SHIMADZU

GCMS-QP2010 Series

Determination of Drugs of Abuse in Oral Fluids

Shimadzu
Gas Chromatograph/Mass Spectrometer

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

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

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A. Opiates

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

i. Extraction Procedure

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

Standards:

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

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

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

Calibration Curve:

i. Negative: 100 µL of deuterated stock solution (200 ng/mL)

ii. 2 ng/mL: 100 µL of deuterated stock solution (200 ng/mL)
25 µL of 20 ng/mL stock solution (dilute 200 ng/mL 1:10)

iii. 4 ng/mL: 100 µL of deuterated stock solution (200 ng/mL)
5 µL of 200 ng/mL stock solution

iv. 10 ng/mL: 100 µL of deuterated stock solution (200 ng/mL)
12.5 µL of 200 ng/mL stock solution

v. 20 ng/mL: 100 µL of deuterated stock solution (200 ng/mL)
25 µL of 200 ng/mL stock solution

vi. 40 ng/mL: 100 µL of deuterated stock solution (200 ng/mL)
50 µL of 200 ng/mL stock solution

vii. 80 ng/mL: 100 µL of deuterated stock solution (200 ng/mL)
100 µL of 200 ng/mL stock solution

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

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

Derivatization

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

ii. Analytical Procedure

Instrument:	Shimadzu GCMS-QP2010
Column:	RTX-XLB (Ultra low bleed, proprietary low polarity phase) 30 m length x 0.25 mm diameter x 0.25 µm film thickness

Injection volume:	2 µL
Injection Temp:	250°C
Injection mode:	Splitless
Column flow:	1.3 mL/min
Linear velocity	43.3 cm/sec
Purge flow:	3 mL/min
Total flow:	49.7 mL/min.

Oven program:	150°C for 1.5 min ramp at 20°C/min to 290°C, hold for 3 min

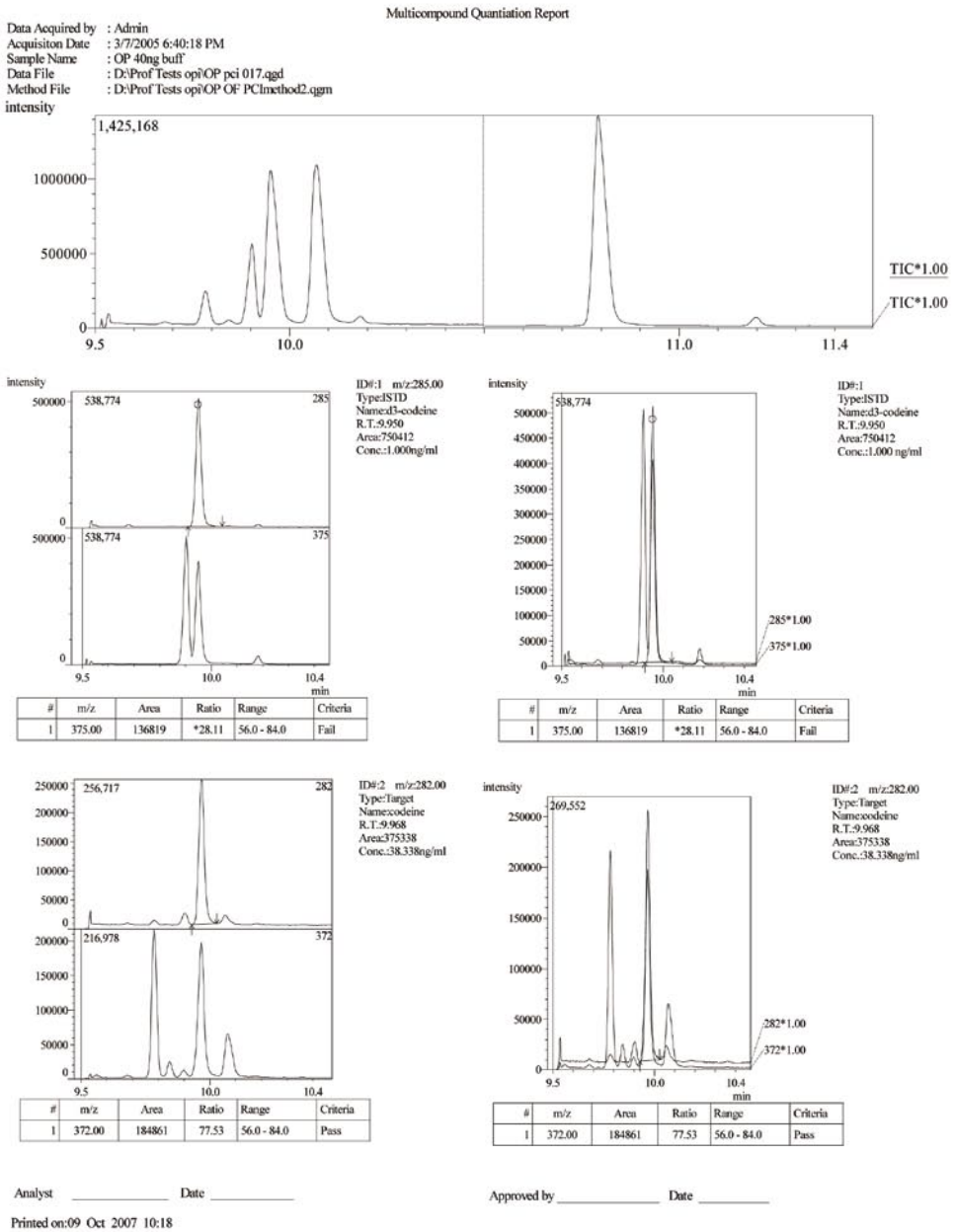
Ion source temperature:	230°C
Interface temperature:	250°C
Mode of operation:	Standard CI mode (positive ion)
Reagent gas:	Methane
Detector gain:	0.8kV above tune
Derivative:	BSTFA
Ions monitored:	375, 285 for deuterated codeine (d3); 372, 282 for codeine; 433, 417 for deuterated morphine (d3); 430, 414 for morphine 402, 343 for deuterated 6-acetylmorphine (d3) 399, 340 for 6-acetylmorphine

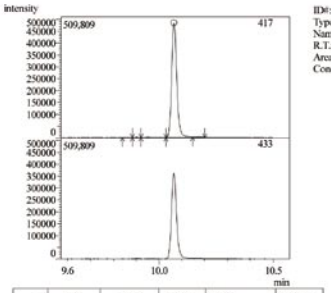
In Electron Impact mode:

Ions monitored: 374.1, 237.1 for deuterated codeine (d3);
371.1, 234.1 for codeine
432.1, 417.1 for deuterated morphine (d3);
429.1, 414.1 for morphine
402.2, 343.2 for deuterated 6-acetylmorphine (d3);
399.2, 340.2 for 6-acetylmorphine

Linearity: 0 – 80 ng/mL; limit of quantitation: 2 ng/mL

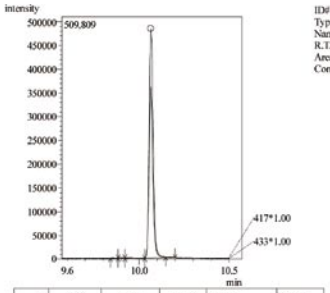
Correlation coefficients: Codeine $r^2 = 0.991$
Morphine: $r^2 = 0.996$
6-acetylmorphine $r^2 = 0.994$





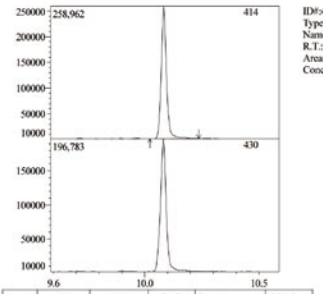
ID#:3 m/z:417.00
Type:ISTD
Name:d3 morphine
R.T.:10.065
Area:765034
Conc.:1.000ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	433.00	345576	75.09	64.0 - 96.0	Pass



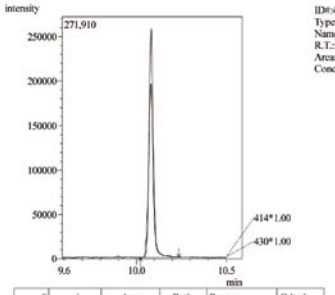
ID#:3
Type:ISTD
Name:d3 morphine
R.T.:10.065
Area:765034
Conc.:1.000 ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	433.00	345576	75.09	64.0 - 96.0	Pass



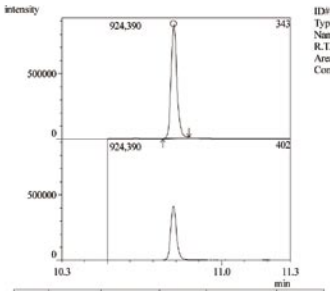
ID#:4 m/z:414.00
Type:Target
Name:morphine
R.T.:10.083
Area:413660
Conc.:37.783ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	430.00	187752	76.11	64.0 - 96.0	Pass



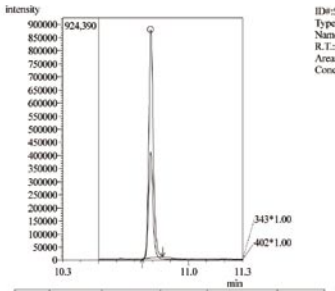
ID#:4 m/z:414.00
Type:Target
Name:morphine
R.T.:10.083
Area:413660
Conc.:37.783ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	430.00	187752	76.11	64.0 - 96.0	Pass



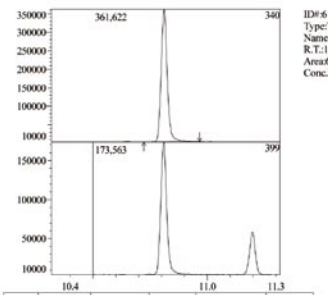
ID#:5 m/z:343.20
Type:ISTD
Name:d3 6-AM
R.T.:10.791
Area:1525779
Conc.:1.000ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	402.30	398373	47.33	32.0 - 48.0	Pass



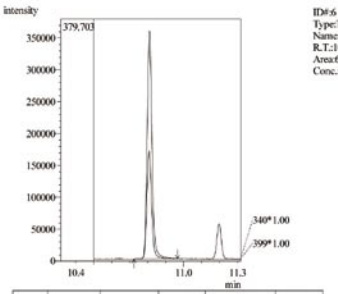
ID#:5
Type:ISTD
Name:d3 6-AM
R.T.:10.791
Area:1525779
Conc.:1.000 ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	402.30	398373	47.33	32.0 - 48.0	Pass



ID#:6 m/z:340.20
Type:Target
Name:6-AM
R.T.:10.811
Area:667791
Conc.:37.814ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	399.30	165803	47.50	32.0 - 48.0	Pass

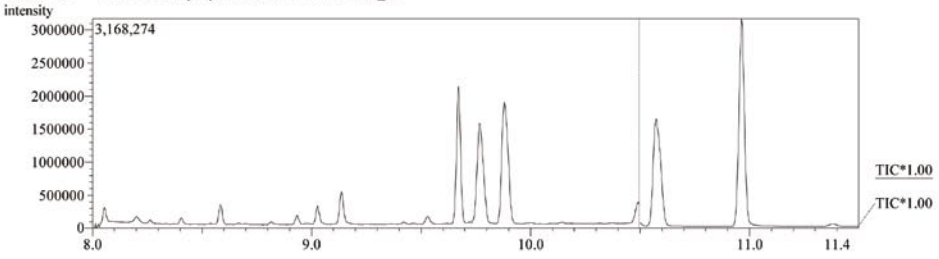


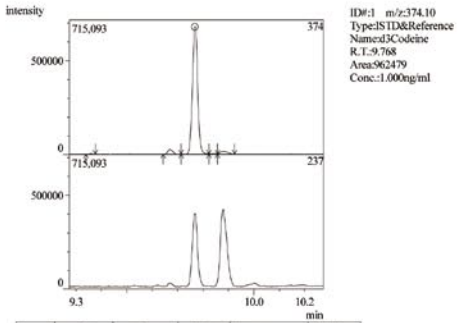
ID#:6 m/z:340.20
Type:Target
Name:6-AM
R.T.:10.811
Area:667791
Conc.:37.814ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	399.30	165803	47.50	32.0 - 48.0	Pass

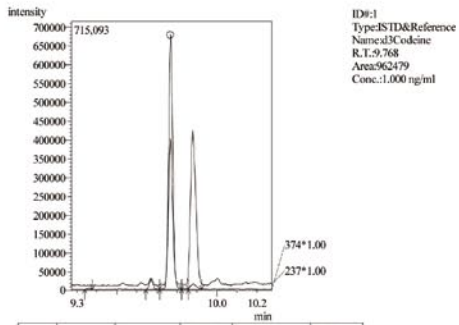
Multicompound Quantiation Report

Data Acquired by : Admin
Acquisiton Date : 5/24/2005 12:07:00 PM
Sample Name : OP 40ng saliva
Data File : D:\Prof Tests opi\OP EI 047.qgd
Method File : D:\Prof Tests opi\Opiate OF buffer curve EI 052405.qgm

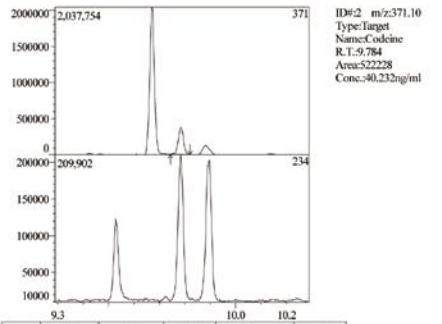




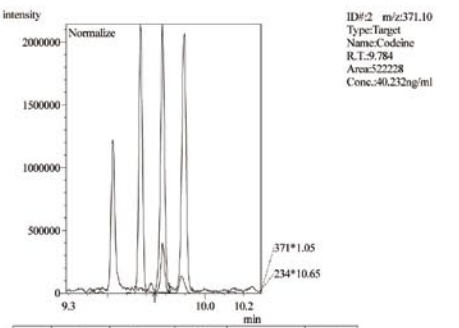
#	m/z	Area	Ratio	Range	Criteria
1	237.10	369229	57.61	30.0 - 70.0	Pass



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



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

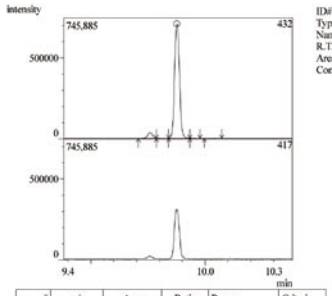


#	m/z	Area	Ratio	Range	Criteria
1	234.10	187358	53.23	30.0 - 70.0	Pass

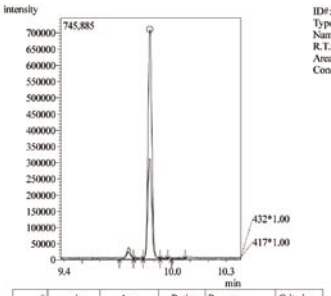
Analyst _____ Date _____

Approved by _____ Date _____

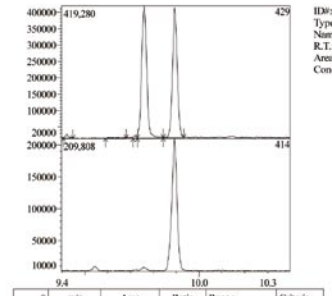
Printed on:09 Oct 2007 11:56



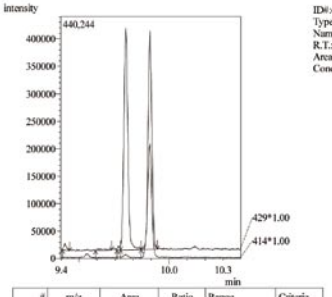
#	m/z	Area	Ratio	Range	Criteria
1	417.10	299799	43.48	20.0 - 60.0	Pass



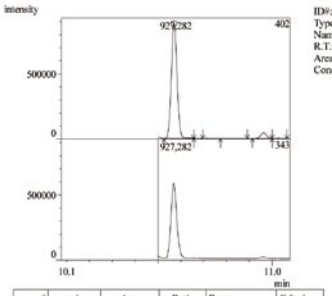
#	m/z	Area	Ratio	Range	Criteria
1	417.10	299799	43.48	20.0 - 60.0	Pass



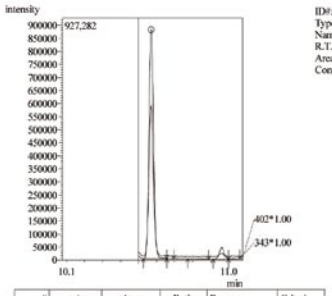
#	m/z	Area	Ratio	Range	Criteria
1	414.10	196101	52.76	30.0 - 70.0	Pass



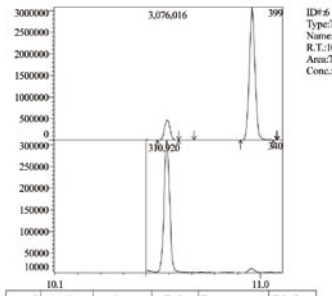
#	m/z	Area	Ratio	Range	Criteria
1	414.10	196101	52.76	30.0 - 70.0	Pass



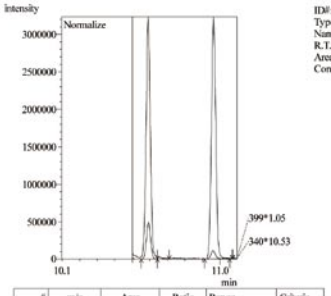
#	m/z	Area	Ratio	Range	Criteria
1	343.20	562311	65.37	40.0 - 80.0	Pass



#	m/z	Area	Ratio	Range	Criteria
1	343.20	562311	65.37	40.0 - 80.0	Pass



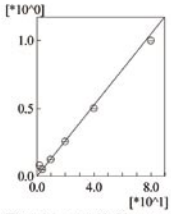
#	m/z	Area	Ratio	Range	Criteria
1	340.20	283571	62.03	40.0 - 80.0	Pass



#	m/z	Area	Ratio	Range	Criteria
1	340.20	283571	62.03	40.0 - 80.0	Pass

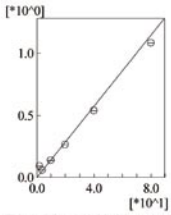
Calibration

ID#:2 Mass:282.00 Name:codeine
f(x)=0.013081*x+0.000000
r2=0.877100



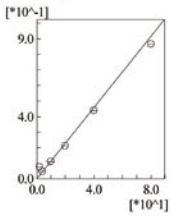
#	Conc. Ratio (ng/ml)	Mean Area Ratio
1	2.000	0.08
2	4.000	0.05
3	10.000	0.12
4	20.000	0.25
5	40.000	0.50
6	80.000	1.00

ID#:4 Mass:414.00 Name:morphine
f(x)=0.014337*x+0.000000
r2=0.880423



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

ID#:6 Mass:340.20 Name:6-AM
f(x)=0.011437*x+0.000000
r2=0.868939



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

B. Cocaine and BZE

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

i. Extraction Procedure

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

Standards:

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

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

Calibration Curve:

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

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

Derivatization

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

ii. Analytical Procedure

GC/MS: Shimadzu GCMS-QP2010

Column: RTX-XLB (Ultra low bleed, proprietary low polarity phase)
30 m length x 0.25 mm diameter x 0.25 µm film thickness

Injection volume:	2 µL
Injection Temp:	260°C
Injection mode:	Splitless
Column flow:	1.32 mL/min
Linear velocity	43.3 cm/sec
Purge flow:	3 mL/min
Total flow:	50.4 mL/min

Oven program: 130°C for 1 min
ramped at 25°C/min to 250°C, held for 3 min
ramped at 30°C/min to 310°C

Ion source temperature:	230°C
Interface temperature:	250°C
Mode of operation:	Standard CI mode (positive ion)
Reagent gas:	Methane
Detector gain:	0.8kV above tune

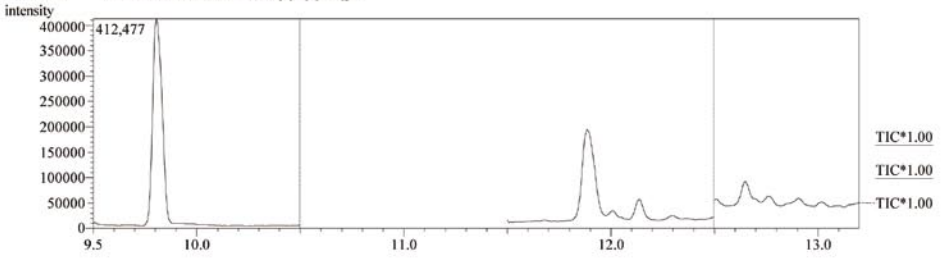
Ions monitored: 307.15,185.15 for deuterated cocaine (d3);
304.15, 182.15 for cocaine;
375.1, 253,1 for deuterated benzoylecgonine (d3);
372.1, 250.1 for benzoylecgonine

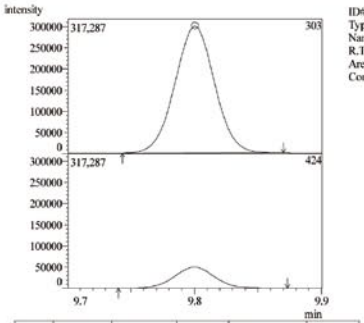
Linearity: 0 – 32 ng/mL; Limit of quantitation: 2 ng/mL

Correlation coefficients: BZE $r^2 = 0.9998$
Cocaine: $r^2 = 0.9985$

Multicompound Quantiation Report

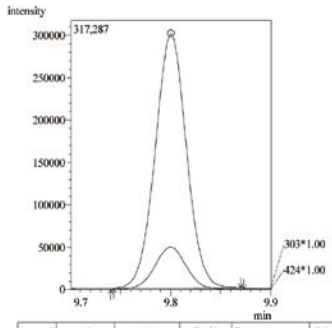
Data Acquired by : Admin
Acquisiton Date : 7/27/2005 8:38:51 PM
Sample Name : 8ng
Data File : D:\Saliva cocaines\coc004.qgd
Method File : D:\Saliva cocaines\saliva cocaine pfpa-pfpoh.qgm





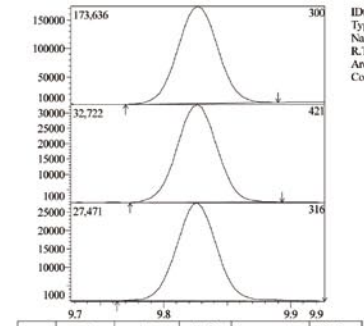
ID#:1 m/z:303.00
Type:ISTD
Name:BZE D3
R.T.:9.800
Area:651489
Conc.:16.000ng/ml

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



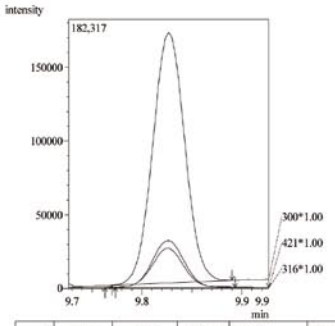
ID#:1
Type:ISTD
Name:BZE D3
R.T.:9.800
Area:651489
Conc.:16.000 ng/ml

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



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

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



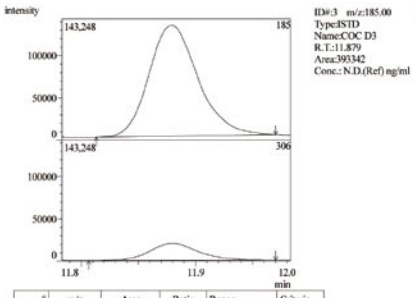
ID#:2 m/z:300.00
Type:Target
Name:BZE
R.T.:9.827
Area:377463
Conc.:7.778ng/ml

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

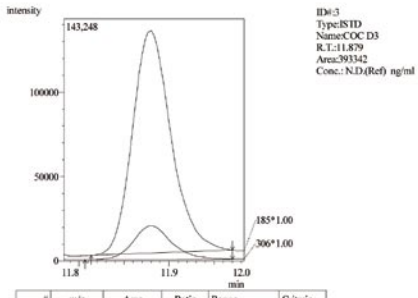
Analyst _____ Date _____

Approved by _____ Date _____

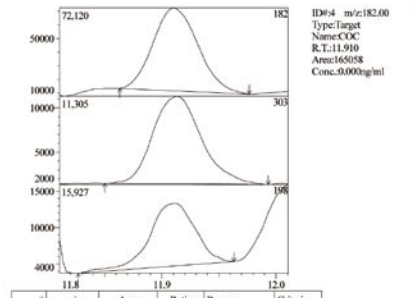
Printed on:22 Oct 2007 12:16



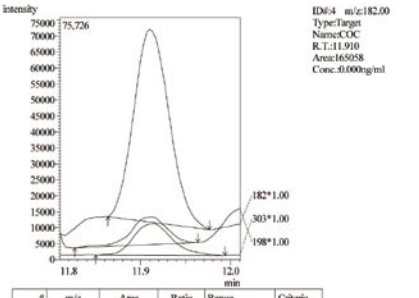
#	m/z	Area	Ratio	Range	Criteria
1	306.05	59467	*15.12	9.6 - 14.4	Fail



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



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



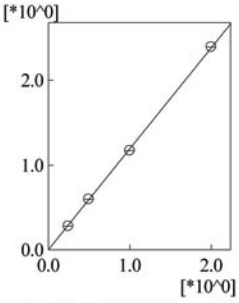
#	m/z	Area	Ratio	Range	Criteria
1	303.05	29240	17.72	14.4 - 21.6	Pass
2	198.00	24216	14.67	10.4 - 15.6	Pass

Calibration

ID#:2 Mass:300.00 Name:BZE

f(x)=1.191832*x+0.000000

rr2=0.999841

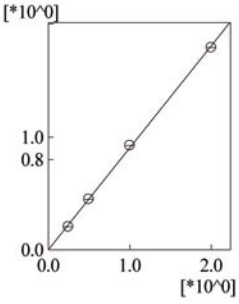


#	Conc. Ratio (ng/ml)	Mean Area Ratio
1	0.250	0.29
2	0.500	0.60
3	1.000	1.17
4	2.000	2.40

ID#:4 Mass:182.00 Name:COC

f(x)=0.898234*x+0.000000

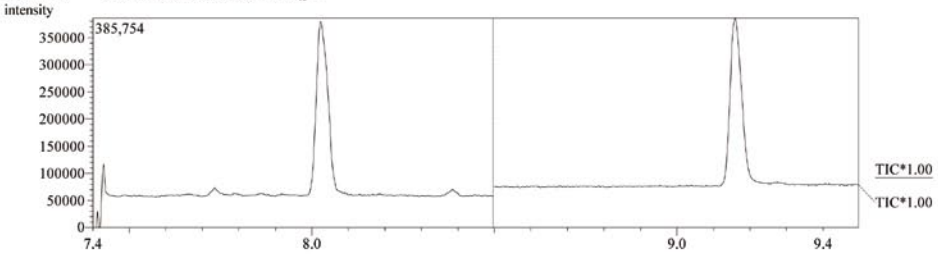
rr2=0.999324

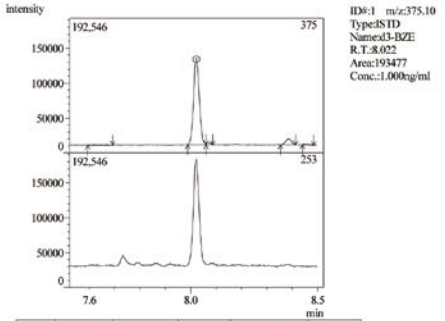


#	Conc. Ratio (ng/ml)	Mean Area Ratio
1	0.250	0.21
2	0.500	0.45
3	1.000	0.92
4	2.000	1.79

Multicompound Quantiation Report

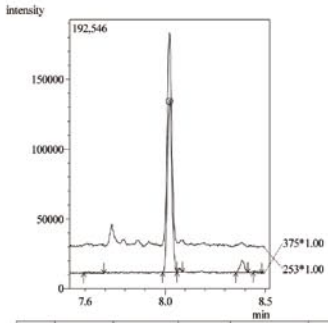
Data Acquired by : Admin
Acquisiton Date : 3/3/2005 6:28:22 PM
Sample Name : coc 8ng/ml
Data File : D:\cocaine saliva DT\coc 003.qgd
Method File : D:\cocaine saliva DT\cocaine HFBA.qgm





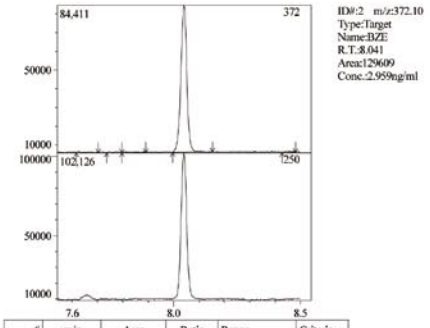
ID#:1 m/z:375.10
Type:ISTD
Name:d3-BZE
R.T.:8.022
Area:193477
Conc.:1.000ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	253.10	144052	121.59	96.0 - 144.0	Pass



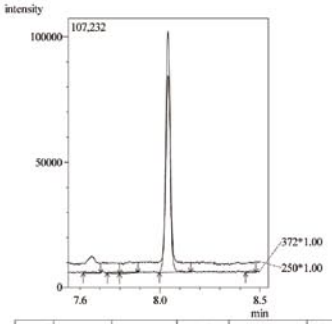
ID#:1
Type:ISTD
Name:d3-BZE
R.T.:8.022
Area:193477
Conc.:1.000 ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	253.10	144052	121.59	96.0 - 144.0	Pass



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

#	m/z	Area	Ratio	Range	Criteria
1	250.10	88836	117.72	96.0 - 144.0	Pass



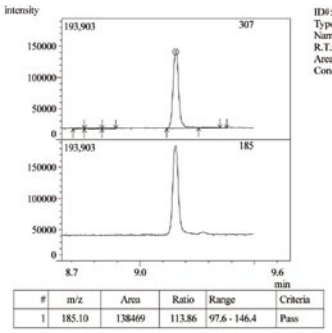
ID#:2 m/z:372.10
Type:Target
Name:BZE
R.T.:8.041
Area:129609
Conc.:2.959ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	250.10	88836	117.72	96.0 - 144.0	Pass

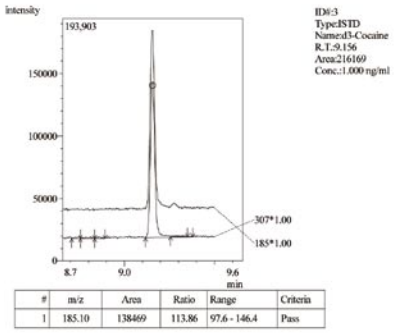
Analyst _____ Date _____

Approved by _____ Date _____

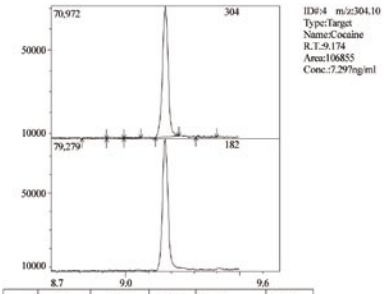
Printed on:22 Oct 2007 12:21



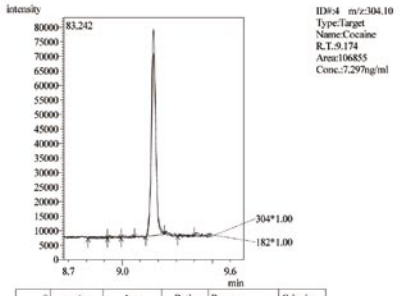
#	m/z	Area	Ratio	Range	Criteria
1	185.10	138469	113.86	97.6 - 146.4	Pass



#	m/z	Area	Ratio	Range	Criteria
1	185.10	138469	113.86	97.6 - 146.4	Pass



#	m/z	Area	Ratio	Range	Criteria
1	182.10	68176	113.18	96.0 - 144.0	Pass



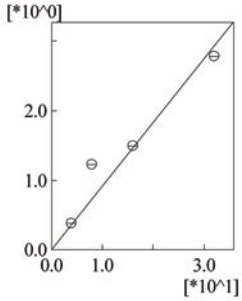
#	m/z	Area	Ratio	Range	Criteria
1	182.10	68176	113.18	96.0 - 144.0	Pass

Calibration

ID#:2 Mass:372.10 Name:BZE

f(x)=0.091169*x+0.000000

r^2=0.950493

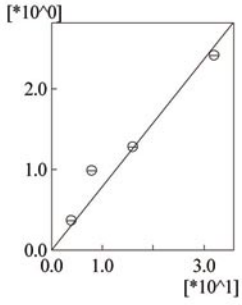


#	Conc. Ratio (ng/ml)	Mean Area Ratio
1	4.000	0.38
2	8.000	1.22
3	16.000	1.49
4	32.000	2.78

ID#:4 Mass:304.10 Name:Cocaine

f(x)=0.078733*x+0.000000

r^2=0.970739



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

C. Phencyclidine

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

i. Extraction Procedure

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

Standards:

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

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

Calibration Curve:

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

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

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

ii. Analytical Procedure

System: Shimadzu GCMS-QP2010

Column: RTX-XLB (Ultra low bleed, proprietary low polarity phase)
30 m length x 0.25 mm diameter x 0.25 µm film thickness

Injection volume:	2 µL
Injection Temp:	250°C
Injection mode:	Splitless
Column flow:	1 mL/min
Linear velocity	36.5 cm/sec
Purge flow:	3 mL/min
Total flow:	38.9 mL/min

Oven program: 60°C for 1 min
ramp at 25°C/min to 200°C, hold for 5.2 min
ramp at 25°C/min to 300°C

Ion source temperature:	230°C
Interface temperature:	250°C
Mode of operation:	Electron Impact
Detector gain:	0.8kV above tune

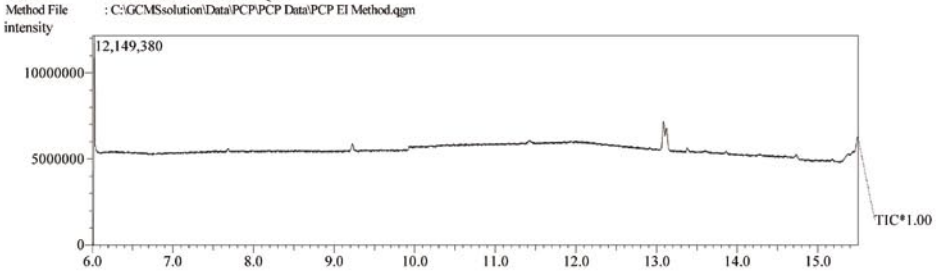
Ions monitored: 205.2, 248.2 for deuterated PCP (d5);
200.2, 243.2, 242.2 for PCP

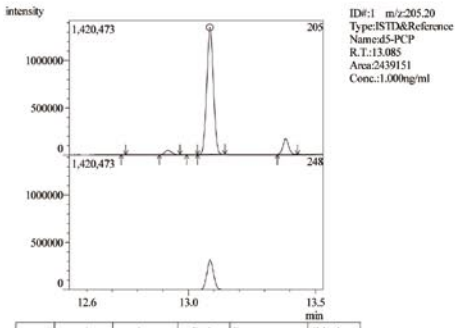
Linearity: 0 – 40 ng/mL; Limit of quantitation: 5 ng/mL

Correlation coefficients: PCP $r^2 = 0.9998$

Multicompound Quantiation Report

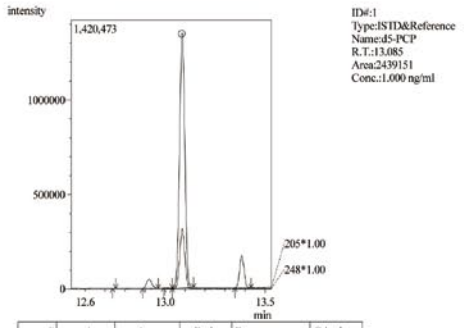
Data Acquired by : Admin
Acquisiton Date : 6/10/2005 12:50:54 PM
Sample Name : 10 ng/mL ext
Data File : D:\PCP\PCP DATA\0101003.QGD
Method File : C:\GCMSsolution\Data\PCP\PCP Data\PCP EI Method.qgm





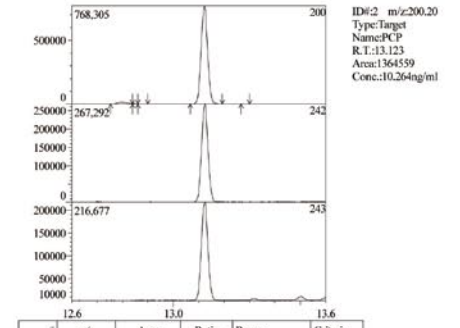
ID#:1 m/z:205.20
Type:ISTD&Reference
Name:d5-PCP
R.T.:13.085
Area:2439151
Conc.:1.000ng/ml

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



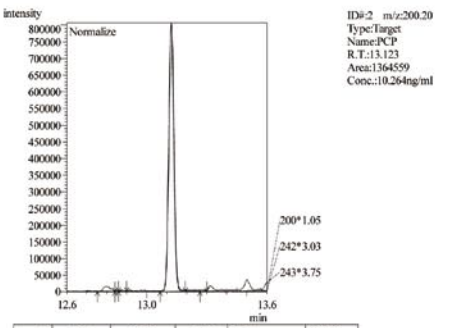
ID#:1
Type:ISTD&Reference
Name:d5-PCP
R.T.:13.085
Area:2439151
Conc.:1.000 ng/ml

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



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

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



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

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

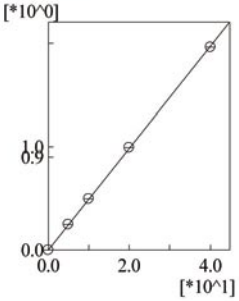
Analyst _____ Date _____

Approved by _____ Date _____

Printed on:22 Oct 2007 14:14

Calibration

ID#:2 Mass:200.20 Name:PCP
f(x)=0.049240*x+0.000000
r^2=0.999932



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

D. Amphetamines

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

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

i. Extraction Procedure

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

Standards:

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

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

Calibration Curve:

i. Negative: 50 µL of deuterated stock solution (250 ng/mL)

ii. 25 ng/mL: 50 µL of deuterated stock solution (250 ng/mL)
25 µL of 250 ng/mL stock solution

iii. 50 ng/mL: 50 µL of deuterated stock solution (250 ng/mL)
50 µL of 250 ng/mL stock solution

iv. 100 ng/mL: 50 µL of deuterated stock solution (250 ng/mL)
100 µL of 250 ng/mL stock solution

v. 200 ng/mL: 50 µL of deuterated stock solution (250 ng/mL)
200 µL of 250 ng/mL stock solution

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

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

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

ii. Analytical Procedure

System: Shimadzu GCMS-QP2010

Column: RTX-XLB (Ultra low bleed, proprietary low polarity phase)
30 m length x 0.25 mm diameter x 0.25 µm film thickness

Injection volume: 2 µL
Injection Temp: 150°C
Injection mode: Splitless
Column flow: 1.3 mL/min
Linear velocity 41.6 cm/sec
Purge flow: 0 mL/min
Total flow: 46.7 mL/min

Oven program: 60°C for 1 min
ramp at 25°C/min to 140°C, hold for 4 min
ramp at 30°C/min to 200°C, hold for 3 min
ramp at 40°C/min to 300°C

Ion source temperature: 220°C
Interface temperature: 250°C
Mode of operation: Electron Impact
Detector gain: 0.8kV above tune

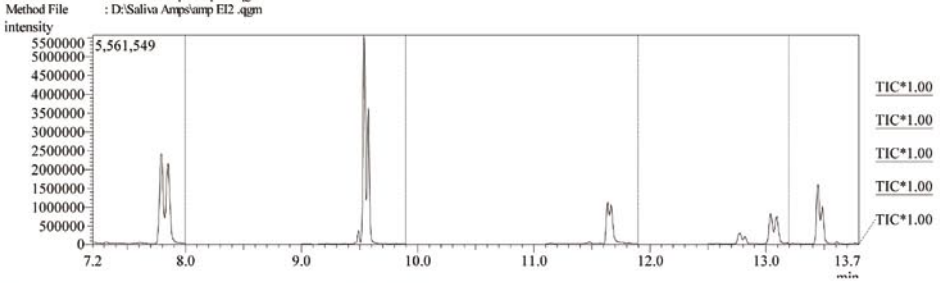
Linearity: 0 – 200 ng/mL; Limit of quantitation: 25 ng/mL
Correlation coefficients: r2 = 0.9998

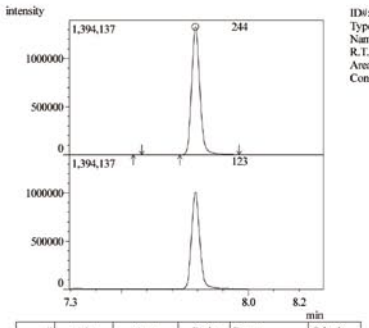
Acquisition Parameter File: Amphetamine Acquisition
Group Entries: Number of Groups: 5

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

Multicompound Quantiation Report

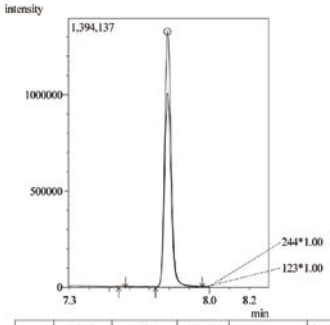
Data Acquired by : Admin
Acquisiton Date : 7/22/2005 2:24:47 PM
Sample Name : 50ng ext saliva
Data File : D:\Saliva Amps\Amp008.qgd
Method File : D:\Saliva Amps\amp EI2 .qgm





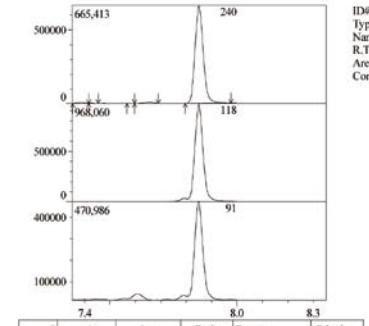
ID#:1 m/z:244.10
Type:ISTD&Reference
Name:Amp d5
R.T.:7.792
Area:3003557
Conc.:100.000ng/ml

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



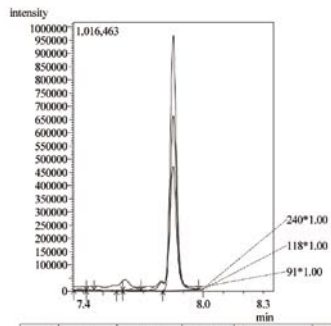
ID#:1
Type:ISTD&Reference
Name:Amp d5
R.T.:7.792
Area:3003557
Conc.:100.000 ng/ml

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



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

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



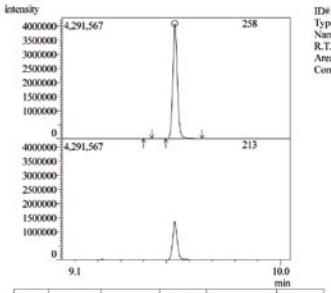
ID#:2 m/z:240.10
Type:Target
Name:Amp
R.T.:7.850
Area:1490283
Conc.:48.413ng/ml

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

Analyst _____ Date _____

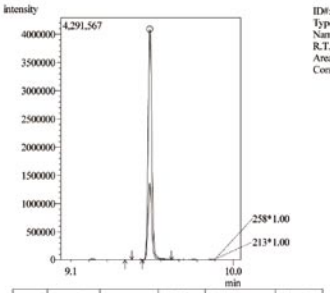
Approved by _____ Date _____

Printed on:22 Oct 2007 14:28



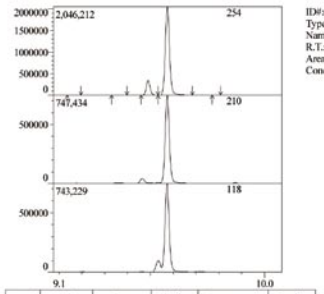
ID#:3 m/z:258.05
Type:ISTD&Reference
Name:Meth d5
R.T.:9.538
Area:5727452
Conc.:100.000ng/ml

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



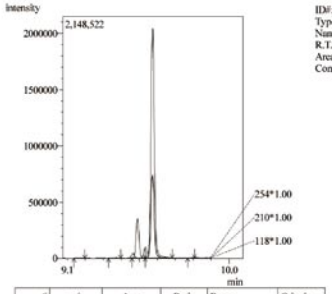
ID#:3
Type:ISTD&Reference
Name:Meth d5
R.T.:9.538
Area:5727452
Conc.:100.000 ng/ml

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



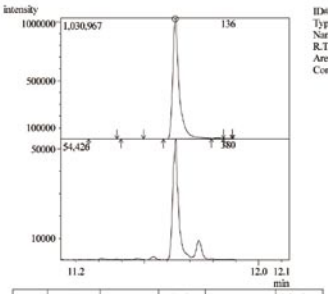
ID#:4 m/z:254.10
Type:Target
Name:Meth
R.T.:9.575
Area:2716094
Conc.:48.700ng/ml

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



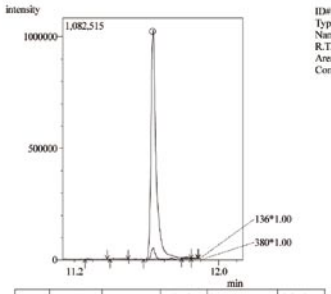
ID#:4 m/z:254.10
Type:Target
Name:Meth
R.T.:9.575
Area:2716094
Conc.:48.700ng/ml

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



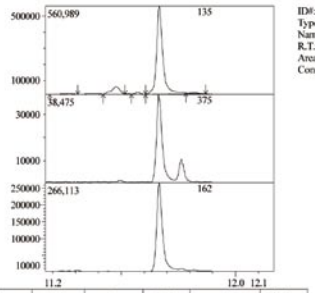
ID#:5 m/z:136.15
Type:ISTD&Reference
Name:MDA d5
R.T.:11.635
Area:2048897
Conc.:100.000ng/ml

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



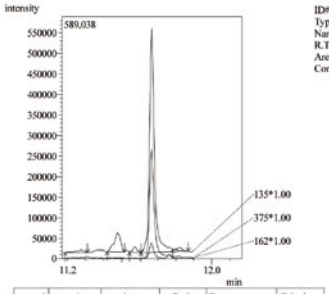
ID#:5
Type:ISTD&Reference
Name:MDA d5
R.T.:11.635
Area:2048897
Conc.:100.000 ng/ml

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



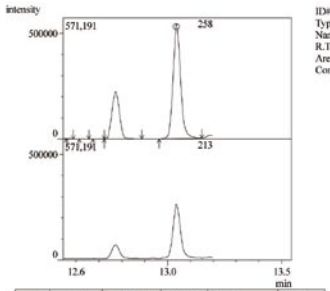
ID#:6 m/z:135.15
Type:Target
Name:MDA
R.T.:11.666
Area:1070892
Conc.:59.130ng/ml

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



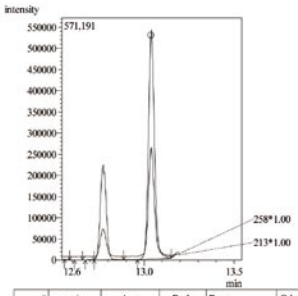
ID#:6 m/z:135.15
Type:Target
Name:MDA
R.T.:11.666
Area:1070892
Conc.:59.130ng/ml

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



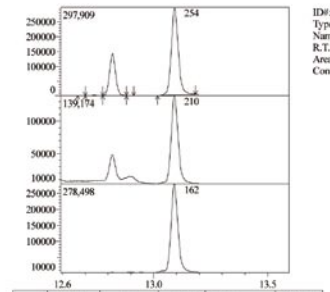
ID#:7 m/z:258.10
Type:ISTD&Reference
Name:MDMA d5
R.T.:13.039
Area:1233181
Conc.:100.000ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	213.05	249995	47.39	36.0 - 54.0	Pass



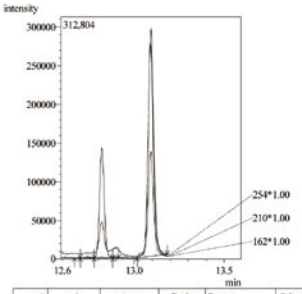
ID#:7
Type:ISTD&Reference
Name:MDMA d5
R.T.:13.039
Area:1233181
Conc.:100.000 ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	213.05	249995	47.39	36.0 - 54.0	Pass



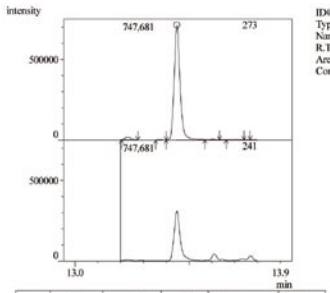
ID#:8 m/z:254.05
Type:Target
Name:MDMA
R.T.:13.093
Area:623577
Conc.:49.728ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	210.05	132363	46.52	36.0 - 54.0	Pass
2	162.15	263071	92.47	72.0 - 108.0	Pass



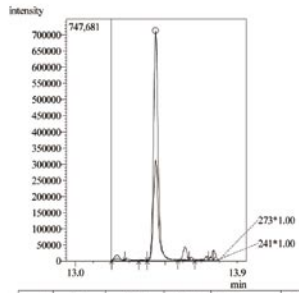
ID#:8 m/z:254.05
Type:Target
Name:MDMA
R.T.:13.093
Area:623577
Conc.:49.728ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	210.05	132363	46.52	36.0 - 54.0	Pass
2	162.15	263071	92.47	72.0 - 108.0	Pass



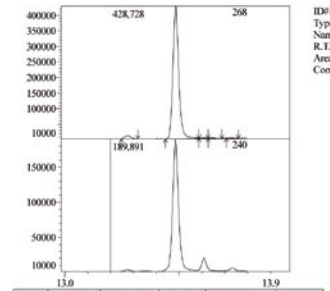
ID#:9 m/z:273.15
Type:ISTD&Reference
Name:MDEA d5
R.T.:13.447
Area:1282110
Conc.:100.000ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	241.10	296445	43.15	32.0 - 48.0	Pass



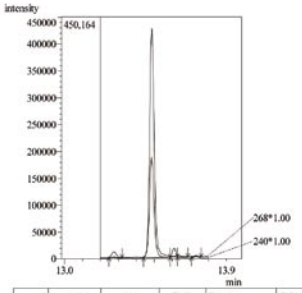
ID#:9
Type:ISTD&Reference
Name:MDEA d5
R.T.:13.447
Area:1282110
Conc.:100.000 ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	241.10	296445	43.15	32.0 - 48.0	Pass



ID#:10 m/z:268.10
Type:Target
Name:MDEA
R.T.:13.485
Area:759874
Conc.:51.784ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	240.10	179213	43.35	34.4 - 51.6	Pass

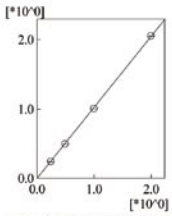


ID#:10 m/z:268.10
Type:Target
Name:MDEA
R.T.:13.485
Area:759874
Conc.:51.784ng/ml

#	m/z	Area	Ratio	Range	Criteria
1	240.10	179213	43.35	34.4 - 51.6	Pass

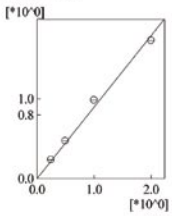
Calibration

ID#:2 Mass:240.10 Name:Amp
f(x)=1.020487*x+0.000000
rr2=0.999958



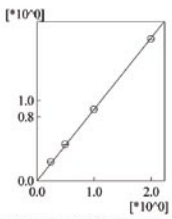
#	Conc. Ratio (ng/ml)	Mean Area Ratio
1	0.250	0.24
2	0.500	0.50
3	1.000	1.01
4	2.000	2.05

ID#:4 Mass:254.10 Name:Meth
f(x)=0.890418*x+0.000000
rr2=0.994056



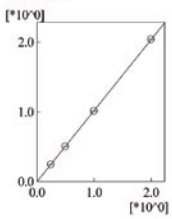
#	Conc. Ratio (ng/ml)	Mean Area Ratio
1	0.250	0.24
2	0.500	0.47
3	1.000	0.98
4	2.000	1.73

ID#:6 Mass:135.15 Name:MDA
f(x)=0.883944*x+0.000000
rr2=0.999984



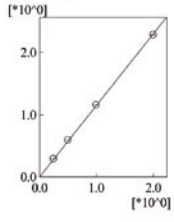
#	Conc. Ratio (ng/ml)	Mean Area Ratio
1	0.250	0.24
2	0.500	0.45
3	1.000	0.89
4	2.000	1.76

ID#:8 Mass:254.05 Name:MDMA
f(x)=1.016862*x+0.000000
rr2=0.999971



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

ID#:10 Mass:268.10 Name:MDEA
f(x)=1.144507*x+0.000000
rr2=0.999914



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

E. THC

Recommended Federal Cut-off: THC 2 ng/mL

i. Extraction Procedure

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

Standards:

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

Calibration Curve:

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

`

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

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

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

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

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

ii. Analytical Procedure

System: Shimadzu GCMS-QP2010

Column: RTX-XLB (Ultra low bleed, proprietary low polarity phase)
30 m length x 0.25 mm diameter x 0.25 µm film thickness

Injection volume:	2 µL
Injection Temp:	250°C
Injection mode:	Splitless
Column flow:	1.39 mL/min
Linear velocity	44.4 cm/sec
Total flow:	50 mL/min
Oven program:	125°C for 0.2 min ramp at 20°C/min to 250°C, hold for 3 min ramp at 30°C/min to 300°C

Ion source temperature:	220°C
Interface temperature:	280°C
Mode of operation:	Electron impact
Detector gain:	0.8kV above tune

Ions monitored:	389, 374 for deuterated THC (d3); 386, 371 for THC

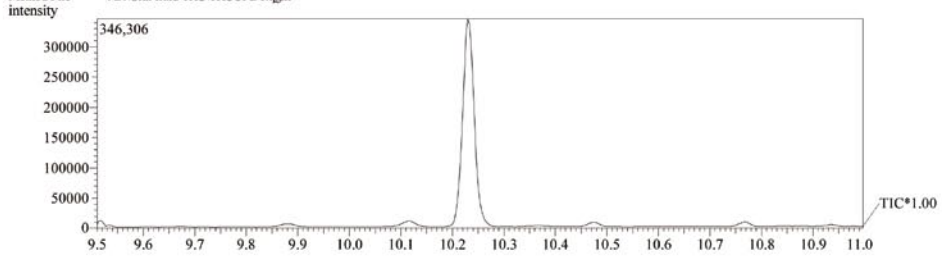
The limit of quantitation of the method was 1 ng/mL

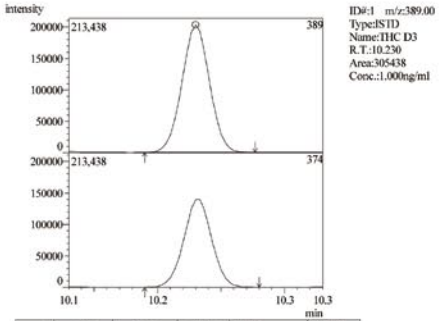
Reference

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

Multicompound Quantiation Report

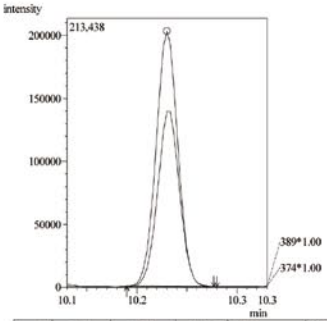
Data Acquired by : Admin
Acquisiton Date : 2/17/2005 4:18:34 PM
Sample Name : 2 ng cal
Data File : D:\Oral fluid THC\03.qgd
Method File : D:\Oral fluid THC\THCOFDT.qgm





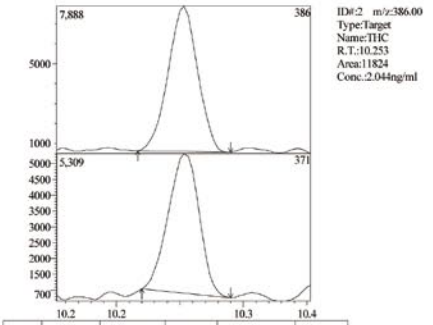
ID#:1 m/z:389.00
Type:ISTD
Name:THC D3
R.T.:10.230
Area:305438
Conc.:1.000ng/ml

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



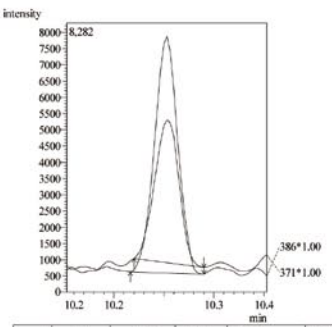
ID#:1
Type:ISTD
Name:THC D3
R.T.:10.230
Area:305438
Conc.:1.000 ng/ml

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



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

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



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

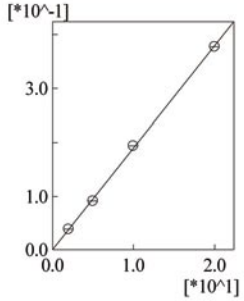
#	m/z	Area	Ratio	Range	Criteria
1	371.00	7448	62.99	48.3 - 89.7	Pass

Analyst _____ Date _____

Approved by _____ Date _____

Printed on:22 Oct 2007 14:42

Calibration
ID#:2 Mass:386.00 Name:THC
f(x)=0.018938*x+0.000000
rr2=0.999598



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

F. Methadone

i. Extraction Procedure

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

Standards:

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

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

Calibration Curve:

i. Negative: 100 µL of deuterated stock solution (200 ng/mL)

ii. 10 ng/mL: 100 µL of deuterated stock solution (200 ng/mL)
12.5 µL of 200 ng/mL stock solution

iii. 20 ng/mL: 100 µL of deuterated stock solution (200 ng/mL)
25 µL of 200 ng/mL stock solution

iv. 40 ng/mL: 100 µL of deuterated stock solution (200 ng/mL)
50 µL of 200 ng/mL stock solution

v. 80 ng/mL: 100 µL of deuterated stock solution (200 ng/mL)
100 µL of 200 ng/mL stock solution

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

ii. Analytical Procedure

System: Shimadzu GCMS-QP2010

Column: RTX-XLB (Ultra low bleed, proprietary low polarity phase)
30 m length x 0.25 mm diameter x 0.25 µm film thickness

Injection volume:	2 µL
Injection Temp:	250°C
Injection mode:	Splitless
Column flow:	1.2 mL/min
Linear velocity	40.8 cm/sec
Total flow:	46.4 mL/min

Oven program: 110°C for 1 min
ramp at 30°C/min to 290°C, hold for 1.5 min

Ion source temperature:	230°C
Interface temperature:	300°C
Mode of operation:	Standard electron impact (EI) mode
Detector gain:	0.8kV above tune

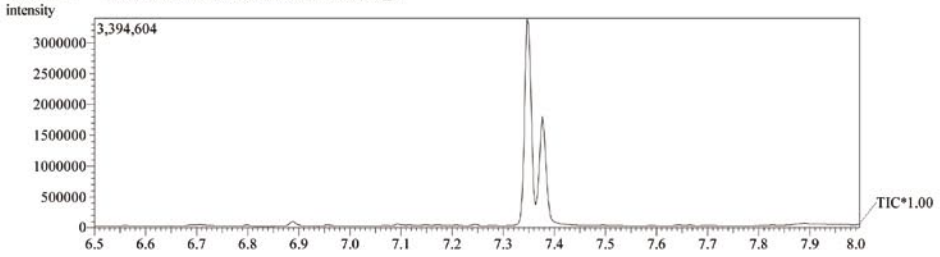
Ions monitored: 303.1, 78.15 for deuterated methadone (d9)
294.0, 72.1 for methadone

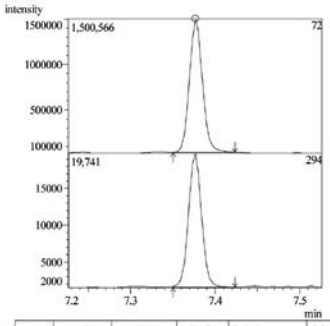
Correlation coefficient: Methadone $r^2 = 0.9999446$

Linearity: 0 – 80 ng/mL; limit of quantitation 10 ng/mL

Multicompound Quantiation Report

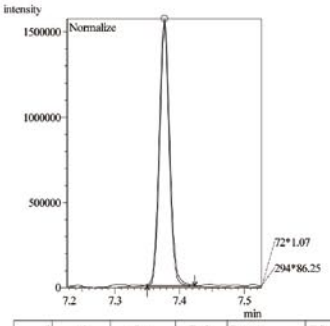
Data Acquired by : Admin
Acquisiton Date : 7/19/2005 4:07:58 PM
Sample Name : 20ng/ml
Data File : D:\Methadone\MED082.qgd
Method File : C:\GCMSsolution\Data\Methadone\METHADONE4.qgm





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

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



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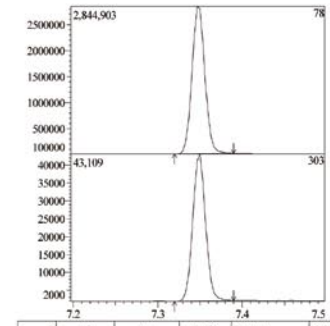
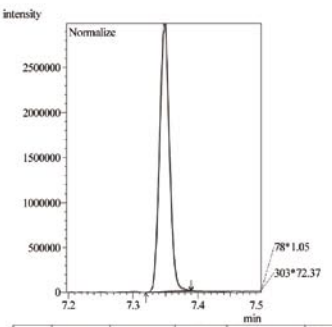


ID#:2 m/z:78.15
Type:ISTD
Name:IMethadone d9
R.T.:7.348
Area:3020157
Conc.:40.000ng/mL

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



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

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

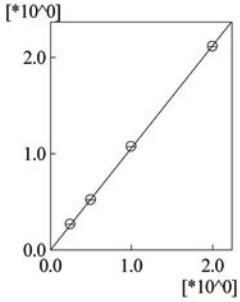
Analyst _____ Date _____

Approved by _____ Date _____

Printed on:22 Oct 2007 14:50

Calibration

ID#:1 Mass:72.15 Name:Methadone
f(x)=1.056983*x+0.000000
rr2=0.999854



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

ID#:2 Mass:78.15 Name:IMethadone d9
f(x)=?
rr2=0.000000

ISTD

About the GCMS-QP2010 Series

- **GCMS-QPQ2010 Plus**
- **GCMS-QP2010S**

The most sensitive GC/MS on the market, Shimadzu's GCMS-QP2010 Plus delivers better performance and reliability with unsurpassed hardware capabilities and powerful, flexible software. Utilizing many of the high-end features of the GCMS-QP2010 Plus, the GCMS-QP2010S offers high throughput and excellent productivity, providing users with an excellent performance-to-cost ratio. The GCMS-QP2010 Series features:

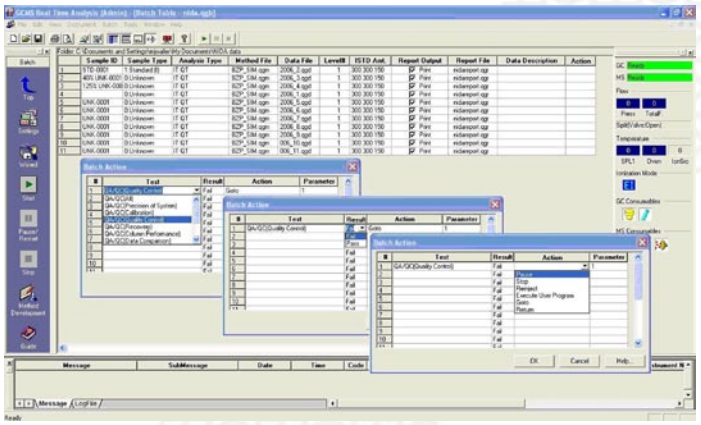
- Extended mass range
- Powerful vacuum
- 20 temperature ramps
- High-performance split-flow TMP
- Patented constant linear velocity

GCMSsolution software combines ease of use and versatility for increased productivity. Features include:

- FASST (Fast Automated Scan/SIM Technology)
- AART (Automatic Adjustment of Retention Time)
- COAST (Creation of Automatic SIM (Scan/SIM) Table)
- Intelligent batch sequencing
- Powerful quantitation
- Flexible, customized reporting

Toxicology Reporting Module

This newly-developed reporting module enables high-throughput drug testing laboratories to address specific productivity and QA/QC requirements. Corrective action plans established by most regulatory methods can be automated and samples may be evaluated during batch sequence injections for a variety of QA/QC parameters. In addition, laboratory productivity is enhanced by automating the data processing and reporting tasks associated with the daily workflow of drug screening analysis.



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