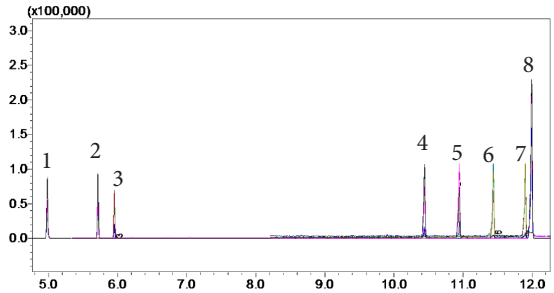

Fig. 1 TIC chromatograms of mixed standards 100 µg/L

Preparation of sample solution

Test sample solution was prepared by transferring 50 mg of test sample into a 20 mL headspace vial containing 0.50 mL of 80% (v/v) acetonitrile in water and 0.50 mL derivatization agent B. Septum was placed and capped immediately.

Linear Calibration Curves and LODs

The mixed standard solutions of 8 sulfonate esters at concentrations 1, 10, 20, 50, 100, 200 µg/L are prepared and analyzed. The concentration is plotted on the x-axis and the peak area is plotted on the y-axis to draw a standard curve, as shown in Fig 3. Correlation coefficients R value and LODs are shown in Table 3.

Repeatability Results

Repeatability was determined by injecting the 10 µg/L standard sample (n=6). Peak area and RSD% are shown in Table 4.

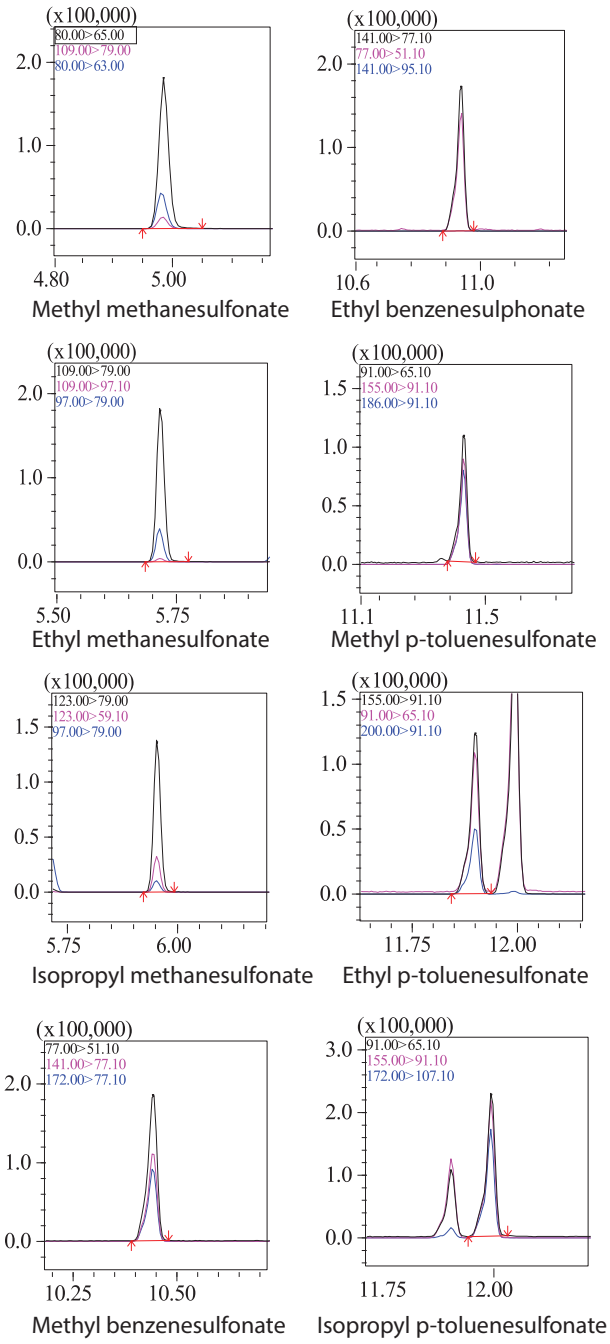

Fig. 2 MRM mass chromatograms of mixed standard solution

Table 3 Correlation coefficient and LOD of each compound

No.	Compound Name	Correlation coefficient R	LOD (µg/L)
1	Methyl methanesulfonate	0.9999	0.02
2	Ethyl methanesulfonate	0.9999	0.02
3	Isopropyl methanesulfonate	0.9998	0.04
4	Methyl benzenesulfonate	0.9998	0.51
5	Ethyl benzenesulphonate	0.9999	0.05
6	Methyl p-toluenesulfonate	0.9999	4.58
7	Ethyl p-toluenesulfonate	0.9998	0.05
8	Isopropyl p-toluenesulfonate	0.9993	0.13

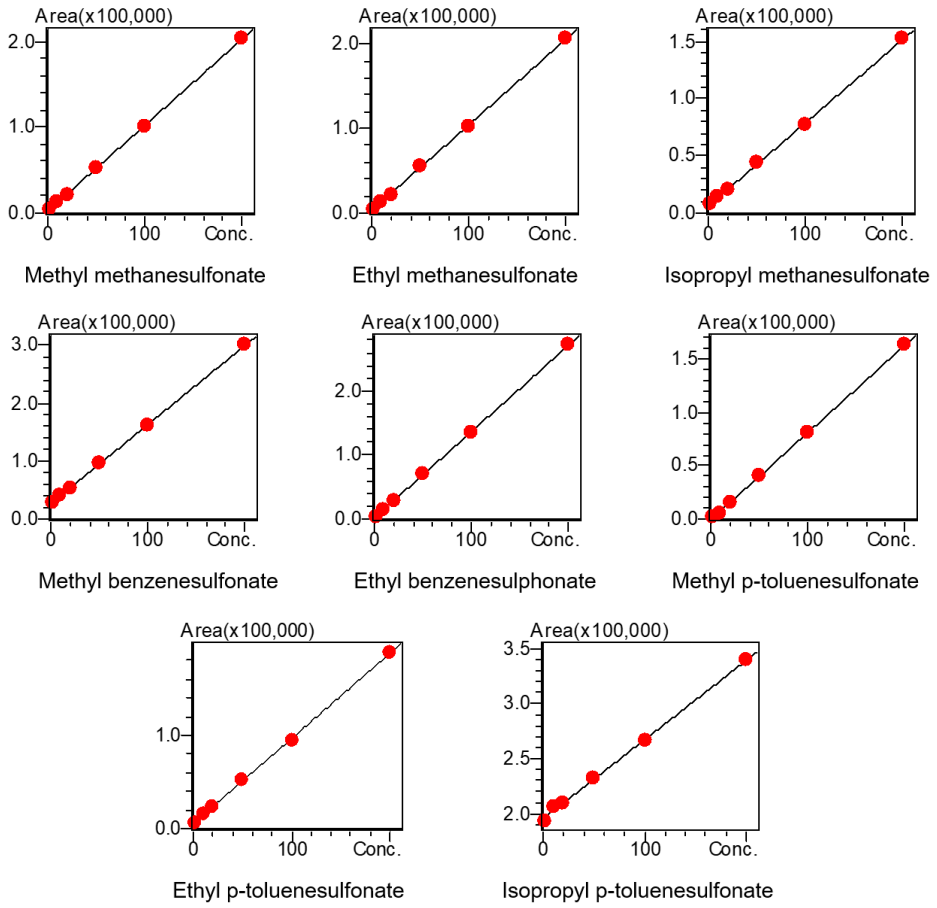

Fig 3 Calibration curves of each compound

Table 4 Repeatability Results

No.	Compound Name	Peak area						RSD% (n=6)
		1	2	3	4	5	6	
1	Methyl methanesulfonate	10311	10905	10554	10739	10998	11149	2.85
2	Ethyl methanesulfonate	11051	11558	11443	11702	11887	11921	2.79
3	Isopropyl methanesulfonate	11648	11598	11141	11060	11661	12106	4.57
4	Methyl benzenesulfonate	32364	34419	31846	33204	32478	32706	2.72
5	Ethyl benzenesulphonate	10475	10685	10996	10811	11308	11490	3.50
6	Methyl p-toluenesulfonate	4768	4841	4967	4873	4644	4565	3.13
7	Ethyl p-toluenesulfonate	9903	10238	10972	10492	10879	10341	3.84
8	Isopropyl p-toluenesulfonate	101566	108335	102831	104506	108168	109131	2.35

Sample Test Results and Recoveries

A commercially available drug was selected and the drug sample was prepared according to the procedure described in the previous section. The analysis of sulfonate esters in the drug sample was conducted using GC-MS/MS. The spiked drug sample of concentrations 1.0 and 10.0 µg/g, were prepared by adding the standard mixture in the sample blank. Recovery was determined by analyzing the spiked drug samples (n = 3 for each concentration). The sample drug test results and the recovery of the spiked sample are determined and shown in Table 5.

Table 5 Sample Testing Results and Spiked Recoveries

No.	Compound Name	Concentration (μg/g)	Spiked concentration (1.0 μg/g)		Spiked concentration (10.0 μg/g)	
			Average recovery (%)	RSD%	Average recovery (%)	RSD%
1	Methyl methanesulfonate	N.D	81.72	3.16	99.14	1.81
2	Ethyl methanesulfonate	N.D	80.05	3.02	101.60	1.63
3	Isopropyl methanesulfonate	N.D	74.21	1.47	99.25	1.32
4	Methyl benzenesulfonate	N.D	74.99	3.65	88.33	0.33
5	Ethyl benzenesulphonate	N.D	74.78	3.53	93.58	1.73
6	Methyl p-toluenesulfonate	N.D	78.93	3.30	93.00	2.39
7	Ethyl p-toluenesulfonate	N.D	74.09	1.64	96.24	0.90
8	Isopropyl p-toluenesulfonate	N.D	79.72	1.16	88.97	1.81

*N.D = Not Detected

Conclusion

This application note describes the analysis of sulfonate esters genotoxic impurities in drugs by Shimadzu triple quadrupole gas chromatography mass spectrometer (GCMS-TQ8040). Linear correlation coefficients R value were greater than 0.999 for all the components over the range of 1 – 200 μg/L. The RSD% of the peak areas of each component for 6 injections were below 5%. The recoveries of each component were 74.21% - 81.72% and 88.33% - 101.60% at the spiked level of 1.0 μg/g and 10.0 μg/g respectively. The described method is highly sensitive, straightforward and easy to perform, and can serve as a reference for the detection of sulfonate esters in drugs.

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Application Data Sheet

No.41

GC-MS
Gas Chromatograph Mass Spectrometer

Analysis of Potential Genotoxic Impurities in Active Pharmaceutical Ingredients (3)
-Analysis of Haloalcohols and Glycidol Part 1-

Haloalcohols (Fig. 1) are used as synthetic materials in pharmaceuticals, and are considered potential genotoxic impurities (PGI). In addition, glycidol (Fig. 1) has been identified as a cancer-causing agent, and has been assigned to Group 2A (probably carcinogenic to humans) in terms of carcinogenic risk by the International Agency for Research on Cancer (IARC). This Application Data Sheet introduces analysis of haloalcohols and glycidol in an active pharmaceutical ingredient (API) using the GC-MS.

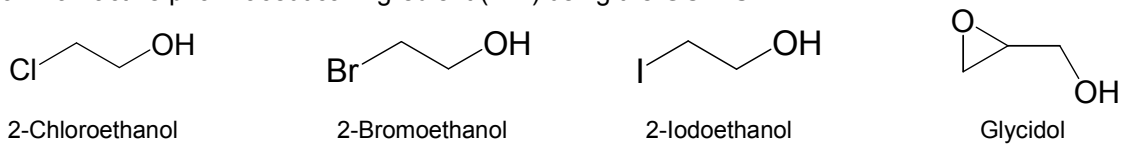

Fig. 1 Compound Structures of Typical Haloalcohols and Glycidol

Experimental

Many APIs are compounds with a high boiling point, and can cause GC-MS and column contamination; therefore, it is critical to extract the target compounds from the API matrix prior to analysis by GC-MS. Haloalcohols and glycidol are highly polar, making them difficult to extract with organic solvents. Accordingly, the target compounds were subjected to trimethylsilyl (TMS) derivatization before a solvent extraction was performed utilizing water and dichloromethane, thereby removing as much of the API as possible [1]. In addition, 1,1,2,2-bromoethanol-D4 was utilized as the internal standard substance, and 50 ng of that was added to 200 µL of solution. Fig. 2 shows the detailed pretreatment procedure.

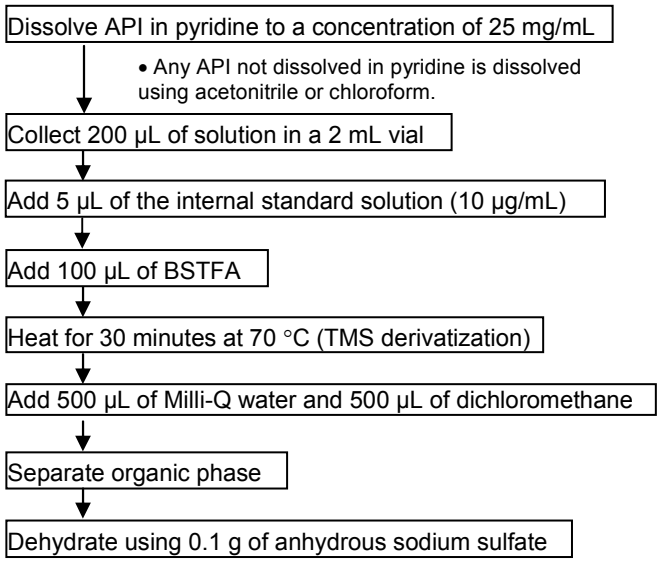

Fig. 2 Sample Preparation Procedure

Analytical Conditions

FASST (Fast Automated Scan/SIM Type), which is capable of simultaneous Scan and SIM measurements, was used as the measurement mode. The analysis conditions are shown in Table 1.

Table 1 Analytical Conditions

GC-MS	: GCMS-QP2010 Ultra
Column	: Rtx-200 (Length 30 m × 0.25 mm I.D., df = 0.25 µm)
Glass Liner	: Deactivated Split insert with glass wool (P/N: 225-20803-01)
[GC]	
Injection Temp.	:280 °C
Column Oven Temp.	:50 °C (5 min) → (10 °C/min) → 100 °C → (20 °C/min) → 320 °C (3 min)
Injection Mode	:Split
Flow Control Mode	:Linear velocity (32.4 cm/sec)
Split Ratio	:30
Injection Volume	:1.0 µL
[MS]	
Interface Temp.	:280 °C
Ion Source Temp.	:230 °C
Measurement Mode	:FASST (simultaneous Scan/SIM measurements)
Scan Mass Range	:*m/z* 30–450
Scan Event Time	:0.2 sec
SIM Event Time	:0.3 sec
SIM Monitoring *m/z*	:
2-chloroethanol-TMS	93, 95
2-bromoethanol-TMS	181, 183
2-bromoethanol-D4-TMS	187
Glycidol-TMS	101, 59
2-iodoethanol-TMS	185, 229

Results

Fig. 3 shows the total ion current chromatogram of a 25 µg/mL standard sample (equivalent to 1000 ng/mg in the pharmaceuticals), and Fig. 4 shows the scan mass spectra.

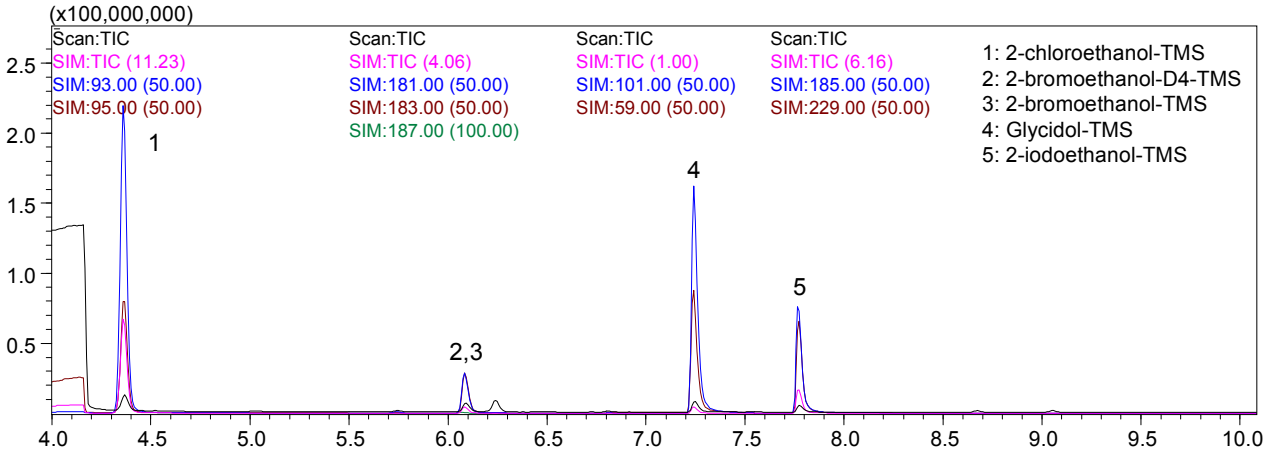

Fig. 3 Total Ion Current Chromatogram

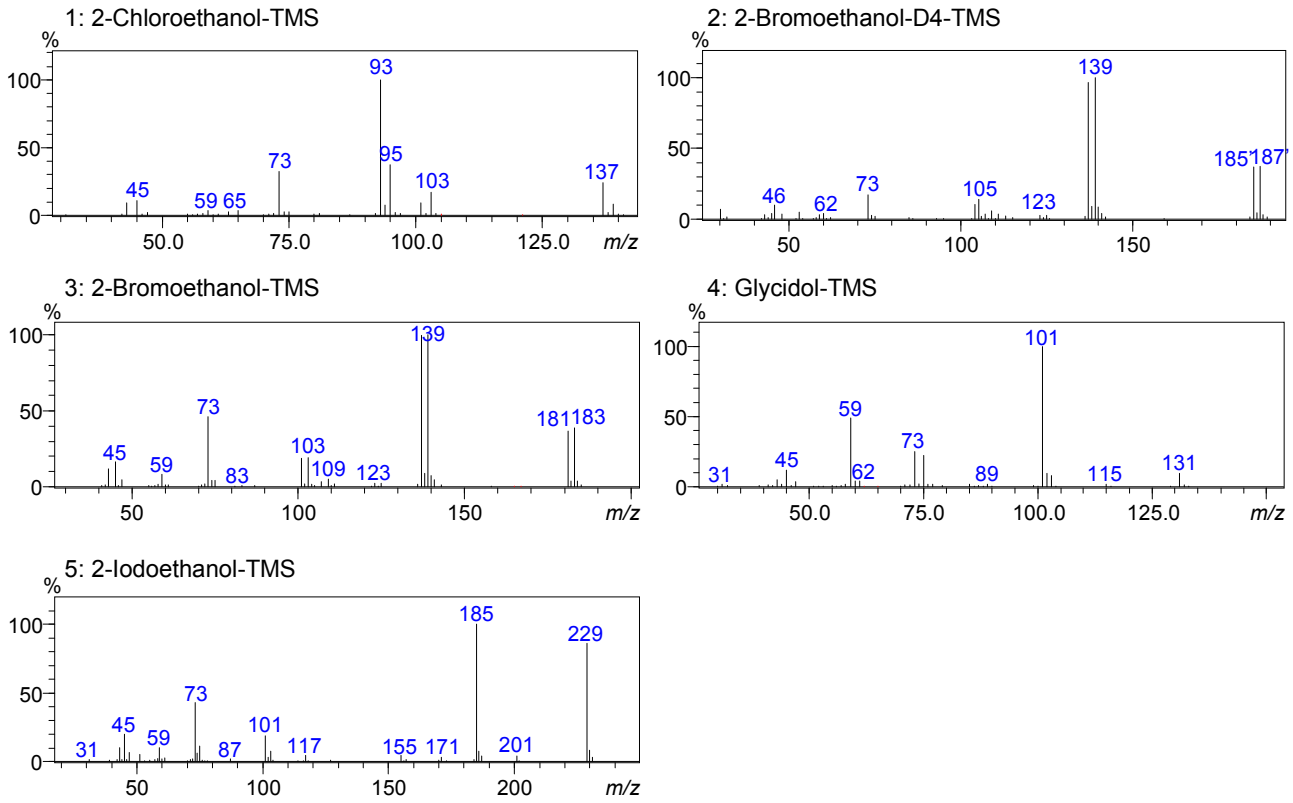

Fig. 4 Scan Mass Spectra of Haloalcohols and Glycidol

Reference

[1] Frank David, Karine Jacq, Pat Sandra, Andrew Baker and Matthew S. Klee: Analysis of potential genotoxic impurities in pharmaceuticals by two-dimensional gas chromatography with Deans switching and independent column temperature control using a low-thermal-mass oven module, Anal Bioanal Chem, 396, 1291-1300 (2010)

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Application Data Sheet

No.42

GC-MS
Gas Chromatograph Mass Spectrometer

Analysis of Potential Genotoxic Impurities in Active Pharmaceutical Ingredients (4)

- Analysis of Haloalcohols and Glycidol Part 2 -

This Application Data Sheet reports on results with respect to a method for quantitating haloalcohols (2-chloroethanol, 2-bromoethanol, and 2-iodoethanol) and glycidol in an active pharmaceutical ingredient (API) utilizing the GCMS system. For the analysis conditions as well as the total ion current chromatogram and mass spectra for the haloalcohols and glycidol, refer to GCMS Application Data Sheet No. 41, "Analysis of Potential Genotoxic Impurities in Active Pharmaceutical Ingredients (3), Analysis of Haloalcohols and Glycidol Part 1."

Experimental

The haloalcohols (2-chloroethanol, 2-bromoethanol, and 2-iodoethanol) and glycidol were dissolved in acetonitrile, and mixed standard solutions (0.025 µg/mL, 0.125 µg/mL, 0.25 µg/mL, 1.25 µg/mL, 2.5 µg/mL, and 25 µg/mL) were prepared. The 200µL of standards were extracted and derivatized as illustrated in Fig. 1[1]. The concentrations of these standard samples were equivalent to 1 ng/mg, 5 ng/mg, 10 ng/mg, 50 ng/mg, 100 ng/mg, and 1,000 ng/mg concentrations in the APIs.
In the recovery test, trazodone, which was confirmed not to contain the target compounds, was dissolved in chloroform and adjusted to 25 mg/mL. 200 µL was extracted, then 25 ng of the haloalcohols and glycidol respectively were added, as the pretreatment shown in Fig. 1. In this case, the concentrations of the haloalcohols and glycidol in the API were both 5 ng/mg.

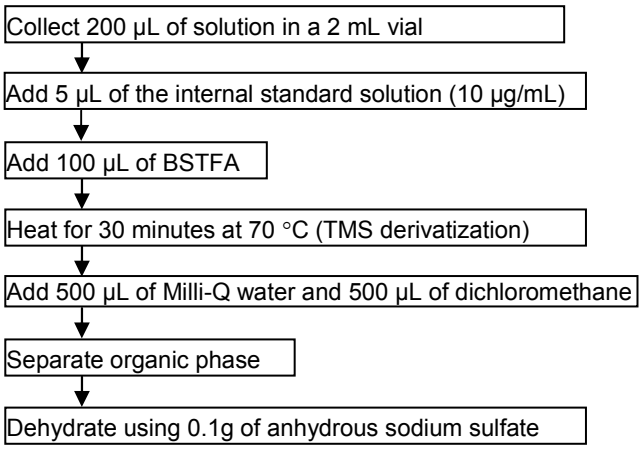

Fig. 1 Sample Preparation Procedure

Sensitivity

Fig. 2 shows the SIM mass chromatograms created by measuring a 0.025 µg/mL standard sample (equivalent to 1 ng/mg in the pharmaceuticals). For each of the compounds investigated, a sensitivity of S/N > 10 was obtained.

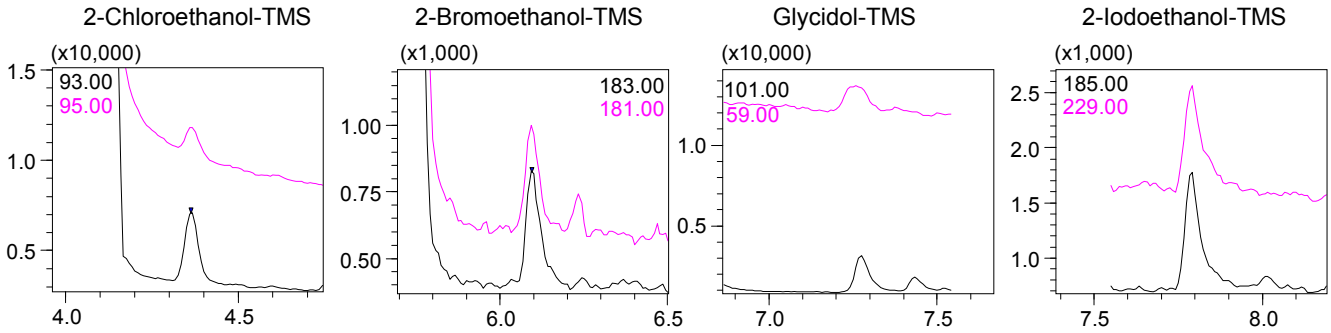

Fig. 2 SIM Mass Chromatograms for 0.025 µg/mL Standard Solution (equivalent to 1 ng/mg in the APIs)

Linearity of the Calibration Curve

Fig. 3 shows the calibration curves created in the concentration range of 0.025 μg/mL to 25 μmg/mL (equivalent to 1 ng/mg to 1,000 ng/mg in the API). The correlation coefficients (R) using 2-bromoethanol-D4-TMS as the internal standard were at least 0.9998, and favorable linearity was obtained.

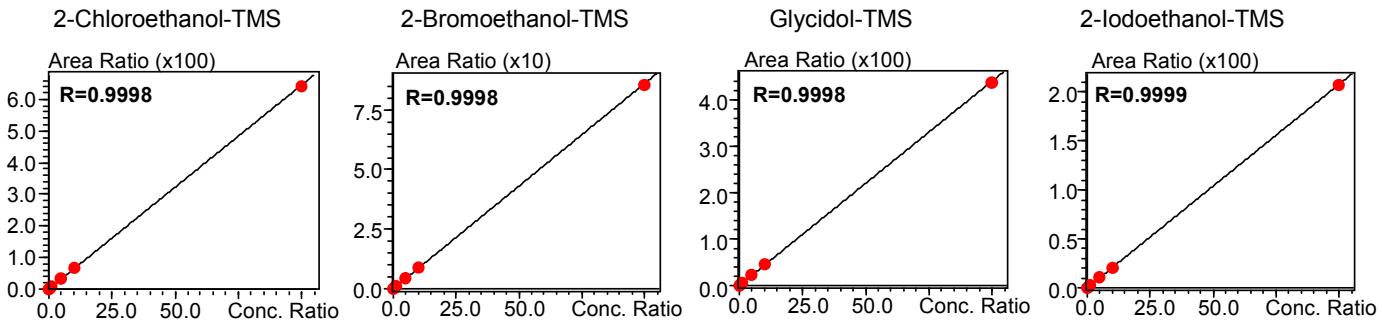

Fig. 3 Calibration Curves of Haloalcohols and Glycidol

Recovery Test

The recovery test was repeated 5 times, and the percent recovery and repeatability were calculated (Table 1). The average recovery for glycidol was poor at 59.7 %, but the recovery of the haloalcohols was at least 84.2 %. Favorable results were obtained, with repeatability (%RSD) of 4.3 % max. for 5 repetitions.

Table 1 Percent Recovery and Repeatability Results

Compound Name	Percent Recovery (%)					Average Recovery (%)	Repeatability %RSD
	No. 1	No. 2	No. 3	No. 4	No. 5		
2-Chloroethanol-TMS	94.6	89.0	89.1	87.0	91.6	90.2	3.2
2-Bromoethanol-TMS	102.7	98.3	99.9	98.4	104.1	100.7	2.6
Glycidol-TMS	60.9	61.7	61.9	56.4	57.4	59.7	4.3
2-Iodoethanol-TMS	84.1	85.3	82.7	82.5	86.4	84.2	2.0

Reference

[1] Frank David, Karine Jacq, Pat Sandra, Andrew Baker and Matthew S. Klee: Analysis of potential genotoxic impurities in pharmaceuticals by two-dimensional gas chromatography with Deans switching and independent column temperature control using a low-thermal-mass oven module, Anal Bioanal Chem, 396, 1291-1300 (2010)

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Application Data Sheet

No.46

GC-MS
Gas Chromatograph - Mass Spectrometer

Analysis of Potential Genotoxic Impurities in Active Pharmaceutical Ingredients (5) - Analysis of Alkyl Halides -

Alkyl halides are used as an alkylating agent for raw ingredients in the synthesis of pharmaceuticals or are generated as a byproduct of drug synthesis. They have been identified as potential carcinogens or genotoxins. This Application Data Sheet shows an example of analyzing 18 alkyl halides using headspace-GC-MS.

Experimental

Standard mixtures were prepared by diluting 18 types of alkyl halides in methanol to 0.2, 2, 10, 20, and 100 μg/mL concentrations. An internal standard solution was prepared by diluting fluorobenzene in methanol to a 20 μg/mL concentration. Test samples were prepared by placing 20 mg of the pharmaceutical ingredients in a 20 mL screw-cap vial (La-Pha-Pack P/N: 18 09 1307), diluting it with 10 mL of Milli-Q water, adding 10 μL of the internal standard solution, and then quickly sealing the vial by screwing on the magnetic screw-cap (La-Pha-Pack P/N: 18 09 1309). Standard aqueous samples were prepared by adding 10 μL of each standard alkali halide mixture and 10 μL of the internal standard solution to 10 mL Milli-Q water. The concentrations of the prepared standard aqueous samples were 0.2, 2, 10, 20, and 100 ng/mL (equivalent to 0.1, 1, 5, 10, and 50 ng/mg concentrations in the active pharmaceutical ingredients), respectively.

Analytical Conditions

FASST (Fast Automated Scan/SIM Type), which is capable of simultaneous Scan and SIM measurements, was used as the measurement mode. The analysis conditions are shown in Table 1.

Table 1: Analytical Conditions

GC-MS	:GCMS-QP2010 Ultra
Autosampler	:AOC-5000 Plus (HS)
Column	:Rtx-1 (60 m length, 0.25 mm I.D., df=1.0 μm)
Glass Insert	:Deactivated Split insert with wool (PN: 225-20803-01)

[AOC-5000 Plus (HS)]

Incubation Temp.	:80 °C
Incubation Time	:30 min
Syringe Temp.	:100 °C
Agitator Speed	:250 rpm
Fill Speed	:500 μL/sec
Pull Up Delay	:500 msec
Inject to	:GC Inj 1
Injection Speed	:500 μL/sec
Pre Inject Delay	:500 msec
Flush Time	:5 min
GC Run Time	:25 min
Injection Volume	:1 mL

[GC]

Injection Temp.	:230 °C
Column Oven Temp.	:40 °C (2 min) → (20 °C/min) → 250 °C (4 min)
Injection Mode	:Split
Carrier Gas	:Helium
Flow Control Mode	:Linear velocity (25.5 cm/sec)
Split Ratio	:10

[MS]

Interface Temp.	:230 °C
Ion source Temp.	:230 °C
Tuning Mode	:High sensitivity
Measurement mode	:FASST (simultaneous Scan/SIM measurements)
Scan Mass Range	:*m/z* 30 - 270
Scan Event Time	:0.05 sec
Scan Speed	:10,000 *u*/sec
SIM Event Time	:0.3 sec

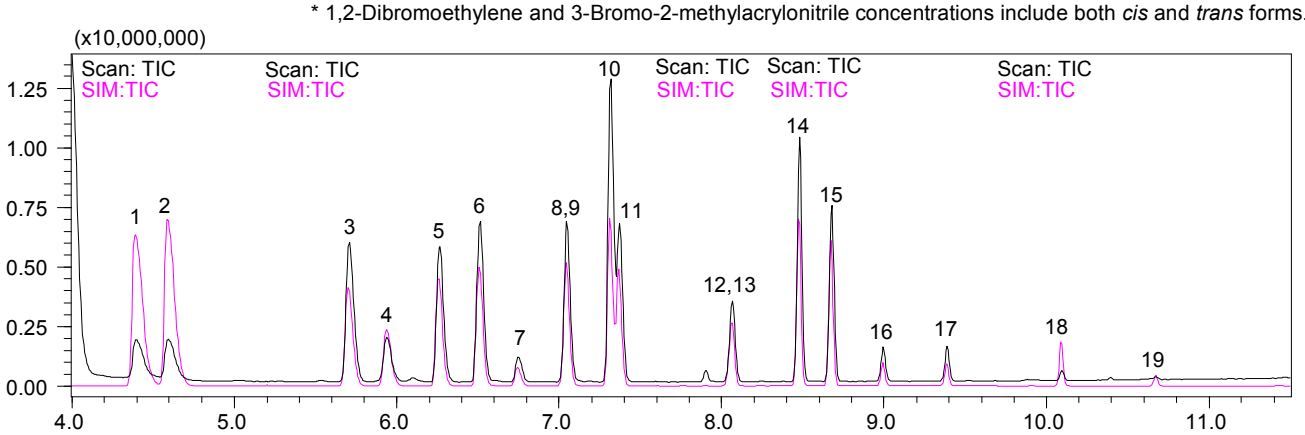
SIM Monitoring *m/z*:

Chloromethane	50, 52	1-Bromopropane	43, 122
Vinyl chloride	62, 64	2-Iodopropane	43, 170
2-Chloropropane	43, 78	Fluorobenzene	96, 70
Iodomethane	142, 127	1-Bromo-2-methylpropene	55, 134
1-Chloropropane	42, 78	1-Iodopropane	43, 170
trans-1,2-Dichloroethylene	61, 96	*trans*-1,2-Dibromoethylene	186, 105
2-Bromopropane	43, 122	*cis*-1,2-Dibromoethylene	186, 105
cis-Dichloroethylene	61, 96	*trans*-3-Bromo-2-methylacrylonitrile	66, 145
2-Chloroacrylonitrile	87, 52	*cis*-3-Bromo-2-methylacrylonitrile	66, 145
1-Chloro-2-methylpropene	55, 90		

Results

The total ion current chromatogram for the 100 ng/mL concentration standard aqueous solution (equivalent to 50 ng/mg concentration* in the active pharmaceutical ingredients) is shown in Fig. 3. The SIM chromatograms for six typical components in the 0.2 ng/mL concentration standard aqueous solution (equivalent to 0.1 ng/mg concentration* in the pharmaceutical) are shown in Fig. 4.

* 1,2-Dibromoethylene and 3-Bromo-2-methylacrylonitrile concentrations include both *cis* and *trans* forms.



ID	Component Name	Retention Time	ID	Component Name	Retention Time
1	Chloromethane	4.391	11	1-Bromopropane	7.367
2	Vinyl chloride	4.590	12	2-Iodopropane	8.049
3	2-Chloropropane	5.700	13	Fluorobenzene (ISTD)	8.068
4	Iodomethane	5.938	14	1-Bromo-2-methylpropene	8.476
5	1-Chloropropane	6.258	15	1-Iodopropane	8.672
6	*trans*-1,2-Dichloroethylene	6.507	16	*trans*-1,2-Dibromoethylene	8.994
7	2-Bromopropane	6.744	17	*cis*-1,2-Dibromoethylene	9.386
8	*cis*-Dichloroethylene	7.041	18	*trans*-3-Bromo-2-methylacrylonitrile	10.088
9	2-Chloroacrylonitrile	7.051	19	*cis*-3-Bromo-2-methylacrylonitrile	10.671
10	1-Chloro-2-methylpropene	7.313			

Fig. 3: Total Ion Current Chromatogram

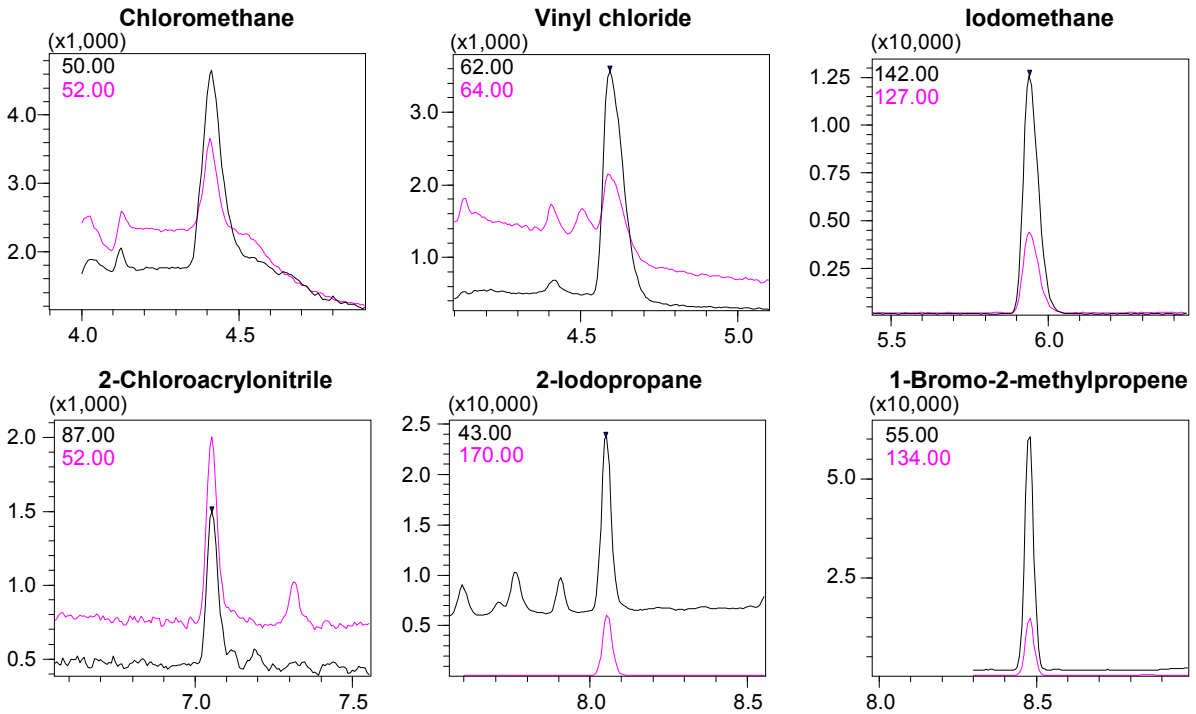


Fig. 4: Typical SIM Mass Chromatograms for 0.1 ng/mg Concentration in Active Pharmaceutical Ingredients

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Application News

No. SSL-CA14-333

Gas Chromatography Mass Spectrometry

Detection of 8 Volatile Nitrosoamines in Drugs by GC-MS/MS Method

Abstract

A method for the simultaneous analysis of 8 volatile nitrosoamines in drugs by GC-MS/MS was developed. Linear correlation coefficients were greater than 0.999 for all the components over the range of 10 – 200 µg/L. The recoveries of each component were 67.0% - 99.7% at the spiked level of 10.0 µg/g. This method can serve as a reference for the detection of volatile nitrosoamines in drugs.

Introduction

Genotoxic impurities refer to compounds that directly or indirectly damage cellular DNA and produce mutagenic and carcinogenic substances. Even at trace concentrations, genotoxic substances can cause damage to human genetic material, which can lead to gene mutations and may promote tumorigenesis. Due to its high toxicity, it poses a strong threat to the safety of medications. In recent years, trace amounts of genotoxic impurities have been found in listed drugs and many medical accidents have occurred. Cases of recalls have occurred from time to time, which has caused huge economic losses to the pharmaceutical factories.

Regulatory agencies such as EMA, ICH, and FDA have made clear regulatory requirements for these genotoxic impurities. At present, there are 1574 carcinogens in the genotoxicity list, of which benzopyrene, aflatoxin, azobenzenes, N-nitrosamines and other substances are highly genotoxic substances.

In this application, a Shimadzu GCMS-TQ8040 triple quadrupole mass spectrometer was used to establish a method for the simultaneous determination of eight volatile nitrosoamines in drugs. The described method is highly sensitive and simple. This can serve as a reference method for the detection of volatile nitrosoamines in drugs.

Experimental

Instrumentation and Analytical Conditions

Triple quadrupole mass spectrometer: GCMS-TQ8040, using the analytical conditions described in Table 1 and 2.

Table 1 Analytical Conditions of GCMS-TQ8040

GC-MS Conditions		
Column	:	SH-Rtx-Wax (30m x 0.25mm x 0.50 µm)
Column Temp. Program	:	50 °C (2 min) → 6 °C/min → 130 °C → 30 °C/min → 220 °C → 5 °C/min → 230 °C (10 min)
Injection Temp.	:	240 °C
Column flow rate	:	2 mL/min
Injection mode	:	Splitless (1 min)
High pressure injection	:	250 kPa (1 min)
Injection volume	:	1 µL
Ion source temp	:	200 °C
Interface temp	:	240 °C
Detector voltage	:	Tuning voltage +0.6 Kv
Acquisition Mode	:	MRM (acquisition parameters : are shown in Table 2).

Table 2 Retention time and MRM parameters of volatile nitrosoamines

No.	Compound Name	Abbreviation	CAS No.	Retention Time (min)	Quantitation Ion	CE	Qualification Ion	CE
1	N-nitrosodimethylamine	NDMA	62-75-9	5.826	74>44	21	74>42	7
2	N-nitrosodiethylamine	NDEA	55-18-5	7.398	102>85	5	102>57	13
3	N-Nitrosodi-n-propylamine	NDPA	621-64-7	10.276	130>113	5	130>88	5
4	N-Nitrosodi-n-butylamine	NDBA	924-16-3	13.911	158>99	9	158>141	5
5	N-Nitrosopiperidine	NPiP	100-75-4	13.952	114>84	9	114>55	20
6	N-Nitrosopyrrolidine	NPir	930-55-2	14.638	100>55	10	100>68	9
7	N-Nitrosomorpholine	NMor	59-89-2	15.669	116>86	5	116>56	12
8	N-Nitrosodiphenylamine	NDPhA	86-30-6	19.797	169>66	23	169>115	30

Sample preparation

The drug sample was ground into fine powder. 0.02 g of the sample drug powder was weighed and placed in a test tube. 2 mL of methanol/ethyl acetate (1:1) solution was added to the test tube. The mixture was vortexed for 2 min, followed by ultrasonicication for 15 min. The extracted mixture was filtered with 0.22 μm organic membrane, and then analyze using the GC-MS/MS.

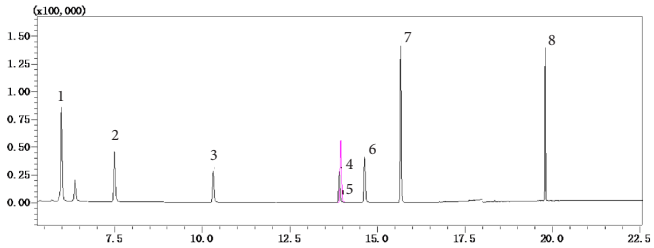

Fig. 1 MRM Chromatograms of standard samples (200 μg/L)

Results and Discussion

Standard chromatograms

The standard chromatogram is shown in Fig 1.

Calibration curves

A series of nitrosamine mixed standard solutions with concentrations of 10, 20, 50, 100, and 200 μg/L were prepared using a methanol/ethyl acetate (1:1) solution. The standard curves and MRM mass chromatograms (10 μg/L) are shown in Fig 2. The limits of detection (LOD) is calculated as a ratio of 3 times the signal-to-noise ratio. The standard curve equations, correlation coefficients, and LODs of the 8 volatile nitrosamine components are shown in Table 3.

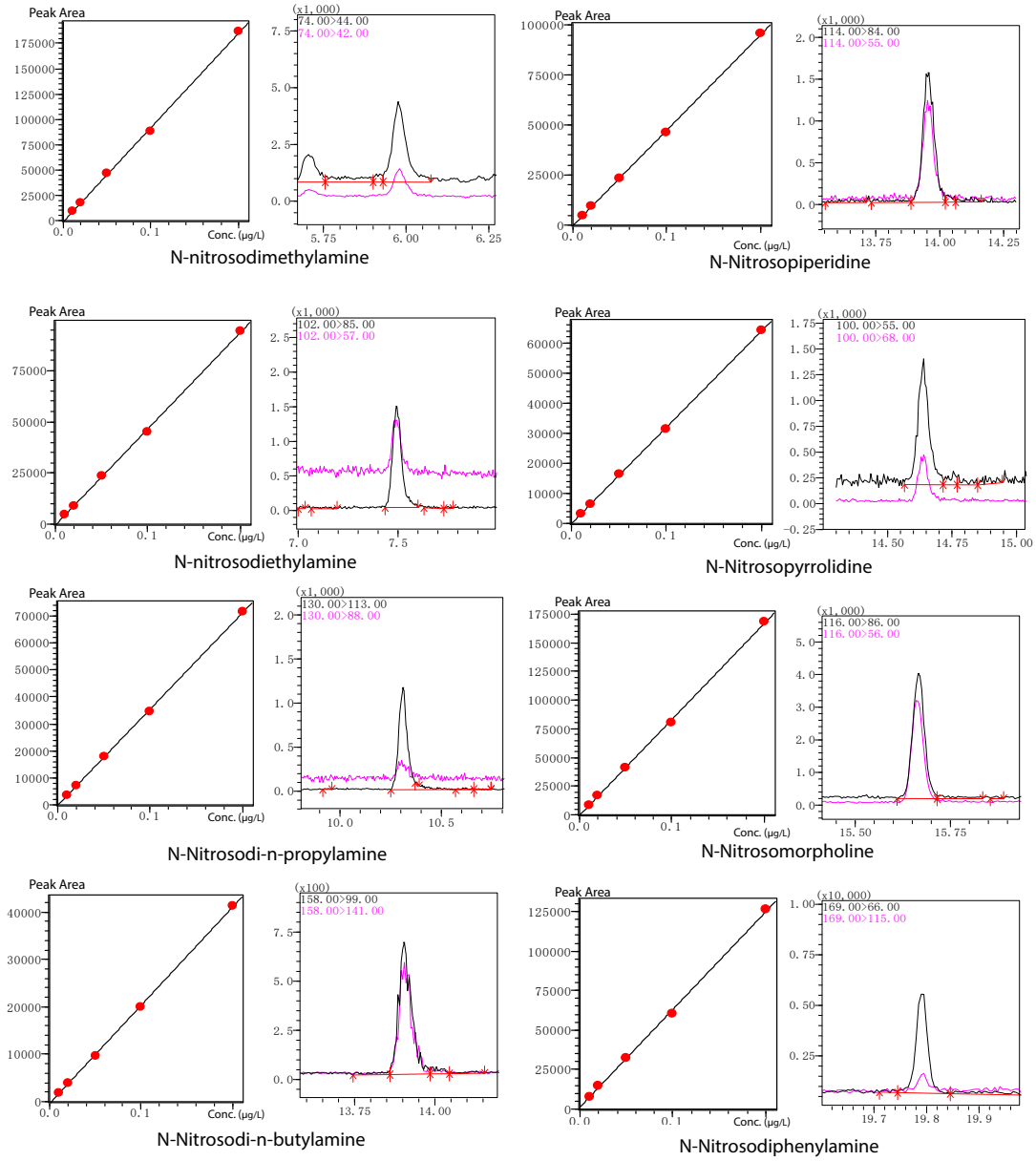

Fig. 2 The standard curves and MRM mass chromatogram (10 μg/L)

Table 3 The standard curve equations, correlation coefficients, LODs, and RSDs (n=6)

No.	Compound Name	Standard Curve Equation	Correlation Coefficient R	LOD (μg/L)	RSD% (10 μg/L)	
					R.T.	Area
1	N-nitrosodimethylamine	Y = 930458.0X - 1149.411	0.9996	0.36	0.08	2.73
2	N-nitrosodiethylamine	Y = 469824.6X - 495.6714	0.9997	0.09	0.07	2.46
3	N-Nitrosodi-n-propylamine	Y = 356113.6X - 116.6333	0.9998	0.07	0.06	2.97
4	N-Nitrosodi-n-butylamine	Y = 206974.8X - 407.4842	0.9998	0.17	0.06	2.54
5	N-Nitrosopiperidine	Y = 477009.0X - 292.2867	0.9998	0.12	0.03	3.12
6	N-Nitrosopyrrolidine	Y = 319466.1X - 15.82242	0.9998	0.56	0.03	2.06
7	N-Nitrosomorpholine	Y = 837290.9X - 609.3064	0.9997	0.07	0.02	1.30
8	N-Nitrosodiphenylamine	Y = 618388.8X + 1285.851	0.9994	0.22	0.01	1.96

Repeatability results

Repeatability was investigated by performing 6 injections of the 10 μg/L nitrosamines mixed standard solutions. The results are shown in Table 3.

Recovery results

Recovery was tested for both the fat-soluble drug and the water-soluble drug (n=6, for each drug). Eight types of volatile nitrosamine were added to the drugs, to give a spiked concentration of 2 μg/g for each nitrosamine. The sample pretreatment was performed for the recovery test and the results are shown in Table 4.

Sample Test

The fat-soluble drug and the water-soluble drug were analyzed, and all the 8 volatile nitrosamines were not detected in both samples. The MRM chromatograms are shown in Fig 3 and Fig 4.

Table 4 Results of the testing samples spiked at 2 μg/g

Compound name	Fat-soluble Drug (2 μg/g)		Water-soluble drugs (2 μg/g)	
	Average recovery (%)	RSD%	Average recovery (%)	RSD%
N-nitrosodimethylamine	74.0	5.53	67.0	9.86
N-nitrosodiethylamine	88.7	3.04	99.7	4.83
N-Nitrosodi-n-propylamine	83.4	1.76	93.4	4.74
N-Nitrosodi-n-butylamine	85.4	1.69	90.0	5.23
N-Nitrosopiperidine	83.7	1.89	87.1	4.89
N-Nitrosopyrrolidine	84.9	4.22	89.8	4.47
N-Nitrosomorpholine	86.6	2.14	85.7	4.64
N-Nitrosodiphenylamine	81.7	6.47	84.4	6.84

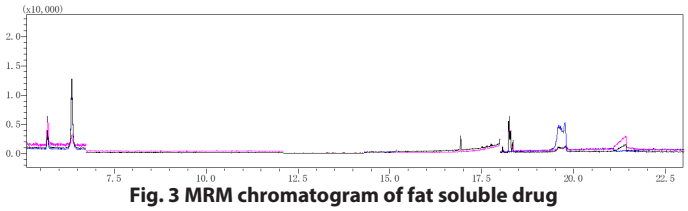

Fig. 3 MRM chromatogram of fat soluble drug

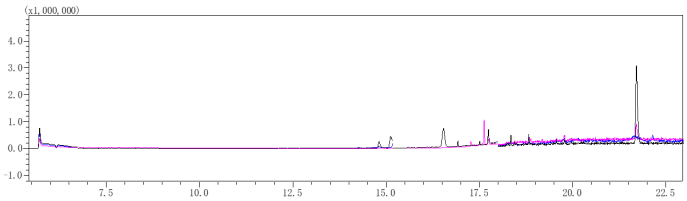

Fig. 4 MRM chromatogram of water-soluble drug

Conclusion

Analytical method of 8 volatile nitrosoamines in drug substances has been established by using Shimadzu triple quadrupole gas chromatography mass spectrometer (GCMS-TQ8040). This method requires simple pretreatment and gives good repeatability. The recoveries of eight volatile nitrosamines were between 67.0 and 99.7%. This method can serve as a reference for the detection of volatile nitrosoamines in drugs.

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Application News

No. L518

High Performance Liquid Chromatography

High Speed Analysis of Pharmaceutical Impurities in Compliance with European Pharmacopoeia Using Nexera-i MT

In recent years, the development of short-time analytical methods for improving analytical task efficiency and productivity is promoting the uptake of an ultra-high-speed analytical technology that uses UHPLC systems and columns packed with microparticles in research and development departments in the pharmaceutical field. This trend also applies to pharmacopoeia. For example, according to "Adjustment of chromatographic condition"[*1] described in the 8th edition of the European Pharmacopoeia (EP), adjustments to parameters in TLC, LC, GC and SFC are only allowed when the system suitability requirements are satisfied. In such a case, revalidation is not required.

This article introduces an example of high speed analysis of pharmaceuticals and related substances in compliance with the EP using the Nexera-i MT integrated high performance liquid chromatograph.

N. Iwata

Allowable Adjustment Range of HPLC

The LC section in "Adjustment of chromatographic condition" is broadly classified into isocratic elution and gradient elution.

For gradient elution, the allowable adjustment range of methods differs from that of isocratic elution because peak-shifting caused by unstable gradient profile of the mobile phase can lead to misidentification and overlapping of multiple peaks. For example, in terms of column particle size, while a reduction of up to 50% is possible for isocratic elution, particle size cannot be adjusted for gradient elution. Furthermore, in the case of gradient elution, it is stated that the elution time of the principal peak must be within ±15 % of that in the testing method. Thus, the adjustments of many parameters are restricted for gradient elution and further high speed analysis is practically impossible. Therefore high speed analysis can only be achieved for isocratic elution.

High Speed Analysis of Ivermectin and Related Substances

Ivermectin, belonging to macrolides, is known as a therapeutic drug for strongyloidiasis, an antiscabietic and an antiparasitic agent for animals. The two main components of ivermectin are H_2B_{1a} (molecular weight: 875) and H_2B_{1b} (molecular weight: 861). The former makes up more than 90 % of its composition.

Table 1 Analytical Conditions

System	:	Nexera-i MT
Column 1 (Conventional)	:	Shim-pack GIST C18 (250 mm L, 4.6 mm I.D., 5 µm)
Flow rate 1	:	1.0 mL/min
Column 2 (High speed)	:	Shim-pack GIST C18 (150 mm L, 4.6 mm I.D., 3 µm)
Flow rate 2	:	1.5 mL/min
Mobile phase	:	A) Water B) Methanol C) Acetonitrile A/B/C=15/34/51 (v/v/v)
Column temp.	:	25 °C
Injection volume	:	20 µL
Detection	:	UV254 nm

In this research we examined reducing the analysis time within the adjustment range allowed by the EP. Table 1 lists the analytical conditions that comply with both the ivermectin related substances testing section[*2] and the allowable adjustment range assigned in the EP. Since the Nexera-i MT used in analysis features both HPLC and UHPLC flow lines, it allows migration between conventional analysis and high speed analysis within a single system. The Shim-pack GIST C18 series was used for the analytical columns. The analytical conditions other than the analytical columns and flow rate are the same as those listed in the EP.

Fig. 1 shows resulting chromatograms of ivermectin standard solution (0.8 mg/mL). The high speed analysis provided approximately 60 % and 40 % reductions of analysis time and mobile phase consumption respectively while maintaining enough separation. Table 2 shows the results of system suitability test. Both conventional analysis and high speed analysis passed the test.

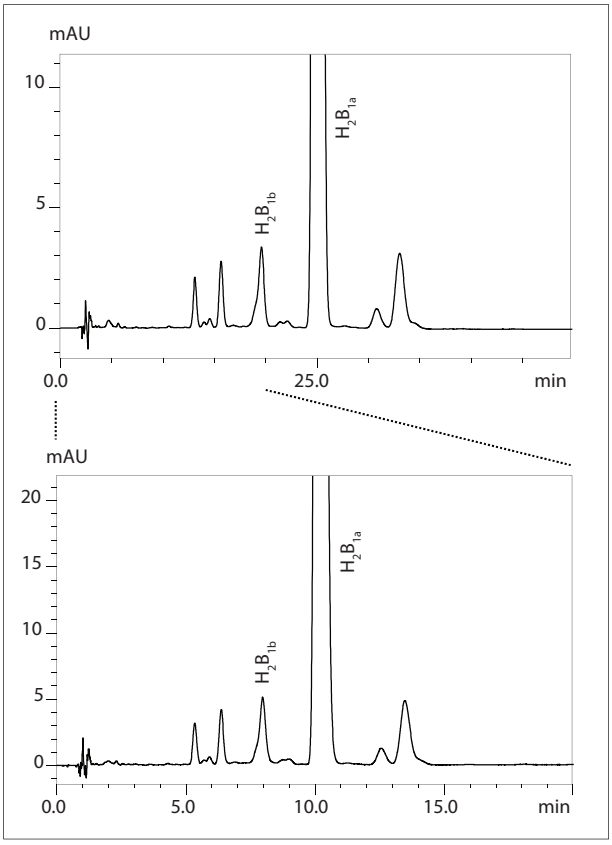

Fig. 1 Chromatograms of Ivermectin Standard Solution
Upper: Conventional Analysis Using HPLC Flow Line (Column 1)
Lower: High Speed Analysis Using UHPLC Flow Line (Column 2)

Table 2 Results of System Suitability Test

System suitability requirements		Results		Judgements
		Conventional	High speed	
Resolution (H_2B_{1b} and H_2B_{1a})	≥ 3.0	5.1	4.7	PASS
Signal-to-noise ratio (0.4 µg/mL)	≥ 10	40	38	PASS
Symmetry factor	≤ 2.5	1.1	1.2	PASS

High Speed Analysis of Diclofenac Sodium and Related Substances

Diclofenac is widely used as an antipyretic and a pain-reliever. Here we introduce an example of high speed analysis of a diclofenac sodium and related substances based on the EP.

Fig. 2 shows the resulting chromatograms of diclofenac standard solution (1.0 mg/mL). Table 3 lists the analysis conditions that comply with both the testing section[*3] of diclofenac sodium related substances and the allowable adjustment range assigned in the EP. The analytical columns used in conventional analysis and high speed analysis were both the same as those used in the analysis of ivermectin. A commercially-available reagent for system suitability testing was used as the reference standard.

In conventional analysis, the mobile phase flow rate assigned in the EP is 1.0 mL/min. Despite adjusting the flow rate to 0.8 mL/min in this research due to the column pressure tolerance, which is within the allowable adjustment range, the obtained results meet the system suitability requirements (Table 4). High speed analysis also passed the system suitability test. The high speed analysis provided approximately 70 % and 40 % reductions of analysis time and mobile phase consumption respectively while maintaining enough separation.

As demonstrated above, Nexera-i MT not only facilitated migration from conventional analysis to high speed analysis but also provided results of an equal level.

Table 3 Analytical Conditions

System	:	Nexera-i MT
Column 1 (Conventional)	:	Shim-pack GIST C18 (250 mm L, 4.6 mm I.D., 5 µm)
Flow rate 1	:	0.8 mL/min
Column 2 (High speed)	:	Shim-pack GIST C18 (150 mm L, 4.6 mm I.D., 3 µm)
Flow rate 2	:	1.4 mL/min
Mobile phase	:	A) Sodium phosphate buffer (pH 2.5) B) Methanol A/B=34/66 (v/v)
Column temp.	:	25 °C
Injection volume	:	20 µL
Detection	:	UV 254 nm

Table 4 Results of System Suitability Test

System suitability requirement		Results		Judgement
		Conventional	High speed	
Resolution (impurity F and Diclofenac)	≥ 4.0	6.8	5.1	PASS

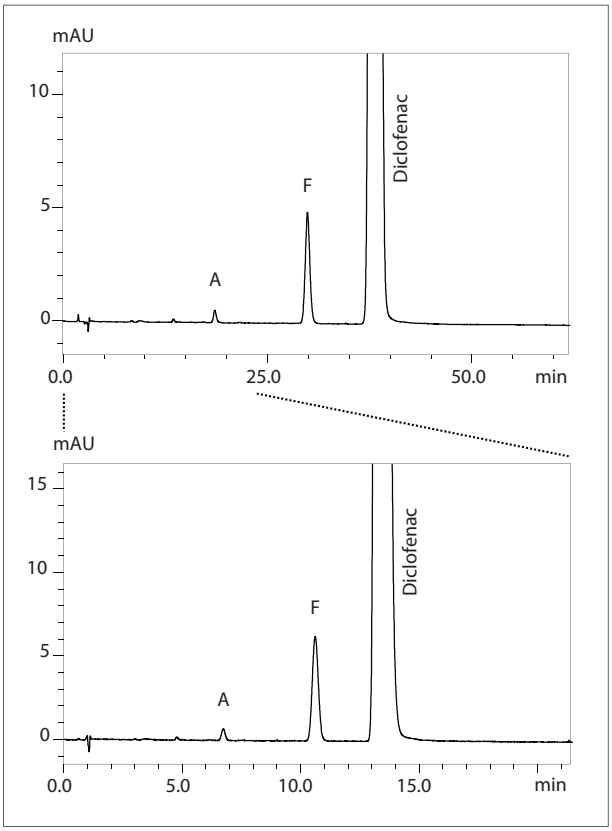

Fig. 2 Chromatograms of Diclofenac Standard Solution
Upper: Conventional Analysis Using HPLC Flow Line (Column 1)
Lower: High Speed Analysis Using UHPLC Flow Line (Column 2)

<References>
*1 European Pharmacopoeia 8.0, 04/2009:20246
2.2.46. Chromatographic separation techniques
*2 European Pharmacopoeia 8.8, 04/2016:1336 "Ivermectin"
*3 European Pharmacopoeia 8.8, 07/2014:1002 "Diclofenac sodium"

First Edition: Apr. 2017

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Application News

No. LC-15-ADI-036

Ultra fast Purification Liquid Chromatography

Isolation and identification of Atorvastatin degradation impurities by UFPLC

• Introduction:

Atorvastatin is an antilipemic drug belonging to the statins class, whose reference drug is Pfizer's Lipitor® (shown in Figure 1). It is used to reduce the levels of lipoproteins rich in cholesterol and reduce the risk of coronary artery disease. The drug in question is commonly sought after by pharmaceutical industries that produce generic drugs, due to the fact that the drug has a high value price, it is consumed globally, and its patent expired in late 2010. Atorvastatin has been found to degrade under acid and basic conditions.

Prominence UFPLC, Ultra Fast Preparative and Purification Liquid Chromatograph (Shown in Figure 2.), which enables fast recovery of highly purified target compounds from complex samples such as organic synthesis reaction mixtures and natural products. Prep LC is a widely used technique in many research development and manufacturing applications, including the synthesis of new drug compounds, the discovery of active components in natural products, and as a mechanism to collect large amounts of unknown compounds in foods and drugs for subsequent structural analysis.

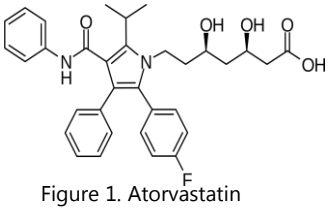


Figure 1. Atorvastatin

Figure 2. UFPLC

• Features

i. Comprehensive Automation of Preparative LC, Concentration, Purification, Elution, Collection and powderization only in 1.5 hours

- ✓Dedicated automation software to assist chemists in prep through collection
- ✓The time of evaporation can be reduced by up to 90% because of collection with organic solvent.

ii. High purity as a Free Base

- ✓Removal of counter ions derived from preparative mobile phase
- ✓De-salting and conversion to free base with Ammonia/Water

iii. Small footprint and Low-initial-cost

- ✓Your lab space can be kept with high functionality by small footprint
- ✓Available in two standard configurations to match your requirement
  - Standard System with one trapping column
  - Advanced System with five trapping columns

• Experimental:

Acid Degradation

200 mg of Atorvastatin API sample was dissolved in 10mL of methanol and added 10 mL of 0.1N Hydrochloric acid and kept at 80^{0}C for 1 hr. After the degradation added few ml of methanol to dissolve residue. This solution was used for analysis on UFPLC for fraction collection. Taken 10µL and diluted with 1mL of Acetonitrile : water (1:1) to make 200 ppm and then injected in HPLC

Analytical Conditions

Mobile phase A	: 0.1% TFAin water
Mobile phase B	: Acetonitrile
Gradient program	: (0.01/ 40, 10.00/50, 15.00/70 20.00/90, 25.00/90, 30.00/40 35.00/40) (Time in mins /B%
Column	: ShimPak C-18 (250X10mm, 5µ)
Flow Rate	: 5.0 ml/min
Wavelength	: 245 nm

• Preparation for Analysis

The degradation sample was diluted with methanol to make the clear solution. After dilution the sample concentration was 10 mg/ml. Before UFPLC analysis diluted samples were analyzed on Nexera system to check the extent of degradation. The fast method was developed on Nexera to check the purity of degradation samples and fractions collected by UFPLC.

• Results and discussion

Automation of Preparative LC, Concentration, Purification, Elution, Collection controlled by dedicated automation software assists chemists in clearly identifying the peaks which are trapped and collected in specific color code. 1D chromatogram is shown in Figure 3 and corresponding area percentages are given in Table 1.

Table 1:Area Percentage

Peak#	Name	Ret. Time	Area	Area%
1	Atorvastatin	4.421	14932410	27.214
2	Impurity H	5.449	17169678	31.292
3	Unknown imp	6.032	22767800	41.494

The UFPLC system is capable of trapping maximum 5 peaks in one injection run on 5 different trap columns. It also rinses the individual trap columns by different rinsing solution to remove salts. It ensures that the compound is in the form of free base before it elutes.

High retention capacity of trap columns can retain compounds of different polarity. Additionally, rinsing the column with an aqueous ammonia solution after trapping allows compounds to be recovered as free bases, which are generally easier to powederize and typically yields greater quality result when used in drug screening and pharmacokinetic studies.

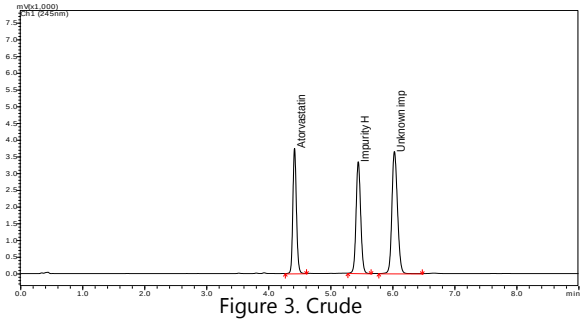


Figure 3. Crude

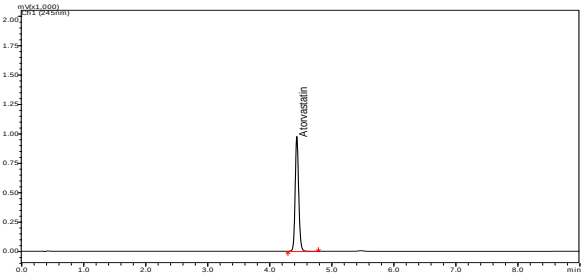


Figure 4. Peak 1

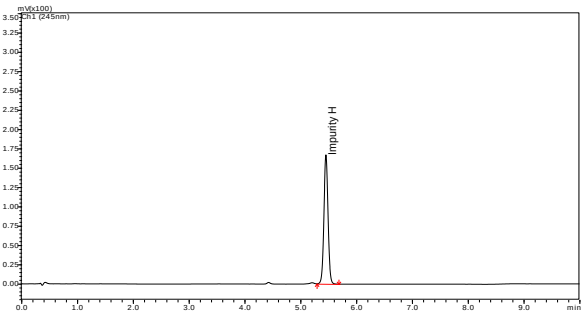


Figure 5. Peak 2

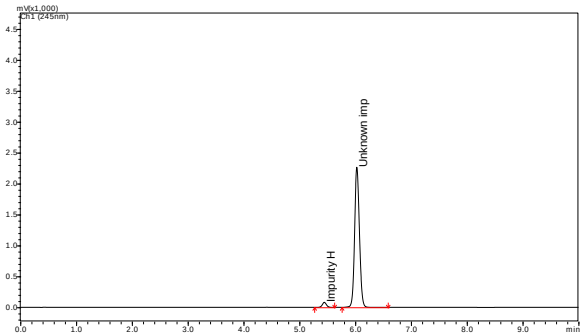


Figure 6. Peak 3

Atorvastatin degradation solution was injected on UFPLC to collect different impurity peak. The fractions were collected as free base after online rinsing and desalting. The collected fractions of individual peaks were injected on Nexera UHPLC system to check the purity. The individual chromatograms are shown in Figure 4,5 & 6. The degradation solution was also injected on LCMSMS as shown in Figure 7 to check the m/z of degradation impurities. The collected purified fractions were also injected on LCMS to confirm the m/z of the impurities.

The individual chromatograms are shown in Figure 8,9 & 10. The two degradation impurities showed m/z of 541.30 and 573.20. These peaks were further subjected to product ion scan to see the structural similarity between Atorvastatin and the degradation impurities (Figure 11 & 13). The fragmentation pattern (Figure 12 & 14) of both the impurities are identical after m/z 318 which indicates the structural similarities between them.

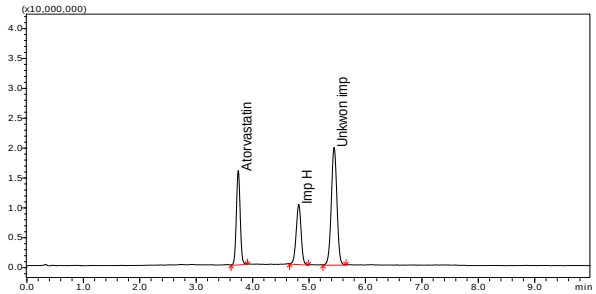


Figure 7. Extracted TIC of crude degradation sample

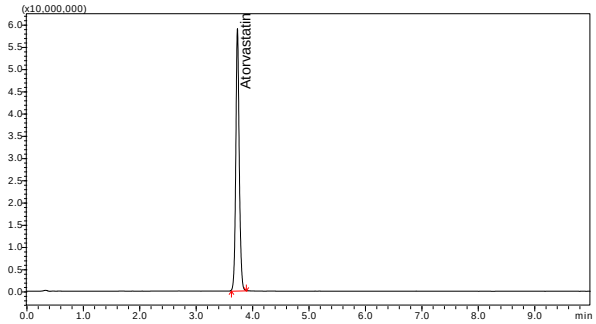


Figure 8. Extracted TIC of Atorvastatin

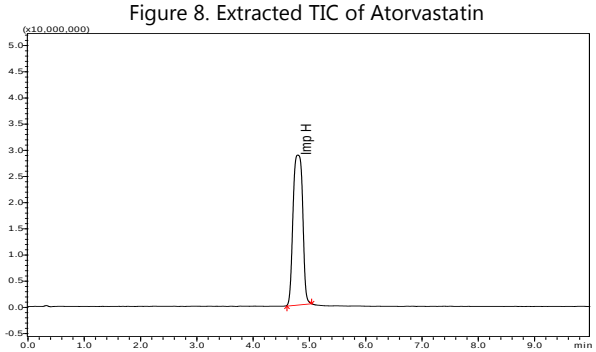


Figure 9. Extracted TIC of imp H

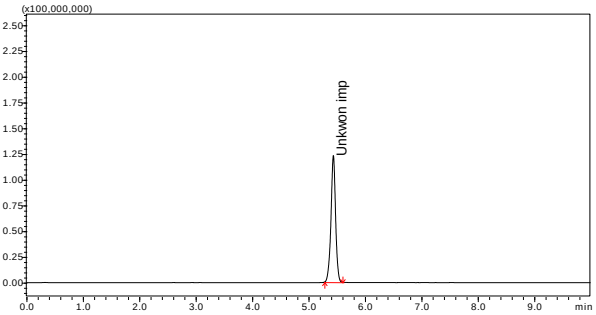


Figure 10. Extracted TIC of Unknown imp

The Prominence UFPLC system utilizes Shimadzu's proprietary purification technology that shortens the time required for fractionation, concentration, purification, and recovery, to about 90 minutes from the conventional eight hours or more (shown in figure 15). The system also enables the recovery of high-purity target compounds. The Prominence UFPLC greatly improves the efficiency of preparative fraction collection and purification workflows in pharmaceutical, food, chemical and other industries as well as research organizations.

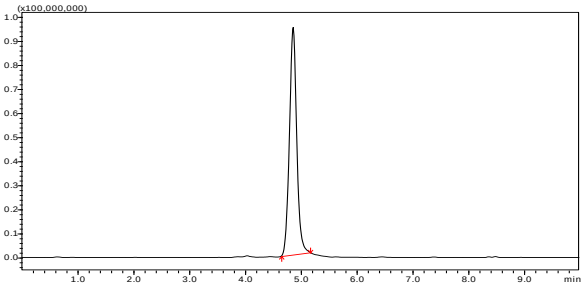


Figure 11. TIC of Product ion scan imp H

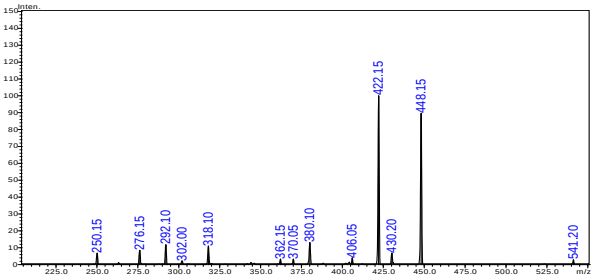


Figure 12. Spectra of Product ion scan imp H

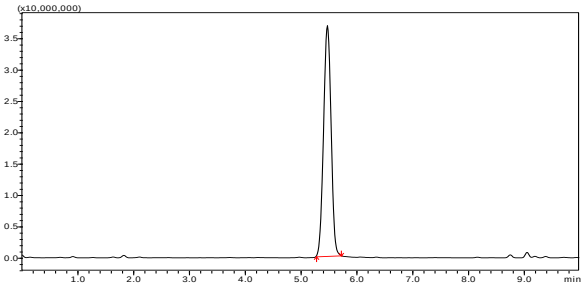


Figure 13. TIC of Product ion scan unknown imp

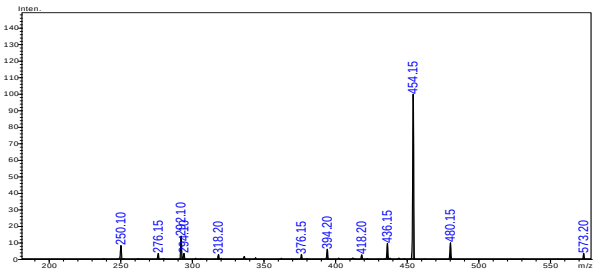
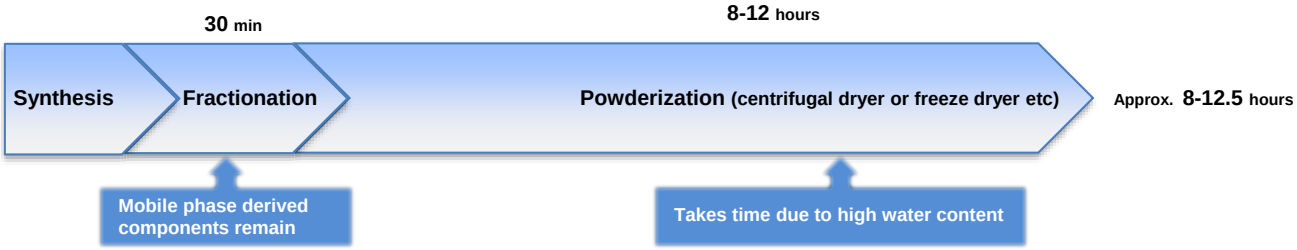


Figure 14. Spectra of Product ion scan unknown imp

>>Existing method



>>Prominence UFPLC

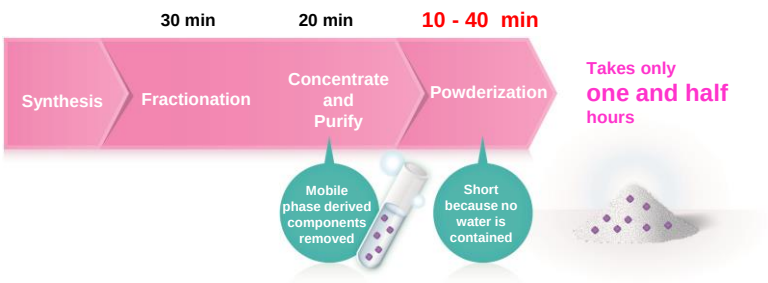
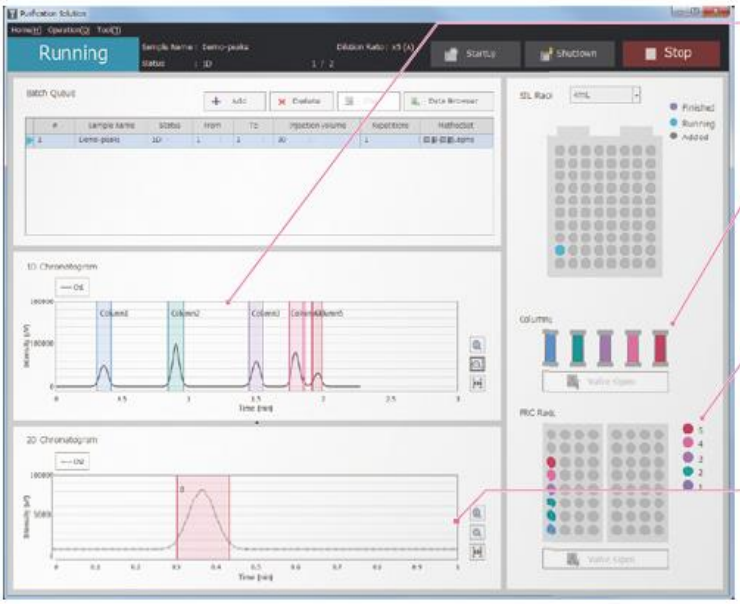


Figure 15. UFPLC purification cycle

With the dedicated Purification Solution software, the analysis status can be quickly confirmed at a glance using the peak tracking function.



Chromatogram

Individual fraction peaks are color-coded.

Trap Column

Trap columns are displayed with the same color as their corresponding faction peaks.

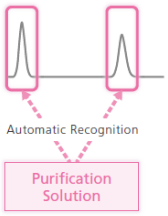
Fraction Collector

This displays which vial in the fraction collector was used to collect the eluate recovered from the trap column.

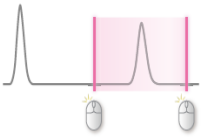
Elution Chromatogram

The chromatogram range is color-coded in accordance with the trap column and fraction collector vial display colors.

To ensure reliable fractionation and purification of precious samples, the Purification Solution software offers three fractionation modes



Automatic Fractionation Mode
In this mode, the software automatically identifies peaks and collects fractions based on parameter settings.



Manual Fractionation Mode
In this mode, the mouse pointer is used to fractionate peaks while viewing the window. When the same sample is concentrated by repeated injections, the first fractionation range is saved and the second and subsequent samples are automatically fractionated using the same fractionation range.

Time-Specified Fractionation Mode
This mode collects fractions based on the retention times in previously acquired data. It is ideal for routinely performed preparative purification processes.

- **Conclusion**

The Prominence UFPLC seamlessly integrates traditional Prep LC with novel fraction trapping for upto five compounds of interest. The instrument is controlled by a dedicated walk-up software designed to empower non-expert users to easily set conditions for chromatographic separation and isolation of target compounds, trapping, purifying, eluting and collecting highly purified compounds in as little as 90mins. For applications involving the isolation of low concentration targets, replicate injection and collection to the same trapping column to increase the amount of compound trapped on column prior to elution is easily accomplished.

The Prominence UFPLC eliminates some of the problems associated with conventional Prep LC, especially poor purity of collected compounds due to mobile phase additives, which become contaminants in the final collected fraction and inhibit powderization. Shimadzu's "Shim-pack C2P-H" trapping column strongly retains target compounds allowing unwanted organic solvents, water and additives to be flushed away in very quick time.

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Application News

No.C97

Liquid Chromatography Mass Spectrometry

Analysis of Impurities in Pharmaceutical Ingredients Using Trap-Free Two-Dimensional HPLC and Triple Quadrupole LC/MS/MS (LCMS-8040)

Controlling and confirming trace impurities contained in products in terms of the type, quantity, and safety has become an increasingly important issue to guarantee the product quality in a wide range of fields, including drugs (final formulations and raw materials, generic drugs), foods (health foods, supplements), and fine chemical products (solvents, paints, surfactants, many other synthetic products).
Mass spectrometers, such as triple quadrupole LC/MS/MS instruments, have been attracting attention as a useful means of measuring trace impurities in products. However, its widespread adoption has been complicated by the fact that HPLC-UV methods, which are commonly used for impurity analysis, use non-volatile mobile phase conditions incompatible with LC/MS analysis. To address this problem, laboratories have attempted to modify these methods to make them compatible with LC/MS. However, due to the risk and difficulty associated with changing method conditions, including changing the order of elution and missing impurities that elute near the principle component, very careful consideration is required.
In this report, we introduce an example of analysis in which trap-free two-dimensional HPLC was used to detect impurities using non-volatile mobile phase conditions, which were then converted without complication to volatile mobile phase conditions online to complete the analysis using the LCMS-8040 triple quadrupole mass spectrometer.

HPLC Analysis Using Non-Volatile Mobile Phase (1st Dimension)

A 1 mg/mL rabeprazole sodium test solution was prepared using commercially available laboratory reagents. The sample was then analyzed according to the method described in the Japanese Pharmacopoeia, shown in Table 1. Since phosphate buffer solution is used as the mobile phase, it cannot be introduced directly into the LC/MS.

Table 1 Analytical Conditions

Column	: Shim-pack VP-ODS (150 mm L. × 4.6 mm I.D., 4.6 µm)
Mobile Phase	: Methanol / 50 mmol/L Phosphate Buffer pH 7.0 (3/2)
Flowrate	: 1.0 mL/min.
Column Temp.	: 30 °C
Injection Volume	: 20 µL
Detection	: UV 290 nm

Analysis was conducted using an instrument configuration consisting of a combination of a trap-free two-dimensional HPLC and an LC/MS/MS, as shown in Fig. 1. The mobile phase flow direction differs depending on the valve position associated with each operation. Referring to Fig. 1, the non-volatile mobile phase flow line is indicated in red, the volatile mobile phase flow line in blue, and the impurity fraction peak capture loop in green.

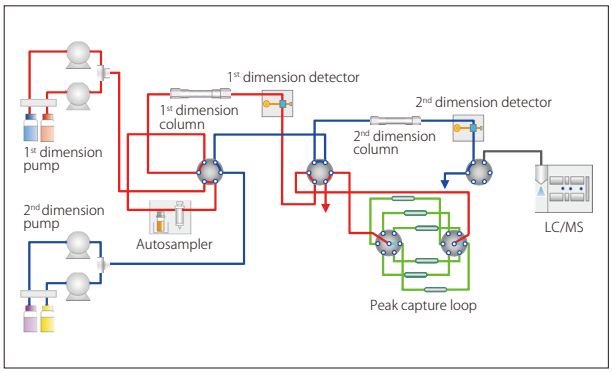

Fig. 1 Flow Diagram

This system was used to analyze a 1 mg/mL rabeprazole sodium solution. The obtained UV chromatogram is shown in Fig. 2. The principle component, rabeprazole, eluted at 5.3 minutes, and several impurity peaks are noticeable in that vicinity before and after that peak. Of these, the four impurity substances shown in the figure were fractionated using the peak capture loop.

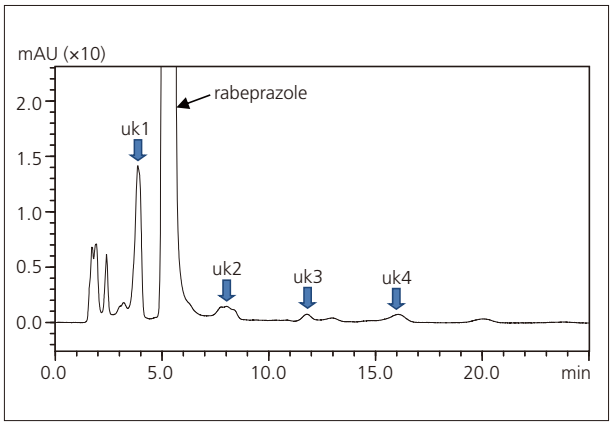

Fig. 2 UV Chromatogram of Rabeprazole Sodium (1st Dimension)

LC/MS Analysis Using MS Compatible Mobile Phase (2nd Dimension)

The impurities (uk1 – uk4) fractionated in the peak capture loop are forced out of the loop by the MS compatible volatile mobile phase due to switching of the valve position and activation of the second dimension pump to introduce each of the peaks into the LC/MS. The conditions used in the second dimension are shown in Table 2.

Table 2 Analytical Conditions (2nd Dimension)

Column	: Shim-pack XR-ODS (50 mm L. × 2.0 mm I.D., 2.2 µm)
Mobile Phase A	: 5 mmol/L Ammonium Acetate – Water
Mobile Phase B	: Methanol
Flowrate	: 0.2 mL/min.
Column Temp.	: 30 °C
Injection Volume	: 20 µL (Loop Volume)
Detection	: UV 290 nm, MS Q3scan (Positive and Negative Mode)

By comparing the UV chromatograms associated with the respective blank measurements and sample measurements, as shown in the LabSolutions LCMS data browser of Fig. 3, it is possible to gain a clear understanding of the elution positions of the target substances from the second dimension column. Further, by examining ions observed in the Q3 analysis results at specific peak elution times, it is possible to deduce the molecular weight of each of the target impurities. In the case of uk-1, as a peak without blank data, *m/z* 376 is observed as positive, and *m/z* 374 is observed as negative, verifying that uk-1 is an impurity with a molecular weight of 375. Table 3 summarizes the results obtained from analysis of the LC/MS data for each impurity.

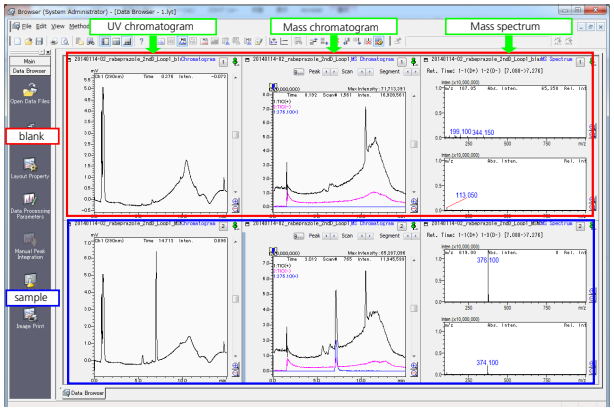

Fig. 3 Analysis Example Using Data Browser (uk-1)

Table 3 Analytical Results of Respective Impurities by LC/MS

compounds	content percentage (%)	*m/z*		deduced MW
		positive	negative	
rabeprazole	99.045	360.20	358.15	359
uk-1	0.433	376.15	374.15	375
uk-2	0.081	394.15	392.10	393
uk-3	0.023	344.20		343
uk-4	0.046	270.20		269

Also, when LC/MS/MS is used, not only can impurity molecular weight information be obtained, but by comparing the fragmentation patterns of the principal component and impurities following the product ion scan, it is possible to predict impurity structures. One example of this is seen in Fig. 4, which shows the product ion scan results for the principal component and uk-1. As the molecular weight of uk-1 was determined from the Q3 scan results to be 375, the mass difference between that and the principal component rabeprazole becomes 16 Da.

Furthermore, by comparing the two product ion scans, it was clear that many of the fragment ions or cleavage positions were the same. From this, it was obvious that uk-1 had a structure similar to that of the principle component. Furthermore, the product ions enclosed in red were specifically observed to have a molecular mass difference of 16 Da. At the position where this ion appears, it is possible to predict the difference in structure. Fig. 4 shows the predicted structure of uk-1.

Thus, by combining trap-free two-dimensional HPLC and LC/MS/MS, it was possible to identify and predict the structure of an impurity peak with high accuracy. At the same time, the non-volatile mobile phase conditions used in the Japanese Pharmacopoeia method could be retained when coupled with LC/MS compatible conditions by the system.

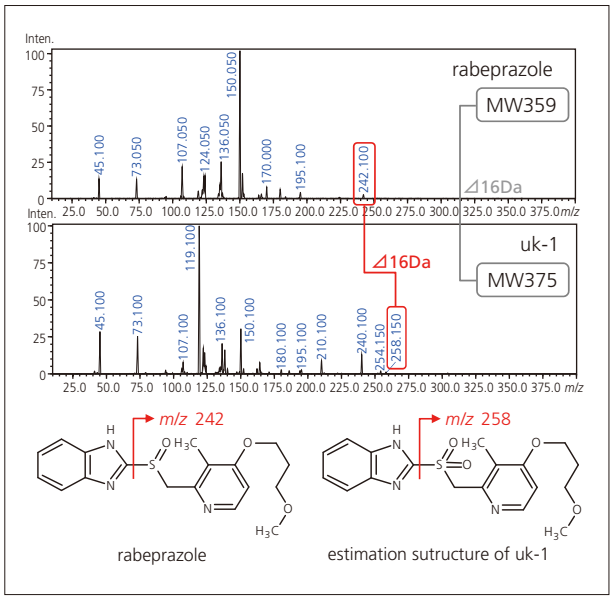

Fig. 4 MS/MS Analysis of Rabeprazole and uk-1

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Application News

No. G290

Gas Chromatograph

Analysis of Residual Solvents in drug products using Nexis GC-2030 combined with HS-20 head space sampler - USP <467> Residual Solvents Procedure A -

Residual solvents in pharmaceuticals are defined as volatile organic compounds used in or generated from the manufacture of drug substances, pharmaceutical additives, or drug products. They are strictly controlled according to risk classifications from Class 1 to Class 3, which are based on the risk to human health.

Headspace GC methods specified in the USP (U.S. Pharmacopeia), General Chapters <467> Residual Solvents, are commonly used for analysis of residual solvents. This Application News presents data obtained using the Shimadzu HS-20 Headspace Sampler and Nexis GC-2030 Gas Chromatograph, from Class 1 and Class 2 standard solutions, in accordance with Water-Soluble Articles, Procedure A, in USP <467> Residual Solvents.

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Fig. 1 Nexis GC-2030 + HS-20

Class1

Fig.2 shows the Class 1 standard solution chromatogram. Procedure A requires that the S/N ratio obtained for 1,1,1-Trichloroethane in this chromatogram be 5 or higher. As shown, the S/N ratio was 220. Even for carbon tetrachloride, which had the lowest sensitivity level, the S/N was 20.

Table 3 indicates the S/N ratio of each peaks and the repeatability of the peak area (n=6).

Table 3 S/N ratio and Repeatability of Class1

No.	Compounds	S/N ratio	%RSD (n=6)
1	1,1-Dichloroethane	320	2.8
2	1,1,1-Trichloroethane	220	2.3
3	Carbon tetrachloride	20	2.9
4	Benzene	170	2.5
5	1,2-Dichloroethane	60	3.4

Instruments and Analytical Conditions

Table 1 GC Method for USP 467 Procedure A

Model	: Nexis GC-2030
Detector	: FID-2030
Headspace Sampler	: HS-20
Column	: SH-Rxi-624 Sil MS (0.32 mm I.D. × 30 m, d.f. = 1.8 µm)
Column Temperature	: 40 °C (20 min) - 10 °C /min - 240 °C (20 min) Total 60 min
Injection Mode	: Split 1 : 5
Carrier Gas Controller	: Constant Linear Velocity (He)
Linear Velocity	: 35 cm/sec
Detector Temperature	: 250 °C
FID H2 Flow Rate	: 40 mL/min
FID Make up Flow Rate	: 30 mL/min (He)
FID Air Flow Rate	: 400 mL/min
Injection Volume	: 1 mL

Table 2 HS-20 Method for USP 467 Procedure A

Oven Temperature	: 80 °C
Sample Line Temperature	: 110 °C
Transfer Line Temperature	: 120 °C
Vial Stirring	: Off
Vial Volume	: 20 mL
Vial Heat-retention Time	: 60 min
Vial Pressurization Time	: 1 min
Vial Pressure	: 75 kPa
Loading Time	: 1 min
Needle Flush Time	: 5 min

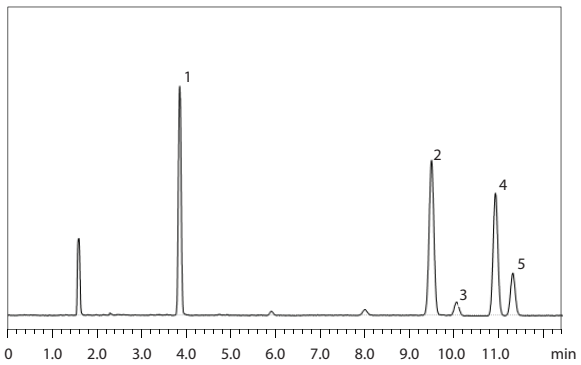
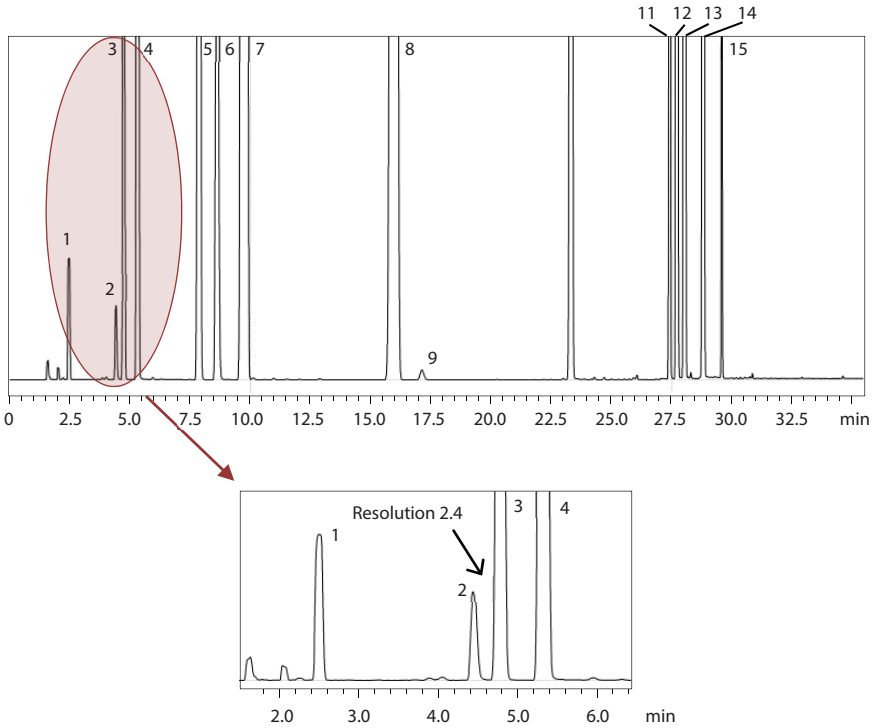


Fig. 2 Chromatogram of WATER-SOLUBLE ARTICLES Class1 Standard Solution by Procedure A

Class2

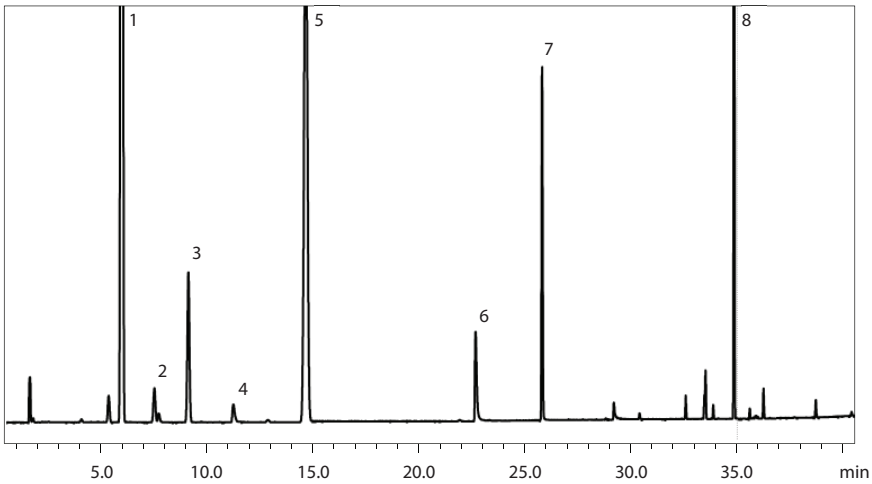
Due to the large number of components in the Class 2 standard solution, it was separated into two mixtures: A and B. Respective measurement results are shown in Fig.3 and Fig.4. Procedure A requires that the resolution for acetonitrile and methylene chloride in the Class 2 standard solution Mixture A chromatogram be 1.0 or greater.

Fig.3 shows that, using the Restek SH-Rxi-624SilMS low-bleed column, the specified peaks are completely separated, with a resolution of 2.4.



1: Methanol
2: Acetonitrile
3: Methylene chloride (DCM)
4: trans-1,2-Dichloroethylene
5: cis-1,2- Dichloroethylene
6: Tetrahydrofuran
7: Cyclohexane
8: Methylcyclohexane
9: 1,4-Dioxane
10: Toluene
11: Chlorobenzene
12: Ethylbenzene
13: m,p-Xylene
14: o-Xylene
15: Cumene

Fig. 3 Chromatogram of WATER-SOLUBLE ARTICLES Class 2A Standard Solution by Procedure A



1: Hexane
2: Nitromethane
3: Chloroform
4: 1,2-Dimethoxyethane
5: Trichloroethene
6: Pyridine
7: Methylbutylketone
8: Tetraline

Fig. 4 Chromatogram of WATER-SOLUBLE ARTICLES Class 2B Standard Solution by Procedure A

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Application News

No.M272

Gas Chromatography Mass Spectrometry

Analysis of Residual Solvents in Pharmaceuticals Using Headspace GC-FID/MS Detector Splitting System

Headspace gas chromatography with flame ionization detection (GC-FID) is often used for residual solvent testing of pharmaceuticals, though the qualitative power of this method is not particularly high. Because gas chromatography mass spectrometry (GC/MS) utilizes MS to perform qualitative analysis based on mass spectra, GC/MS can be used to estimate and identify individual peaks detected in the expected vicinity of a target solvent as well as other unknown peaks.
We describe an example of residual solvent test of a pharmaceutical using a detector splitting system that simultaneously obtains FID and MS data in a single measurement.

Sample Preparation

According to Water-Soluble Articles, Procedure A, in USP <467>, we prepared a class 1 standard solution, class 2 standard solution A, class 2 standard solution B, test solution, and class 1 system suitability solution. An active pharmaceutical ingredient was used for the test solution sample.

Analytical Conditions

The image of the Shimadzu GCMS-QP2020/FID detector splitting system is shown in Fig. 1, and analytical conditions are shown in Table 1. Headspace conditions were determined based on USP <467>. The column outlet was split between FID and MS, and MS was performed in scanning mode. Using Shimadzu's Advanced Flow Technology Software to determine the splitting ratio, the flowrate ratio was optimized to FID:MS of 1:1.

Table 1 Analytical Conditions

Headspace Sampler	: HS-20
GCMS	: GCMS-QP2020
Hydrogen Flame Ionization Detector Splitting System	: FID-2010Plus
HS	
Mode	: Loop (volume 1 mL)
Oven Temp.	: 80 °C
Sample Line Temp.	: 90 °C
Transfer Line Temp.	: 105 °C
Gas Pressure for Vial Pressurization	: 76.4 kPa
Vial Equilibrating Time	: 45 min
Vial Pressurizing Time	: 2.0 min
Pressure Equilibrating Time	: 0.1 min
Load Time	: 0.5 min
Load Equilibrating Time	: 0.1 min
Injection Time	: 0.5 min
Needle Flushing Time	: 15.0 min
APC Pressure	: 20 kPa
GC	
Column	: SH Rxi-624sil MS (30 m × 0.32 mm I.D., 1.8 µm)
Injection Mode	: Split (split ratio 1:5)
Control Mode	: Constant Pressure (89.4 kPa)
Carrier Gas	: He
Oven Temp.	: 40 °C (20 min) → 10 °C/min → 240 °C (20 min)
Restrictor (FID)	: 1.1 m × 0.25 mm
Restrictor (MS)	: 1.5 m × 0.20 mm
APC Pressure	: 20 kPa
FID	
Temp.	: 250 °C
Make-Up Flowrate	: 30 mL/min (He)
Hydrogen Flowrate	: 40 mL/min
Air Flowrate	: 400 mL/min
MS	
Ion Source Temp.	: 200 °C
Interface Temp.	: 250 °C
SCAN Range	: *m/z* 29 to 250
Event Time	: 0.3 sec

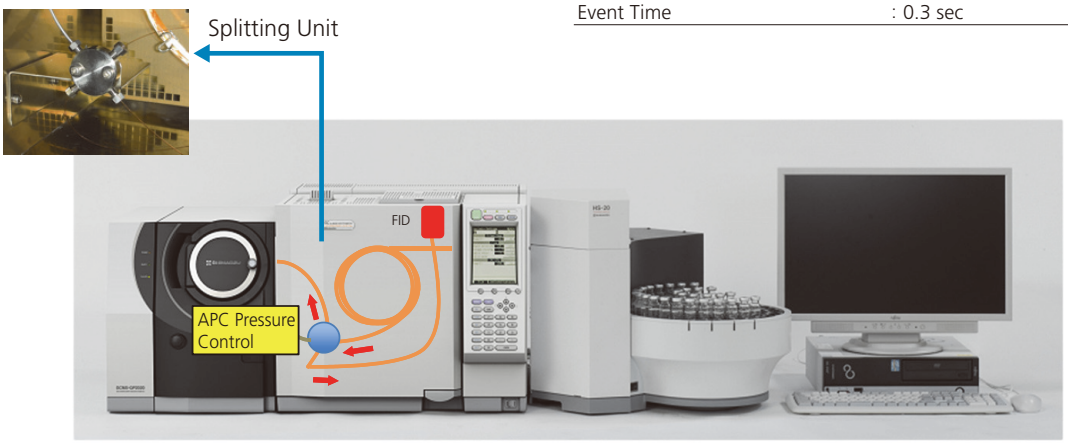

Fig. 1 System Image

Results

Fig. 2 to 5 show the FID and MS chromatograms obtained for class 1 standard solution, class 2 standard solution A, class 2 standard solution B, and class 1 system suitability solution.

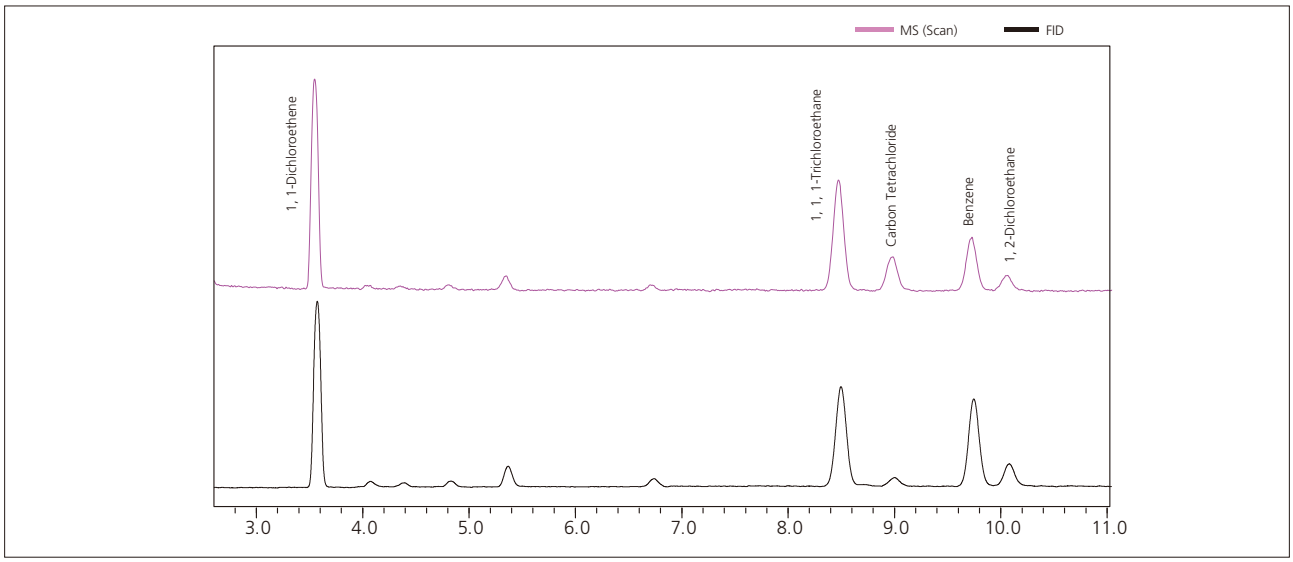

Fig. 2 Chromatograms of Class 1 Standard Solution

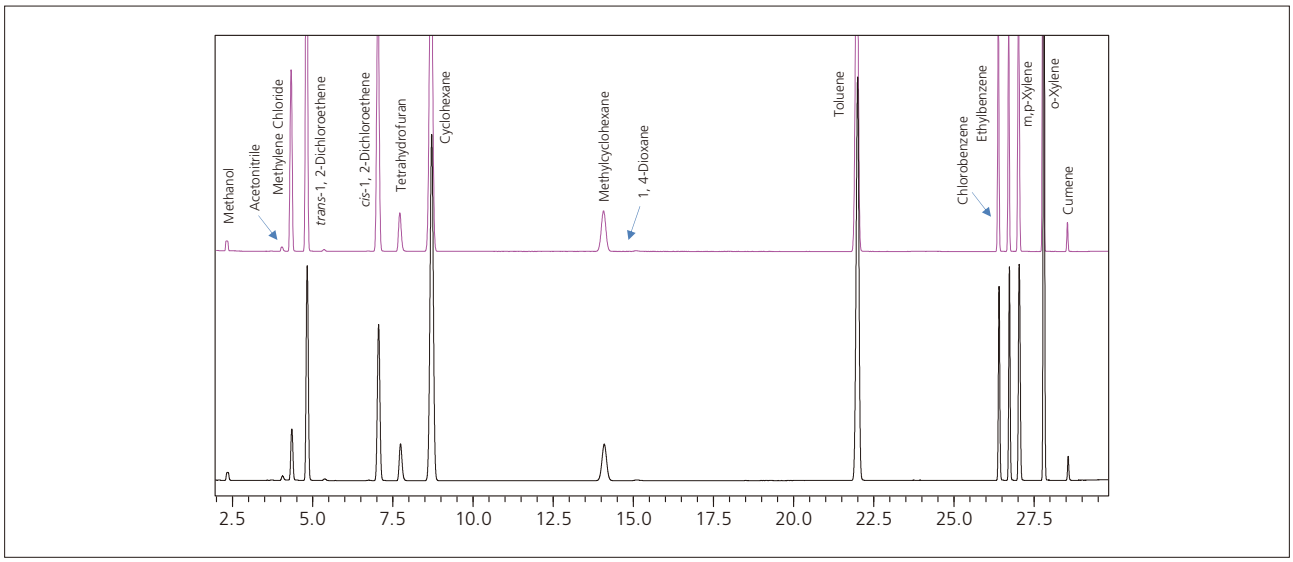

Fig. 3 Chromatograms of Class 2 Mixture A Standard Solution

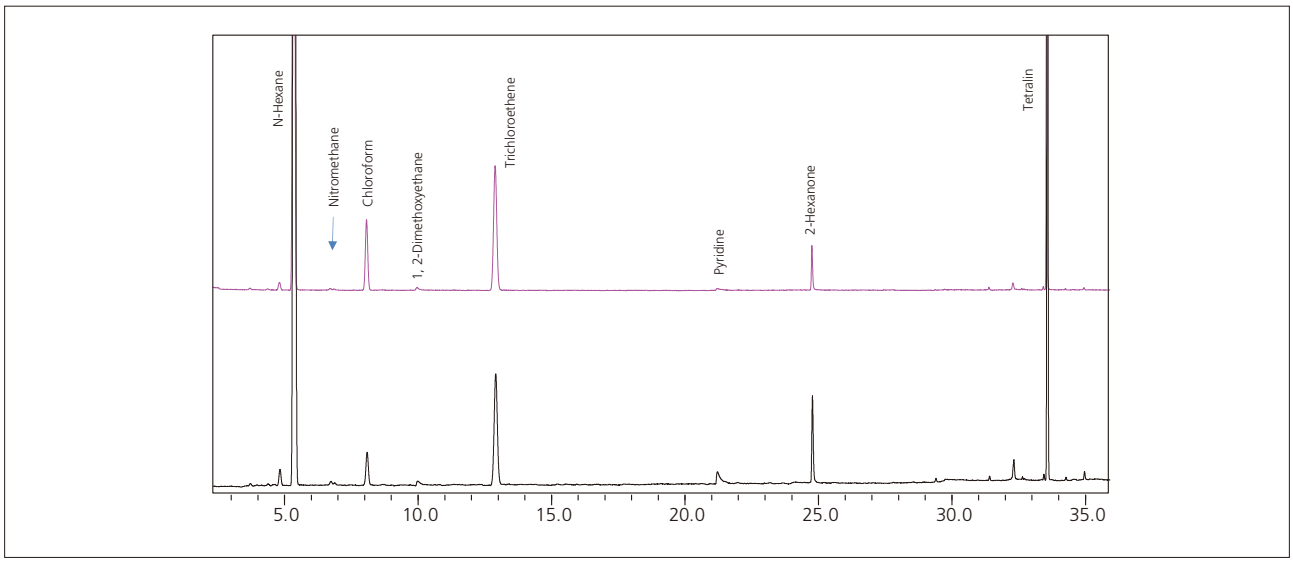

Fig. 4 Chromatograms of Class 2 Mixture B Standard Solution

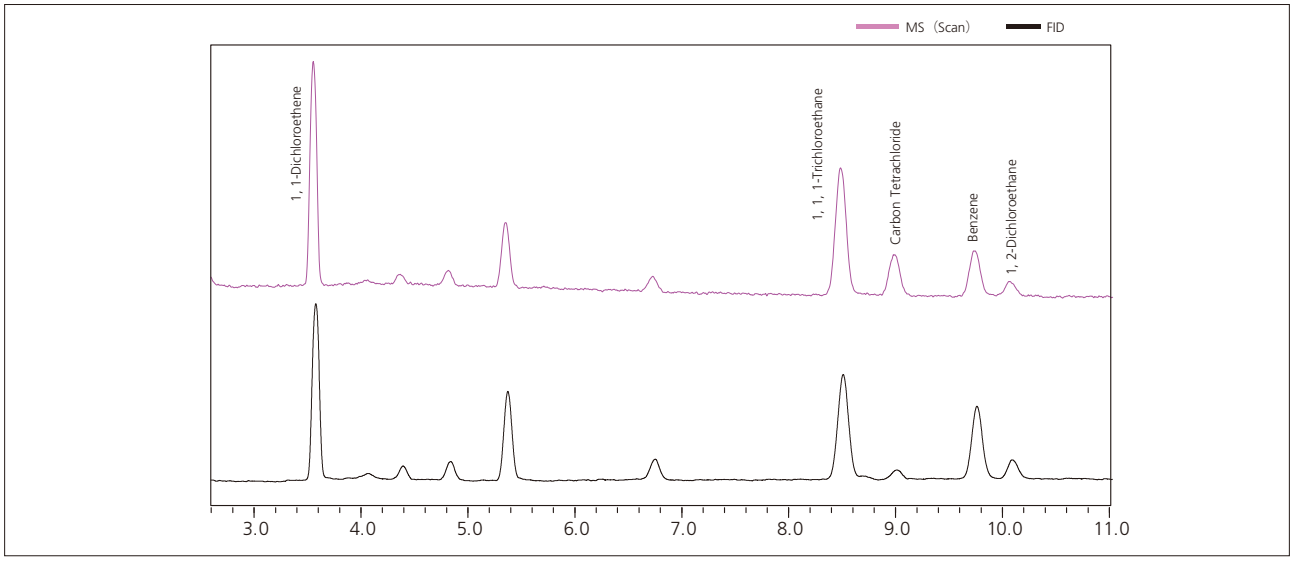

Fig. 5 Chromatograms of Class 1 System Suitability Solution

To check the mass spectra of the peaks detected by FID, the peak retention times in chromatograms obtained by FID and MS must match as closely as possible. Looking at Fig. 2 to 4 show all the peak retention times are lined up, from the earliest to the latest constituent.
When using a detector splitting system, the two detectors must detect the same peaks detected by normal gas chromatography. In other words, detector splitting systems are expected to have the equivalent system performance as a normal analytical system. Procedure A in USP <467> states the two items below concerning system suitability. We attempted to confirm the two items below for the detector splitting system, and for the repeatability of class 1 standard solution analysis.

(1) Detector confirmation
The S/N ratio of 1, 1, 1-trichloroethane in class 1 standard solution is 5 or higher; the S/N ratio of each peak in class 1 system suitability solution is 3 or higher.

(2) System performance
The peak resolution between acetonitrile and dichloromethane in class 2 standard solution is 1.0 or higher.

The results (FID S/N ratios) of analyzing class 1 standard solution and class 1 system suitability solution with the detector splitting system are shown in Table 2, and the repeatability results (FID repeatability) of analyzing class 1 standard solution are shown in Table 3. These results show the detector splitting system meets the performance required of a standard system. The peak resolution of acetonitrile and dichloromethane in class 2 standard solution was 2.37, showing this system is also suitable in terms of resolution.

Table 2 Signal-to-Noise Ratio in Class 1 Standard Solution and System Suitability Solution

Compound	Standard solution	Solution for system suitability test
1, 1-Dichloroethene	221.9	141.4
1, 1, 1-Trichloroethane	117.6	82.2
Carbon tetrachloride	10.2	7.6
Benzene	106.3	56.8
1, 2-Dichloroethane	26.4	14.2

Table 3 Repeatability in Class 1 Standard Solution (n=6)

Compound	Relative standard deviation (%)
1, 1-Dichloroethene	1.6
1, 1, 1-Trichloroethane	2.2
Carbon tetrachloride	1.8
Benzene	3.5
1, 2-Dichloroethane	2.9

The results (chromatograms) of analyzing active pharmaceutical ingredients in the detector splitting system are shown in Fig. 6, and the mass spectra of detected peaks are shown in Fig. 7 to 9. Peaks a and b, based on their respective mass spectra (Fig. 7 and 8), were estimated to be ethyl acetate and butanol. Both these constituents are low toxicity class 3 solvents.

Though its peak strength is smaller than that observed in the standard solution, a peak was also detected at the elution position of o-xylene (c). Checking the mass spectrum of this peak (Fig. 9) showed it differed from the mass spectrum of xylene (peak d, Fig. 10), and was estimated to be dibutyl ether.

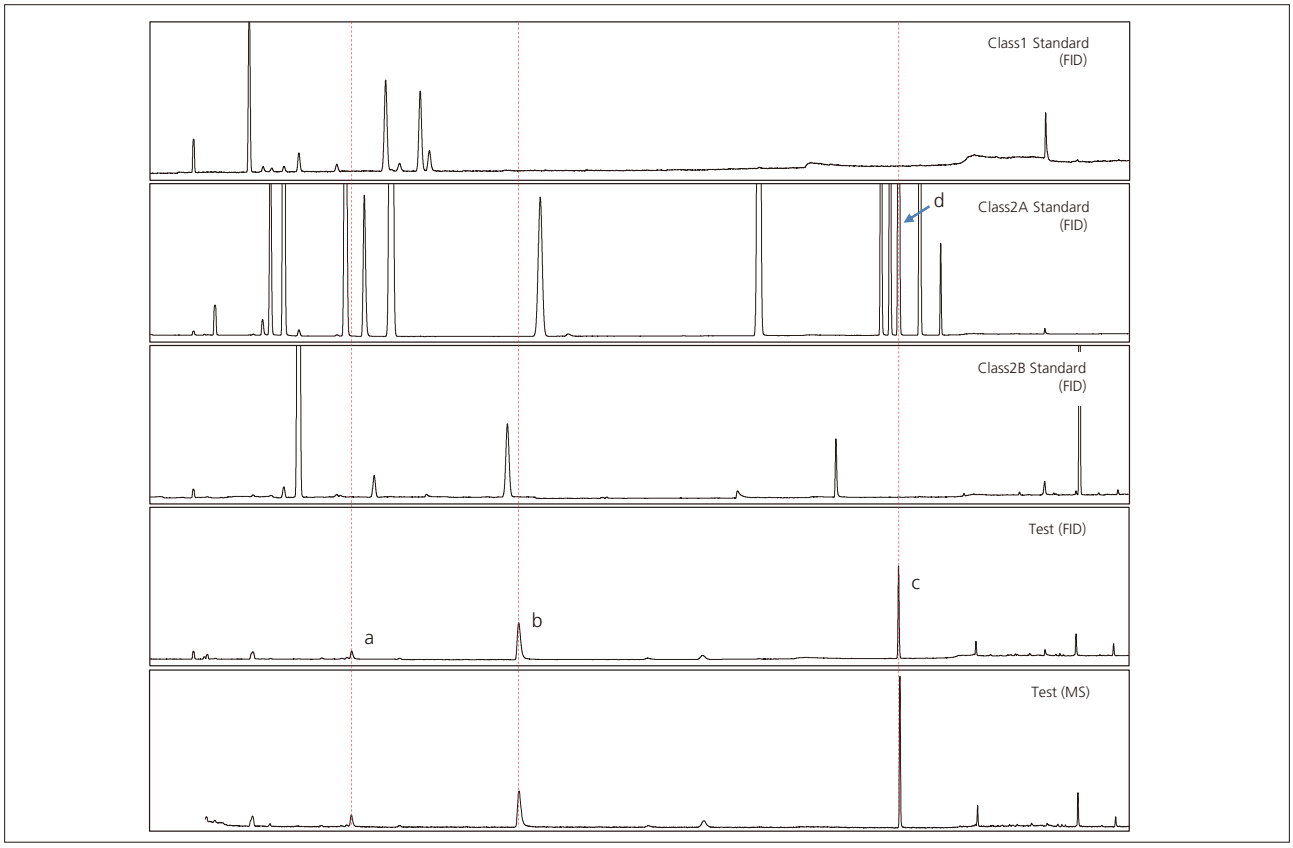

Fig. 6 Chromatograms of Standard Solutions and Test Solutions

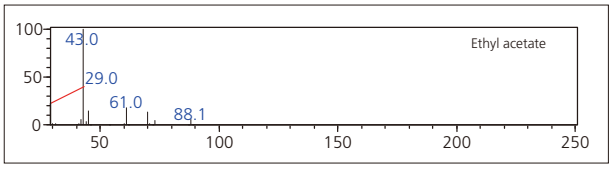

Fig. 7 Mass Spectrum of Peak a

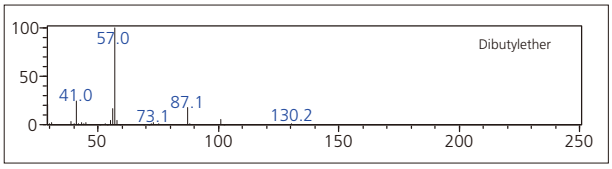

Fig. 9 Mass Spectrum of Peak c

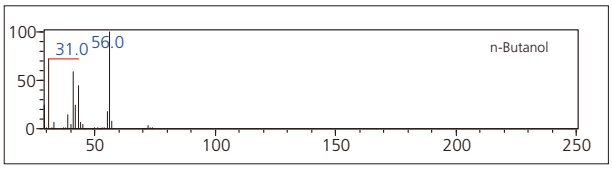

Fig. 8 Mass Spectrum of Peak b

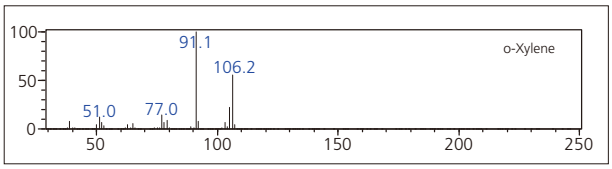

Fig. 10 Mass Spectrum of Peak d

Conclusion

An FID and MS detector splitting system obtains FID and MS data simultaneously in a single analysis, and can be used as a simpler method of confirming constituent identity. This system shows promise for use in residual solvent testing of pharmaceuticals.

Note: Reference USP <467>
This data was obtained by a method that does not conform to the pharmacopoeia, as analytical conditions based on USP <467> was modified before use.

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PO-CON1464E

Analysis of styrene leached from polystyrene cups using GCMS coupled with Headspace (HS) sampler

ASMS 2014 TP763

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Introduction

Worldwide studies have revealed the negative impacts of household disposable polystyrene cups (Figure 1) on human health and environment.
Molecular structure of styrene is shown in Figure 2. Styrene is considered as a possible human carcinogen by the WHO and International Agency for Research on Cancer (IARC).[1] Migration of styrene from polystyrene cups containing beverages has been observed.[2] Styrene enters into our body through the food we take, mimics estrogens in the body and can therefore disrupt normal hormonal functions. This could also lead to breast and prostate cancer.
The objective of this study is to develop a sensitive, selective, accurate and reliable method for styrene determination using low carryover headspace sampler, HS-20 coupled with Ultra Fast Scan Speed 20,000 u/sec, GCMS-QP2010 Ultra to assess the risk involved in using polystyrene cups.

Figure 1. Polystyrene cup

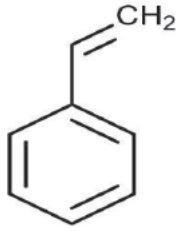

Figure 2. Structure of styrene

Method of Analysis

Extraction of styrene from polystyrene cups

This study was carried out by extracting styrene from commercially available polystyrene cups and recoveries were established by spiking polystyrene cups with standard solution of styrene. Solutions were prepared as follows,

1) Standard Stock Solution:
   1000 ppm of styrene standard stock solution in DMF: Water-50:50 (v/v) was prepared. It was further diluted with water to make 100 ppm and 1 ppm of standard styrene solutions.
2) Calibration Curve:
   Calibration curve was plotted using standard styrene solutions in the concentration range of 1 to 50 ppb with water as a diluent. 5 mL of each standard styrene solution was transferred in separate 20 mL headspace vials and crimped with automated crimper.
3) Sample Preparation:
   150 mL of boiling water (around 100 °C)[1] was poured into polystyrene cups. The cup was covered with aluminium foil and kept at room temperature for 1 hour. After an hour, 5 mL of sample from the cup was transferred into the 20 mL headspace vial and crimped with automated crimper.

Method was partly validated to support the findings by performing reproducibility, linearity, LOD, LOQ and recovery studies. For validation, solutions of different concentrations were prepared using standard stock solution of styrene (1000 ppm) as mentioned in Table 1.

Table 1. Method validation parameters

Parameter	Concentration (ppb)
Linearity	1, 2.5, 5, 10, 20, 50
Accuracy / Recovery	2.5, 10, 50
Precision at LOQ level	1
Reproducibility	50

HS-GCMS Analytical Conditions

Figure 3 shows the analytical instrument, HS-20 coupled with GCMS-QP2010 Ultra on which samples were analyzed with following instrument parameter.

Figure 3. HS-20 coupled with GCMS-QP2010 Ultra by Shimadzu

HS-GCMS analytical parameters

Headspace parameters

- Sampling Mode : Loop
- Oven Temp. : 80.0 °C
- Sample Line Temp. : 130.0 °C
- Transfer Line Temp. : 140.0 °C
- Equilibrating Time : 20.00 min
- Pressurizing Time : 0.50 min
- Pressure Equilib. Time : 0.10 min
- Load Time : 0.50 min
- Load Equilib. Time : 0.10 min
- Injection Time : 1.00 min
- Needle Flush Time : 10.00 min
- GC Cycle Time : 23.00 min

Chromatographic parameters

• Column	: Rxi-5Sil MS (30 m L x 0.25 mm I.D., 0.25 µm)		
• Injection Mode	: Split		
• Split Ratio	: 10.0		
• Carrier Gas	: Helium		
• Flow Control Mode	: Linear Velocity		
• Linear Velocity	: 36.3 cm/sec		
• Pressure	: 53.5 kPa		
• Column Flow	: 1.00 mL/min		
• Total Flow	: 14.0 mL/min		
• Total Program Time	: 12.42 min		
• Column Oven Temp.	: Rate (°C /min)	Temperature (°C)	Hold time (min)
		50.0	0.00
	40.00	200.0	1.00
	30.00	280.0	5.00

Mass Spectrometry parameters

• Ion Source Temp.	: 200 °C
• Interface Temp.	: 230 °C
• Ionization Mode	: EI
• Event Time	: 0.20 sec
• Mode	: SIM
• m/z	: 104,103 and 78
• Start Time	: 1.00 min
• End Time	: 5.00 min

Results

Fragmentation of styrene

Mass spectrum of styrene is shown in Figure 4. From the mass spectrum, base peak of m/z 104 was used for quantitation where as m/z 103 and 78 were used as reference ions.
SIM chromatogram of 50 ppb standard styrene solution with m/z 104, 103 and 78 is shown in Figure 5.
Method validation data is summarized in Table 2. Figures 6 and 7 show overlay of SIM chromatograms for m/z 104 at linearity levels and calibration curve respectively.

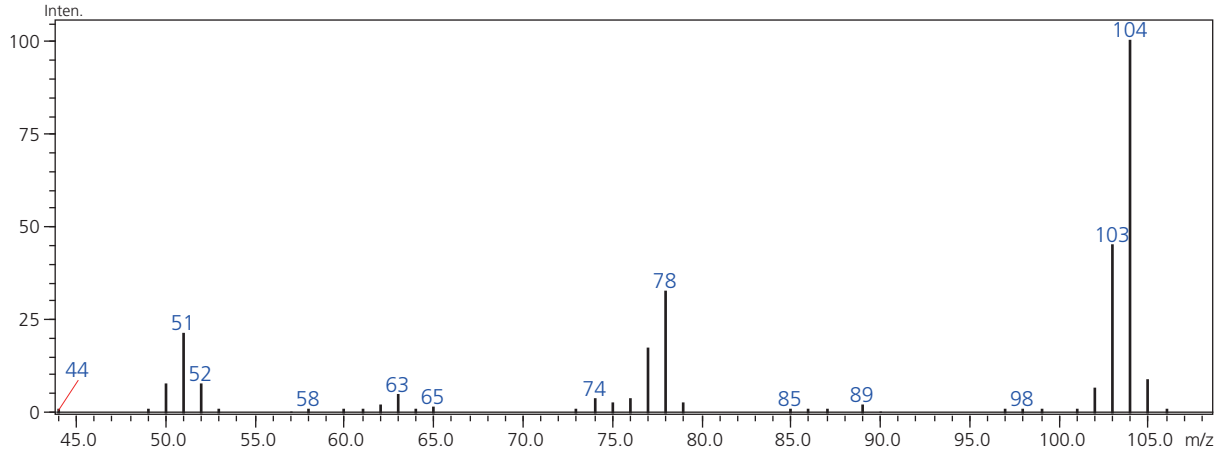

Figure 4. Mass spectrum of styrene

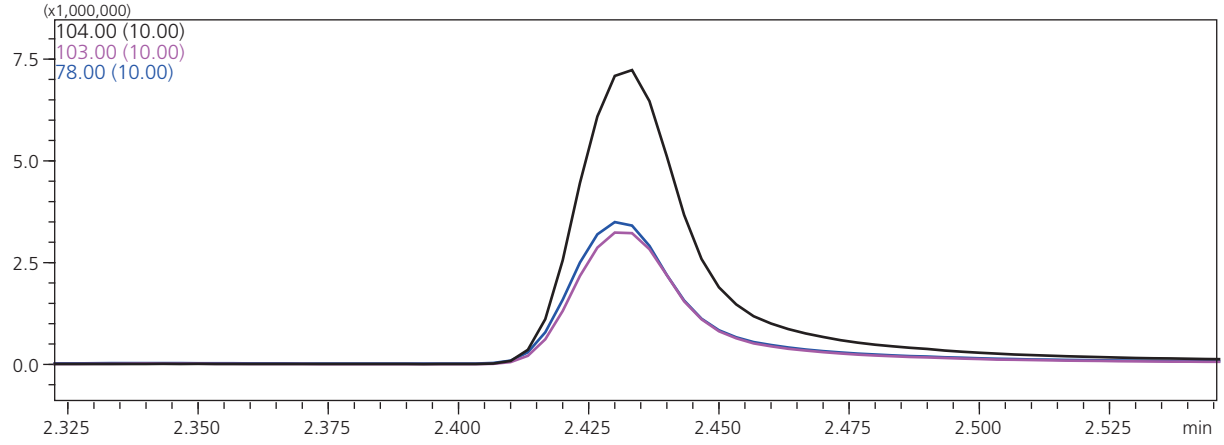

Figure 5. SIM chromatogram of 50 ppb standard styrene solution

Summary of validation results

Table 2. Validation summary

Sr. No.	Compound Name	Parameter	Concentration in ppb	Result
1	Styrene	Reproducibility (% RSD)	50	% RSD : 1.74 (n=6)
2		Linearity* (R^2)	1 – 50	R^2 : 0.9996
3		LOD	1 – 50	LOD : 0.2 ppb
4		LOQ		LOQ : 1 ppb
5		Precision at LOQ	1	S/N ratio : 38 (n=6)
				% RSD : 3.2 (n=6)

* Linearity levels – 1, 2.5, 5, 10, 20 and 50 ppb.

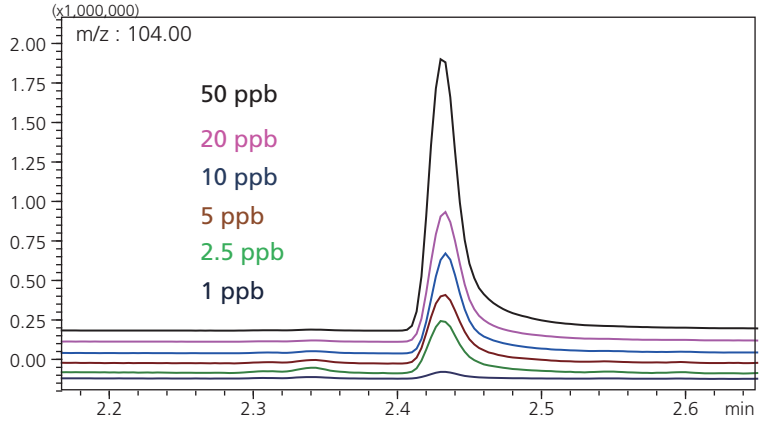

Figure 6. Overlay of SIM chromatograms for m/z 104 at linearity levels

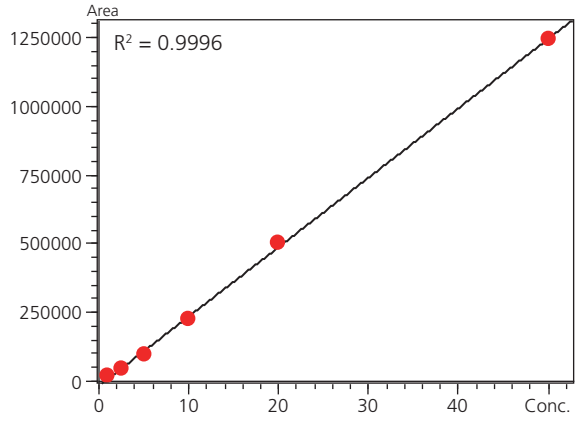

Figure 7. Calibration curve for Styrene

Quantitation of styrene in polystyrene cup sample

Analysis of leachable styrene from polystyrene cups was done as per method described earlier. Recovery studies were carried out by spiking 2.5, 10 and 50 ppb of standard styrene solutions in polystyrene cups. Figure 8 shows overlay SIM chromatogram of spiked and unspiked samples. Table 3 shows the summary of results.

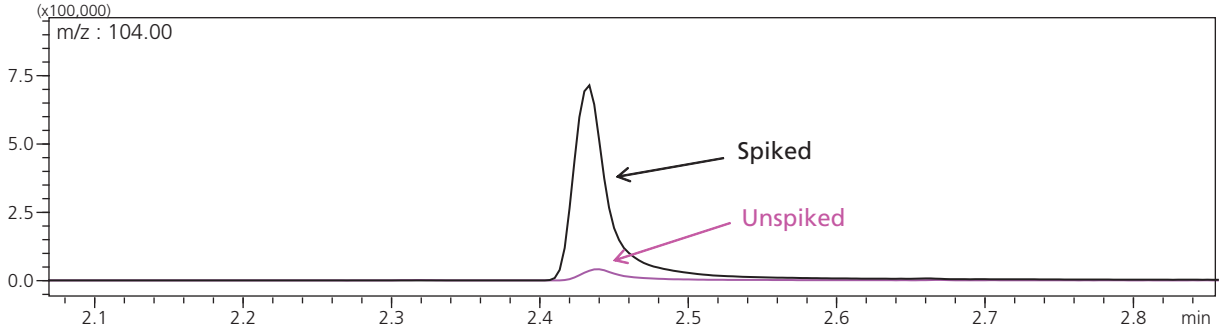

Figure 8. Overlay SIM chromatograms of spiked and unspiked samples

Table 3. Summary of results for sample analysis

Sr. No.	Sample Name	Parameter	Observed Concentration in ppb	Spiked Concentration in ppb	% Recovery
1	Unspiked sample	Precision	9.8	NA	NA
2	Spiked polystyrene cups	Recovery	12.0	2.5	88.0
			18.5	10	87.0
			55.9	50	92.2

Conclusion

- HS-GCMS method was developed for quantitation of styrene leached from polystyrene cup. Part method validation was performed. Results obtained for reproducibility, linearity, LOQ and recovery studies were within acceptable criteria.
- With low carryover, the characteristic feature of HS-20 headspace, reproducibility even at very low concentration level could be achieved easily.
- Ultra Fast Scan Speed 20,000 u/sec is the characteristic feature of GCMS-QP2010 Ultra mass spectrometer, useful for quantitation of styrene at very low level (ppb level) with high sensitivity.

References

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Application News

No. SSL-CA14-059

Gas Chromatography Mass Spectrometry

Simultaneous determination of residual solvents in pharmaceutical packaging materials using headspace-GC/MS

Abstract

A highly sensitive and precise method utilizing Headspace-GCMS-QP2010 Ultra has been developed for the analysis of residual solvents in pharmaceutical packaging materials. The established method is rapid and easy to perform and is the preferred method for residual solvent analysis in quality control laboratories.

Introduction

Common pharmaceutical packaging consists of plastics and thin metal foils such as blister packs and bottles. Manufacturing of these packaging materials produces residual solvents. Furthermore, inks and adhesives (in the form of prescription labels) printed on the pharmaceutical packaging may migrate and transfer to these residual solvents. These organic volatile solvents are in direct contact with the pharmaceutical drugs, thereby increasing the risk of contamination, and the possible harm to human safety. Therefore, a careful assessment of residual solvents, and quality control of the pharmaceutical product is crucial.

In 2012, US FDA announced for pharmaceutical manufacturers to avoid the use of dibutyl phthalates (DBP) and di(2-ethylhexyl) phthalates (DEHP) in pharmaceutical drugs and biologic products. In the meanwhile, due to PAEs are also commonly used as plasticizers for packaging materials, and their presence in pharmaceutical packaging may result in leaching and the eventual contamination of PAEs in drugs. Consequently, according to the latest 2015 edition of the Chinese Pharmacopoeia, guideline issued that prohibited the use of diethyl phthalate (DEP) as pharmaceutical excipients in Chinese Pharmacopoeia regulated products. This application note established a rapid, simple and highly-sensitive method for the detection of these phthalates in pharmaceutical products.

Experimental

Instrumentation and Analytical Conditions Headspace Sampler: HS-20
GC-MS: GCMS-QP2010 Ultra
The analytical conditions used are shown in Table 1.

Table 1 Analytical Conditions of HS-20 and GCMS-QP2010 Ultra

Headspace conditions		
Vial equilibration	:	80 °C
Sample line temperature	:	160 °C
Transfer line temperature	:	170 °C
Vial warming time	:	30 min
Injection time	:	0.5 min
Injection volume	:	1 mL
GC-MS conditions		
Column	:	Rtx-624 60 mx0.32 mmx1.8 µm
Column temp. Program	:	35 °C (2min) → 20 °C/min → 200 °C
Injection mode	:	Split
Split ratio	:	10:1
Control mode	:	Constant linear velocity
Linear velocity	:	36.0 cm/sec
Ion source temp	:	230 °C
Interface temp	:	230 °C
Acquisition mode	:	SIM Mode

Sample preparation

Each packaging sample, of appropriate dimensions, was shredded and placed into a 20 mL headspace vial. Septum was placed and capped immediately.

Results and Discussion

Standard chromatograms

The 16 volatile organic solvents (residual solvents) investigated in this study are listed in Table 2. The mixed stock solution of the volatile organic solvents at 10 000 µg/ml was prepared and further diluted to 500 µg/ml. 10 µl of the 500 µg/ml mixed stock solution (5 µg) was added to the headspace vial, capped and analyzed immediately. The total ion current chromatogram (TIC) for the residual solvents (5 µg) were detected as shown in Figure 1. The retention time and MS parameters of the residual solvents are tabulated in Table 2.

Linearity and repeatability

Mixed stock solution of the residual solvents at 10 000 µg/ml was diluted with water to prepare calibration solutions of concentrations 1, 2, 5, 10, 20, 50, 100, 200 and 500 µg/ml. 10 µl of these standard calibration

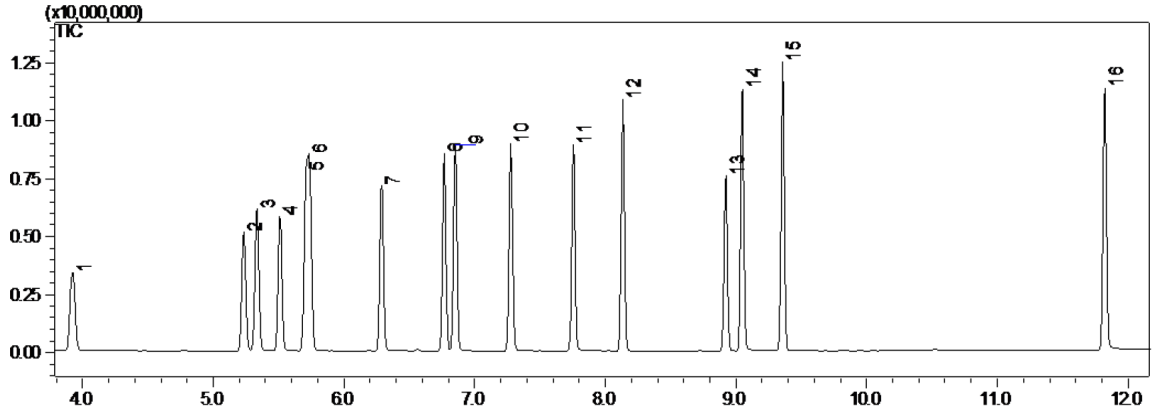

Fig. 1 Total ion current chromatogram (TIC) for 5 µg of stock solution

Table 2. Mass spectrometry parameters for the GC-MS determination of target compounds

No.	Compound name	Retention time (min)	CAS	Quantifier Ion	Qualifier Ion 1	Qualifier Ion 2
1	Methanol	3.915	67-56-1	31	32	29
2	Acetone	5.228	67-64-1	43	58	42
3	Isopropanol	5.329	67-63-0	45	43	29
4	Acetonitrile	5.504	75-05-8	41	40	39
5	Dichloromethane	5.702	75-09-2	49	84	86
6	2-Methyl-2-propanol	5.732	75-65-0	59	31	41
7	1-Propanol	6.281	71-23-8	31	29	42
8	Ethyl acetate	6.761	79-20-9	43	29	45
9	2-Butanol	6.846	78-92-2	45	59	31
10	2-Methyl-1-propanol	7.27	78-83-1	43	41	42
11	1-Butanol	7.751	71-36-3	56	31	41
12	Ethyl propionate	8.128	109-60-4	43	61	73
13	Toluene	8.917	108-88-3	91	92	65
14	1-Pentol	9.04	71-41-0	42	55	41
15	Butyl acetate	9.351	123-86-4	43	56	41
16	2-Ethyl-hexanol	11.815	104-76-7	57	41	43

solutions were added to the respective GC headspace vials to give 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2 and 5 µg. The 9 calibration solutions were analyzed, quantified and plotted to give the calibration curves. Calibration curves of some residual solvents are shown in Figure 2. The Instrument Detection Limit (IDL), at S/N ratio of 3, of each compound was determined by injecting 0.2 µg standard mixture. The method calibration performance for the 16 residual solvents such as R and RSD of the retention time and area were also determined and shown in Table 3.

Sample analysis

A pharmaceutical packaging sample (Sample 1, capsule packaging material of dimensions 1.6cm × 2.7cm) were prepared and pretreated as described in the procedure 1.3 above. The sample was analyzed thrice, quantified and the amount of residual solvents are tabulated in Table 4.

Another pharmaceutical packaging sample (Sample 2, granule packaging material of dimensions 4.5cm × 5cm) was selected and prepared as indicated in procedure 1.3 described above. The prepared sample 2 was analyzed twice and the results are shown in Table 5.

Conclusion

This article introduces the analysis of 16 residual solvents utilizing Shimadzu GCMS-QP2010 Ultra with HS-20 headspace sampler. These volatile organic compounds demonstrated good linearity over the range 0.1~5µg. In summary, this study has proven a simple, rapid and sensitive GC-MS method for the routine analysis of residual solvents in pharmaceutical packaging materials.

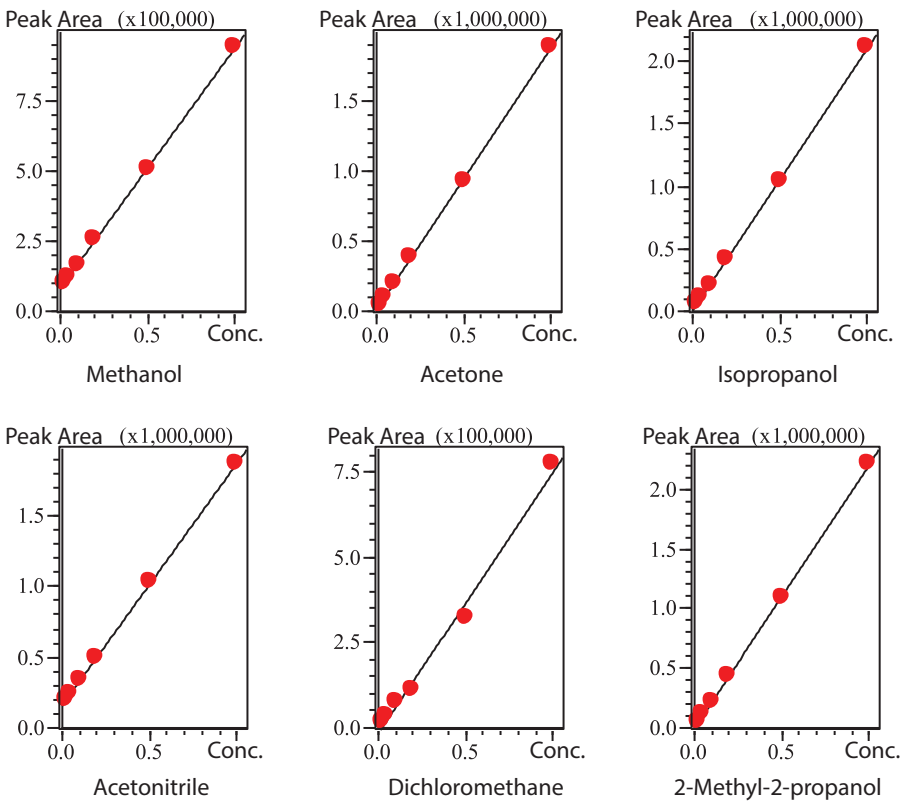

Figure 2. Calibration curves of some residual solvents

Table 3. Method validation data

No.	Compound name	R	RSD% (Area ratio)	RSD% (Retention time)	IDLs (µg)
1	Methanol	0.9999	1.04	0.01	0.0002
2	Acetone	0.9999	3.72	0.01	0.0007
3	Isopropanol	0.9999	1.81	0.00	0.0001
4	Acetonitrile	0.9999	3.30	0.01	0.0001
5	Dichloromethane	0.9953	8.20	0.00	0.0002
6	2-Methyl-2-propanol	0.9999	3.22	0.01	0.0002
7	1-Propanol	0.9998	3.04	0.00	0.0004
8	Ethyl acetate	0.9999	4.52	0.01	0.0028
9	2-Butanol	0.9998	3.39	0.00	0.0191
10	2-Methyl-1-propanol	0.9998	3.22	0.01	0.0211
11	1-Butanol	0.9999	6.77	0.00	0.0005
12	Ethyl propionate	0.9998	4.92	0.01	0.0002
13	Toluene	0.9876	11.69	0.01	0.0002
14	1-Pentol	0.9994	5.77	0.01	0.0005
15	Butyl acetate	0.9996	5.20	0.01	0.0004
16	2-Ethyl-hexanol	0.9995	8.89	0.01	0.0001

Table 4. Sample results (capsule packaging materials)

No.	Compound name	Sample1-1	Sample 1-2	Sample 1-3	Average results	RSD%
1	Methanol	0.12	0.11	0.10	0.11	5.89
2	Acetone	0.03	0.03	0.03	0.03	11.73
3	Isopropanol	0.03	0.03	0.03	0.03	4.07
4	Acetonitrile	N.D	N.D	N.D	-	-
5	Dichloromethane	0.02	0.02	0.02	0.02	0.77
6	2-Methyl-2-propanol	N.D	N.D	N.D	-	-
7	1-Propanol	N.D	N.D	N.D	-	-
8	Ethyl acetate	0.13	0.14	0.11	0.12	10.25
9	2-Butanol	N.D	N.D	N.D	-	-
10	2-Methyl-1-propanol	0.21	0.20	0.23	0.21	7.53
11	1-Butanol	0.07	0.06	0.07	0.07	5.27
12	Ethyl propionate	N.D	N.D	N.D	-	-
13	Toluene	0.05	0.05	0.05	0.05	0.60
14	1-Pentol	0.02	0.02	0.02	0.02	1.21
15	Butyl acetate	N.D	N.D	N.D	-	-
16	2-Ethyl-hexanol	0.22	0.25	0.23	0.23	5.43

N.D.: Not detected

Table 5. Sample results (granule packaging materials)

No.	Compound name	Sample 2-1	Sample 2-2	Average results
1	Methanol	0.06	0.07	0.07
2	Acetone	N.D	N.D	N.D
3	Isopropanol	N.D	N.D	N.D
4	Acetonitrile	N.D	N.D	N.D
5	Dichloromethane	0.03	0.03	0.03
6	2-Methyl-2-propanol	N.D	N.D	N.D
7	1-Propanol	N.D	N.D	N.D
8	Ethyl acetate	N.D	N.D	N.D
9	2-Butanol	N.D	N.D	N.D
10	2-Methyl-1-propanol	N.D	N.D	N.D
11	1-Butanol	0.02	0.02	0.02
12	Ethyl propionate	N.D	N.D	N.D
13	Toluene	0.05	0.06	0.05
14	1-Pentol	0.02	0.02	0.02
15	Butyl acetate	N.D	N.D	N.D
16	2-Ethyl-hexanol	0.02	0.02	0.02

N.D.: Not detected

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Application News

No. SSL-CA09-520

Gas Chromatography Mass Spectrometry

Simultaneous Determination of Phthalate Esters in Pharmaceuticals Using GC-MS

Abstract

In this application note, a method was demonstrated for the rapid trace-level quantitation of sixteen phthalate esters using Shimadzu GCMS-QP2010 Ultra. Sample preparation was easily performed using ultrasonic extraction. Excellent linearity, where R = 0.9943 - 0.9996 over the range 0.1 to 5 µg/mL, and good repeatability (RSD < 5.0%, n = 5) were obtained. The limit of detection for the phthalates are mainly in the range of 0.43 – 15.0 µg/mL and the spiked recovery are all in the acceptable range (73.15 – 111.06%). The described GC-MS method provides a simple, rapid and highly-sensitive method for the detection and identification of phthalate esters in pharmaceuticals.

Introduction

Phthalate esters (PAEs, also known as phthalates) are a group of synthetic chemicals with widespread industrial applications. Some phthalates have shown no toxic effects while others are known to be endocrine disruptors. Studies on these toxic PAEs have shown that it interferes with the processes of the natural hormones, disrupts the developmental and reproductive functions and causes cancer.

With the increasing occurrence of contamination of PAEs in food, it has also raised high concern over the issue of these chemicals in pharmaceuticals.

In 2012, US FDA announced for pharmaceutical manufacturers to avoid the use of dibutyl phthalates (DBP) and di(2-ethylhexyl) phthalates (DEHP) in pharmaceutical drugs and biologic products. However, based on the 2010 edition of the Chinese Pharmacopoeia, DEP is legally approved as inert in drug production and classified as safe. Furthermore, these PAEs are also commonly used as plasticizers for packaging materials. Their presence in pharmaceutical packaging may result in leaching and the eventual contamination of PAEs in drugs.

This application note established a rapid, simple and highly-sensitive method for the detection of these phthalates in pharmaceutical products.

Experimental

Instrumentation and Analytical Conditions

GC-MS: GCMS-QP2010 Ultra

The analytical conditions used are shown in Table 1.

Table 1 Analytical Conditions GCMS-QP2010 Ultra

GC-MS conditions		
Column	:	Rxi-5Sil MS 30m×0.25mm×0.25µm
Column temp. Program	:	60 °C (1 min) → 20 °C/min → 220 °C (0 min) → 5 °C/min → 280 °C
Injection temp	:	280 °C
Injection mode	:	Splitless (1 min)
Carrier Gas	:	Helium Gas
Control mode	:	Constant linear velocity
Linear velocity	:	36.5 cm/sec
Ion source temp	:	200 °C (EI)
Interface temp	:	280 °C
Injection Volume	:	1 mL
Acquisition mode	:	Scan Mode
Mass Range	:	35 ~ 500

Sample preparation

Grind drug sample into a fine homogenous powder

↓

Add 10 mL n-hexane, and extract for 30 minutes using ultrasonication

↓

Centrifuge and extract 1.0 mL of the extracted supernatant into a 1.5 mL GC vial

Results and Discussion

Standard chromatograms

16 PAEs were separated and determined using GC-MS. Table 2 shows the types of PAEs and their corresponding GC-MS retention time. The chromatogram of the mixed standard PAEs at 5 µg/mL is shown in Figure 1.

Table 2. Types of PAEs and their corresponding retention time

No.	Retention time (min)	Compound name
1	7.855	Dimethyl phthalate (DMP)
2	8.730	Diethyl phthalate (DEP)
3	10.540	Diisobutyl phthalate (DIBP)
4	11.330	Dibutyl phthalate (DBP)
5	11.645	Bis(2-methoxyethyl) phthalate (DMEP)
6	12.380	Bis(4-Methyl-2-pentyl)phthalate (BMPP)
7	12.750	Bis(2-ethoxyethyl) phthalate (DEEP)
8	13.195	Di-N-pentyl phthalate (DPP)
9	15.435	Dihexyl phthalate (DHXP)
10	15.580	Butyl benzyl phthalate (BBP)
11	17.040	Bis(2-n-butoxyethyl) phthalate (DBEP)
12	17.760	Dicyclohexyl phthalate (DCHP)
13	17.920	Bis(2-ethylhexyl) phthalate (DEHP)
14	18.090	Dipentyl phthalate (DPP)
15	20.510	Di-n-octyl phthalate (DNOP)
16	21.200	Dinonyl phthalate (DNP)

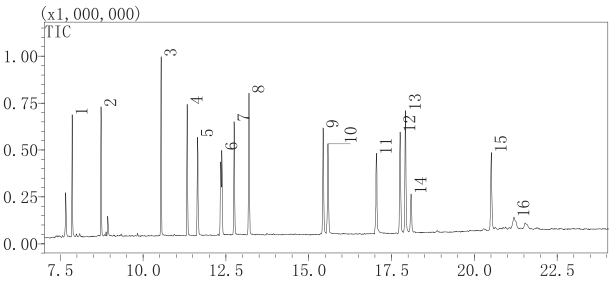

Fig. 1 Total ion current chromatograms (TIC) of 16 phthalate esters at 5 µg/mL

Calibration curves

Mixed standard solutions were prepared by diluting sixteen PAE standards with n-hexane to obtain a series of calibration standards at concentrations of 0.1, 0.2, 0.5, 1, 2, and 5 µg/mL. The calibration curves are shown in Figure 2.

Instrument detection limit and repeatability results

The Instrument Detection Limit (IDL) of each compound, (S/N ratio = 3), was determined by injecting 0.1 µg/mL standard mixture.. The method performance, such as the correlation coefficient, IDL for each compound was satisfactory and tabulated in Table 3.

Spiked recovery test

Two commercially available pharmaceutical samples were used for the spiked recovery test: (1) compounded anti-cough tablets (FuFang JieGeng ZhiKe Pian) and (2) compounded bismuth aluminate tablets. Mixed PAE standard solution at 0.5 µg/mL were spiked to the two drug samples, and pretreatment was conducted as described previously. The spiked recovery test was repeated thrice and the recovery and its repeatability were calculated and shown in Table 4.

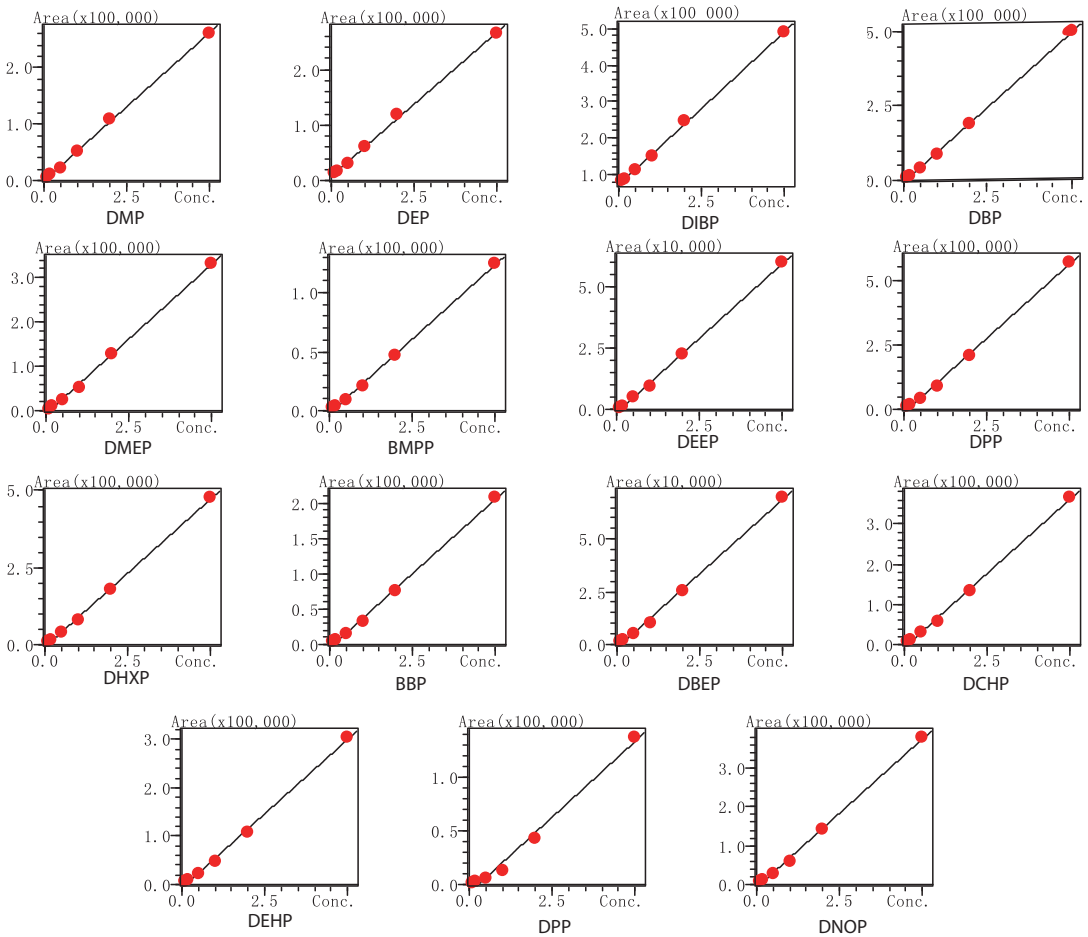

Fig. 2 Standard calibration curves of some PAEs

Table 3. IDLs and repeatability of area ratio (n=5)

No.	Compound name	R	IDL (μg/L)	%RSD
1	DMP	0.9995	1.03	2.19
2	DEP	0.9993	0.82	2.44
3	DIBP	0.9996	0.43	3.57
4	DBP	0.9997	0.64	2.32
5	DMEP	0.9991	1.18	4.21
6	BMPP	0.9993	2.69	4.80
7	DEEP	0.9991	4.43	4.74
8	DPP	0.9990	0.67	3.73
9	DHXP	0.9992	0.90	4.79
10	BBP	0.9989	2.25	4.01
11	DBEP	0.9984	7.05	4.90
12	DCHP	0.9991	1.36	3.62
13	DEHP	0.9988	1.61	3.11
14	DPP	0.9943	2.75	12.00
15	DNOP	0.9989	1.38	3.60
16	DNP	0.9952	15.0	4.38

Conclusion

A GC-MS method was developed for the analysis of phthalate esters in pharmaceuticals and drug products using Shimadzu GCMS-QP2010 Ultra. Excellent linearity was obtained over the calibration range 0.1 - 5 μg/L and IDLs were calculated to be mainly in the range of 0.43-4.43μg/mL and recoveries were obtained in the acceptable range of 73.15 - 111.06%. Additionally, the method has been tested and applied to real pharmaceutical samples.

Table 4. Recovery for the spiked samples

No.	Compound name	Compound Anti-Cough Tablets			Compound Bismuth Aluminate Tablets		
		Results (μg/mL)	Average Recovery Ratio (%, n=3)	%RSD (%, n=3)	Results (μg/mL)	Average Recovery Ratio (%, n=3)	%RSD (%, n=3)
1	DMP	ND	93.17%	1.70	ND	106.38%	0.83
2	DEP	ND	76.72%	2.39	ND	77.28%	1.34
3	DIBP	ND	97.31%	4.42	ND	99.39%	0.68
4	DBP	ND	97.37%	4.35	ND	111.06%	1.14
5	DMEP	ND	100.29%	2.58	0.17	87.63%	2.54
6	BMPP	ND	98.82%	9.67	ND	106.21%	1.98
7	DEEP	ND	103.93%	2.84	ND	107.86%	2.91
8	DPP	ND	90.77%	5.09	ND	101.53%	1.99
9	DHXP	ND	84.84%	4.72	ND	94.71%	1.85
10	BBP	0.12	86.36%	4.49	ND	94.72%	1.21
11	DBEP	0.49	89.37%	8.48	ND	92.35%	3.93
12	DCHP	ND	88.83%	4.32	ND	98.23%	1.10
13	DEHP	0.17	98.82%	2.42	0.13	89.37%	2.86
14	DPP	ND	95.30%	5.38	ND	92.53%	3.69
15	DNOP	ND	78.12%	4.31	ND	85.66%	2.70
16	DNP	ND	73.15%	8.06	ND	81.75%	5.00

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Application News

No.J119

Inductively Coupled Plasma Mass Spectrometry

Analysis of ICH Q3D Guideline for Elemental Impurities in Drug Products Using ICPMS-2030

Outline of ICH Q3D Guideline for Elemental Impurities in Drug Products

The ICH Q3D Guideline for Elemental Impurities sets permitted daily exposures (PDEs) for 24 elemental metal impurities of which toxicity is a concern, including the so-called big four (lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As)), and residual catalyst metals that are added intentionally during active pharmaceutical ingredient synthesis. Table 1 shows the ICH Q3D guideline of PDE for elemental impurities.
The PDE levels shown for metal impurities must be converted to concentrations in order to evaluate the presence of metal impurities in drug products and their components.
We describe an example of analysis of 24 elements included in the ICH Q3D guideline using inductively coupled plasma mass spectrometry.

Sample

・Tablet (daily dose: 1 tablet [0.2 g])

Sample Preparation

After placing one tablet (daily dose: one tablet [0.20 g]), hydrochloric acid 0.5 mL, and nitric acid 5 mL in a quartz decomposition vessel, decomposition was performed using a microwave sample pretreatment system.
After decomposition, hydrochloric acid 0.1 mL was added, the mixture was made up to 20 mL with pure water, and this solution was used for analysis (100-fold dilution). At this point, Sc, Ga, Y, and Te internal standard elements were added (as an analytical solution concentration of 10 µg/L). After decomposition treatment of a sample, the elements to be measured were added to the sample to create a spike-and-recovery test solution.

Instrument and Analytical Conditions

Shimadzu's ICPMS-2030 inductively coupled plasma mass spectrometer was used for analysis. Analytical conditions are shown in Table 2.
In addition to being highly sensitive, the ICPMS-2030 uses a helium gas collision system that greatly reduces the spectral interference caused by argon and chlorine. Use of Eco mode and a mini-torch drastically reduces running costs associated with gas usage, compared to previous ICP-MS systems.

Table 1 Permitted Daily Exposure Levels for Elemental Impurities in ICH Q3D

Class	Element	Oral Preparations µg/day	Injected Preparations µg/day	Inhaled Preparations µg/day	Class	Element	Oral Preparations µg/day	Injected Preparations µg/day	Inhaled Preparations µg/day
1	As	15	15	2	2B	Pt	100	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 Analytical Conditions

Instrument	: ICPMS-2030
High-frequency output	: 1.2 kW
Plasma gas flowrate	: 8.0 L/min
Auxiliary gas flowrate	: 1.1 L/min
Carrier gas flowrate	: 0.60 L/min
Sample introduction	: Nebulizer 10
Chamber	: Cyclone chamber (electronic cooling)
Plasma torch	: Mini-torch
Collision gas	: He

Analysis

Quantitative analysis and spike and recovery testing were performed for 24 elements included in ICH Q3D guideline. Analyses were performed by the calibration curve method and internal standard method.

Analytical Results

Table 3 shows the analytical results for tablet preparations. Good spike and recovery results were obtained for all samples. Detection limits converted into sample concentrations also met all permitted concentration levels.

Conclusions

We successfully and accurately analyzed 24 elements included in the ICH Q3D guideline in a short period of time using the ICPMS-2030.

[References]
- Guideline for Elemental Impurities in Drug Products (PFSB/ELD Notification No. 4, September 30, 2015) [In Japanese]
- ICH Q3D Guideline for Elemental Impurities (December 16, 2014)
- General Tests, Supplement I to the Japanese Pharmacopoeia Sixteenth Edition

Table 3 Analytical Results for Tablet Preparations

Element	Oral Preparation PDE	*1 Permitted Concentration	*2 Detection Limit Converted for Tablet Preparations (3 σ)	Measured Result (in Tablet Preparation)	Spiked Concentration (in Tablet Preparation)	Spike and Recovery (%)
	µg	µg/g	µg/g	µg/g	µg/g	%
Ag	150	750	0.001	N.D.	0.1	107
As	15	75	0.002	N.D.	0.2	101
Au	100	500	0.001	N.D.	0.2	91
Ba	1400	7000	0.002	0.013	0.2	96
Cd	5	25	0.003	N.D.	0.2	96
Co	50	250	0.0006	N.D.	0.4	101
Cr	11000	55000	0.003	0.017	0.4	104
Cu	3000	15000	0.04	0.15	0.4	102
Hg	30	150	0.006	N.D.	0.2	100
Ir	100	500	0.0005	N.D.	0.2	98
Li	550	2750	0.01	N.D.	0.2	93
Mo	3000	15000	0.001	N.D.	0.2	107
Ni	200	1000	0.003	0.156	0.4	101
Os	100	500	0.007	N.D.	0.2	92
Pb	5	25	0.001	0.003	0.2	105
Pd	100	500	0.006	N.D.	0.2	104
Pt	100	500	0.003	N.D.	0.2	99
Rh	100	500	0.0008	0.003	0.2	101
Ru	100	500	0.002	N.D.	0.2	98
Sb	1200	6000	0.0009	0.007	0.2	98
Se	150	750	0.01	N.D.	0.2	98
Sn	6000	30000	0.002	N.D.	0.2	98
Tl	8	40	0.0005	N.D.	0.2	103
V	100	500	0.002	N.D.	0.4	100

*1: Permitted concentration: PDE level based on a daily intake of 0.2 g, which refers to a permitted concentration for oral preparations.
*2: Detection limit converted for tablet preparations (3 σ): Detection limit in measured solution (3 σ) × Dilution ratio (100)
N.D.: Not detected

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Application News

No.J123

Inductively Coupled Plasma Mass Spectrometry

Analysis of Toxic Elements in Supplements as per USP 2232 Using the ICPMS-2030

Introduction

The United States Pharmacopeia (USP) 2232 designates permitted daily exposure (PDE) levels for four elements for which toxicity is a concern in dietary supplements.
PDE values need to be converted to concentrations when evaluating metallic impurities in formulations or their structural components.
Compliance with these regulations is also obligatory for supplements imported to the U.S. from other nations.

Here, we introduce a quantitative analysis performed on arsenic, cadmium, mercury, and lead in supplements using the Shimadzu ICPMS-2030 ICP mass spectrometer.

Sample

Four commercially available supplements
(1) One 0.331 g tablet (three tablets/day)
(2) One 0.350 g tablet (one tablet/day)
(3) One 0.201 g tablet (one tablet/day)
(4) One 0.380 g tablet (six tablets/day)

Sample Preparation

We used the microwave digestion method that could decompose samples faster than the typically used wet digestion method. Since this method uses a closed vessel, it also has the advantage that loss of volatile elements such as arsenic is minimal. In this study, the sample was decomposed using the Milestone General ETHOS-One.

One tablet sample, 0.5 mL of hydrochloric acid, and 6.5 mL of nitric acid were added to a quartz vessel of the microwave digestion system for sample preparation. The mixture was then decomposed by the microwave digestion system.
After sample decomposition, pure water was added to bring the measurement solution to a volume of 30 mL. At this point, Ga, In, and Bi (at a 10 µg/L concentration in measurement solution) were added as the internal standard elements.
Table 1 shows the sample decomposition conditions using the microwave digestion system for sample preparation.

Table 1 Sample Decomposition Conditions Using the Microwave Digestion System for Sample Preparation

STEP	Temperature (℃)	Time (min)	Power (W)
1	50	2	1000
2	30	3	0
3	180	25	1000
4	150	1	0
5	180	4	1000
6	180	15	1000

Instrument and Analytical Conditions

The Shimadzu ICPMS-2030 mass spectrometer with standard attachments as shown in Table 2 was used for measurements.
In addition to providing high sensitivity, the ICPMS-2030 is equipped with a collision system using helium gas, which reduces interference from argon and chlorine significantly.

Table 2 Analytical Conditions

Instrument	: ICPMS-2030
High-frequency output	: 1.2 kW
Plasma gas flowrate	: 8.0 L/min
Auxiliary gas flowrate	: 1.10 L/min
Carrier gas flowrate	: 0.65 L/min
Nebulizer	: Nebulizer 10
Chamber	: Cyclone chamber (electronically cooled)
Plasma torch	: Mini torch
Collision gas	: He

Analysis

The calibration curve method was used for quantitative analysis of the elements arsenic, cadmium, mercury, and lead.
To verify the analysis results, a spike recovery test sample was created by adding a standard solution of measurement elements after the sample decomposition. Quantitative analysis was performed in the same way using this sample.

Analytical Results

Table 3 shows the permitted daily exposure (PDE) levels, maximum permitted concentrations, and detection limits. The analysis results and spike recovery rates are shown in Table 4. These show favorable recovery rates and confirm that the results obtained were appropriate. The sensitivity was also evidently sufficient, even at the maximum intake of 10 g per day.

Conclusion

Using the ICPMS-2030, it is possible to perform an analysis for controlling the heavy metals arsenic, cadmium, mercury, and lead in supplements.

[Reference]
USP 2232 Elemental Contaminants in Dietary Supplements

Table 3 PDE Levels, Maximum Permitted Concentrations, and Detection Limits for Elemental Impurities Specified in USP 2232

Name of Element	As	Cd	Hg	Pb
PDE (µg/day)	15	5	15	10
Maximum Permitted Concentration*1 (µg/g)	1.5	0.5	1.5	1
Maximum Permitted Concentration in Measurement Solution*2 (µg/g)	3	1	3	2
Detection Limit*2 (µg/L)	0.009	0.003	0.003	0.001

*1: When the maximum intake per day is 10 g
*2: When a 0.2 g sample is pretreated and diluted to a volume of 100 mL

Table 4 Analytical Results for Supplements (concentration in solid sample, µg/g)

Name of Element / Sample name	As	Cd	Hg	Pb
①	0.21	0.029	N.D.	0.035
②	0.08	0.018	N.D.	0.009
③	0.11	0.008	N.D.	0.037
④	0.29	0.071	N.D.	0.095
Recovery rate (%)	97	98	92	101

N.D.: Not detected
Spike recovery rate (%) = { (Analysis value for the spike recovery test sample − Analysis value) / Spike concentration} × 100

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