WA20259

The Development of LC/MS Methods for Determination of Polar Drugs of Abuse in Biological Samples

Michael S. Young and Kevin M. Jenkins

Waters Corporation, 34 Maple Street, Milford, MA 01757

© 2002 Waters Corp.

Introduction

The use of certain classes of drugs known collectively as "club drugs" has been increasing worldwide. This term refers to drugs being used by young adults at all-night dance parties such as "raves" or "trances," dance clubs, and bars. Among the more popular drugs used for this purpose are gamma-hydroxybutyrate (GHB) and 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy). Disturbingly, there have been reports of the criminal use of GHB to facilitate sexual assault (date rape). The most common methods of analysis for these compounds utilize GC or GC-MS analysis after a cumbersome derivatization step. In this presentation, we will demonstrate LC-MS methods for the rapid and straightforward determination of GHB and MDMA and metabolites in biological fluids. Also, we will discuss the application of these methods for the determination of other drugs of abuse.

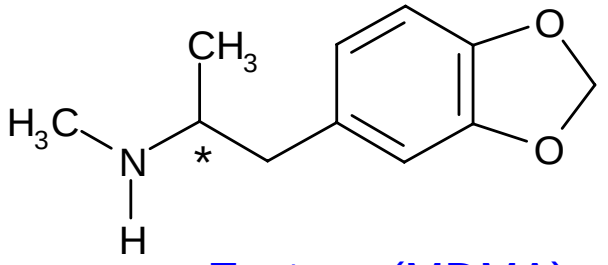
© 2002 Waters Corp.

MDMA (Ecstasy) and Metabolites

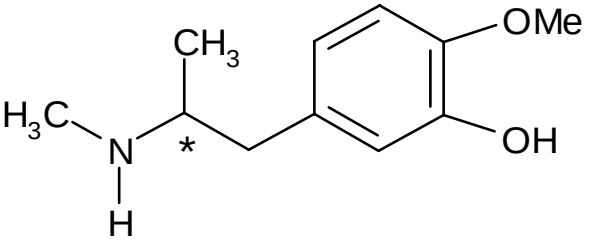
An LC-MS method was developed for determination of MDMA (3,4-methylenedioxymethamphetamine) and its metabolites (MDA, 3,4, methylenedioxyamphetamine and HMMA, N,α-dimethyl-(3-methoxy-4-hydroxybenzene) ethanamine in urine. A mixed-mode cation-exchange SPE cartridge (Oasis® MCX) was utilized for extraction and cleanup prior to LC-MS analysis. The concentration range investigated spanned from 0.10 to 20 μg/mL with recoveries ranging from 88-108% for all analytes. Complete resolution of MDMA, MDA and HMMA as well as the internal standard was accomplished in less than 10 minutes. The quantitation limits (LOQ) were: MDMA = 0.06 μg/mL; MDA = 0.12 μg/mL; and HMMA 0.05 μg/mL.

© 2002 Waters Corp.

What is Ecstasy?



Ecstasy (MDMA)



Ecstasy Metabolite (HMMA)

Ecstasy (MDMA) is a synthetic, psychoactive drug with both stimulant (amphetamine-like) and hallucinogenic (mescaline-like) properties.

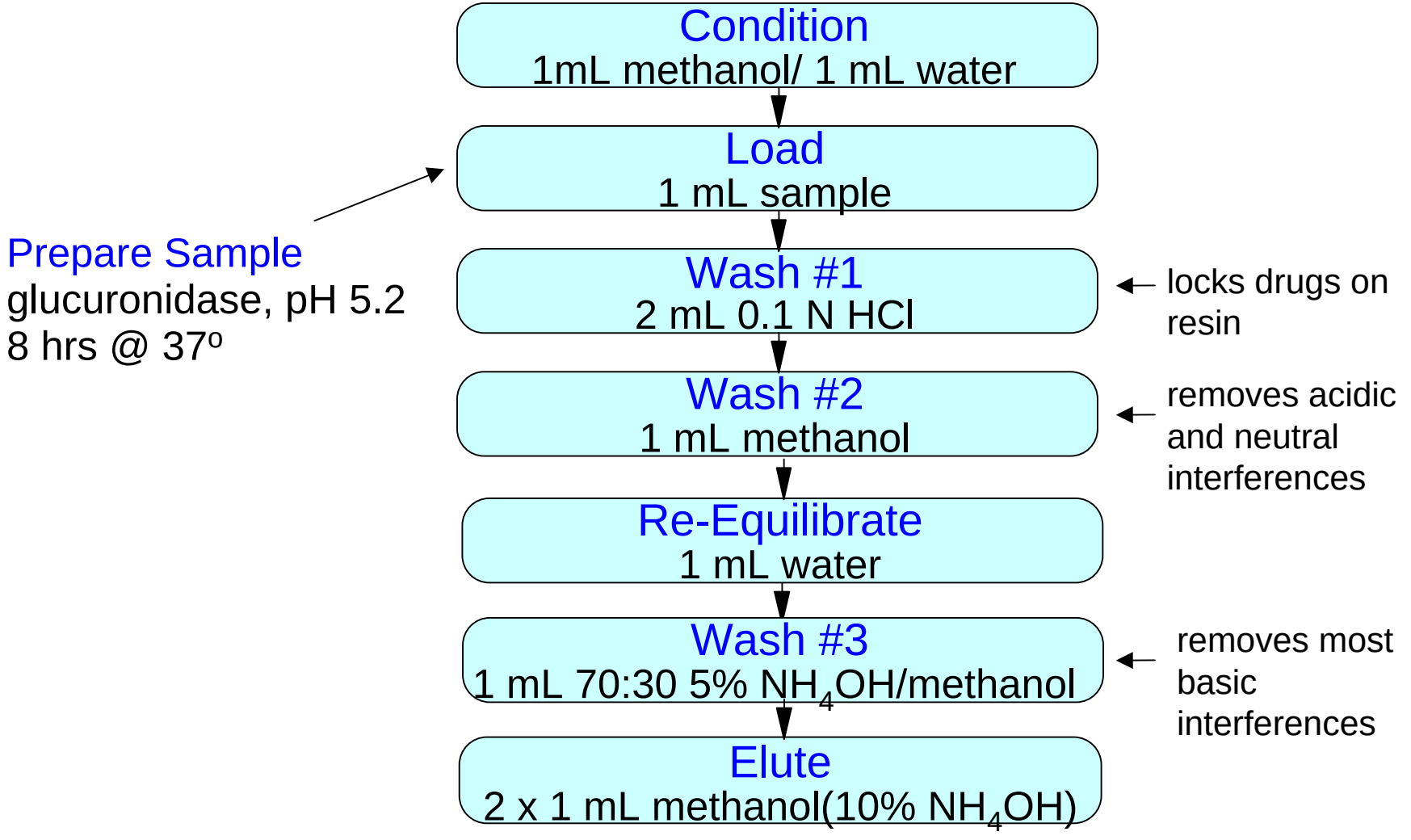
Its chemical structure (3-4 methylenedioxymethamphetamine) is similar to methamphetamine, methylenedioxyamphetamine (MDA), and mescaline.

MDMA is neurotoxic. In high doses it can cause a sharp increase in body temperature (malignant hyperthermia) leading to muscle breakdown and kidney and cardiovascular system failure.

© 2002 Waters Corp.

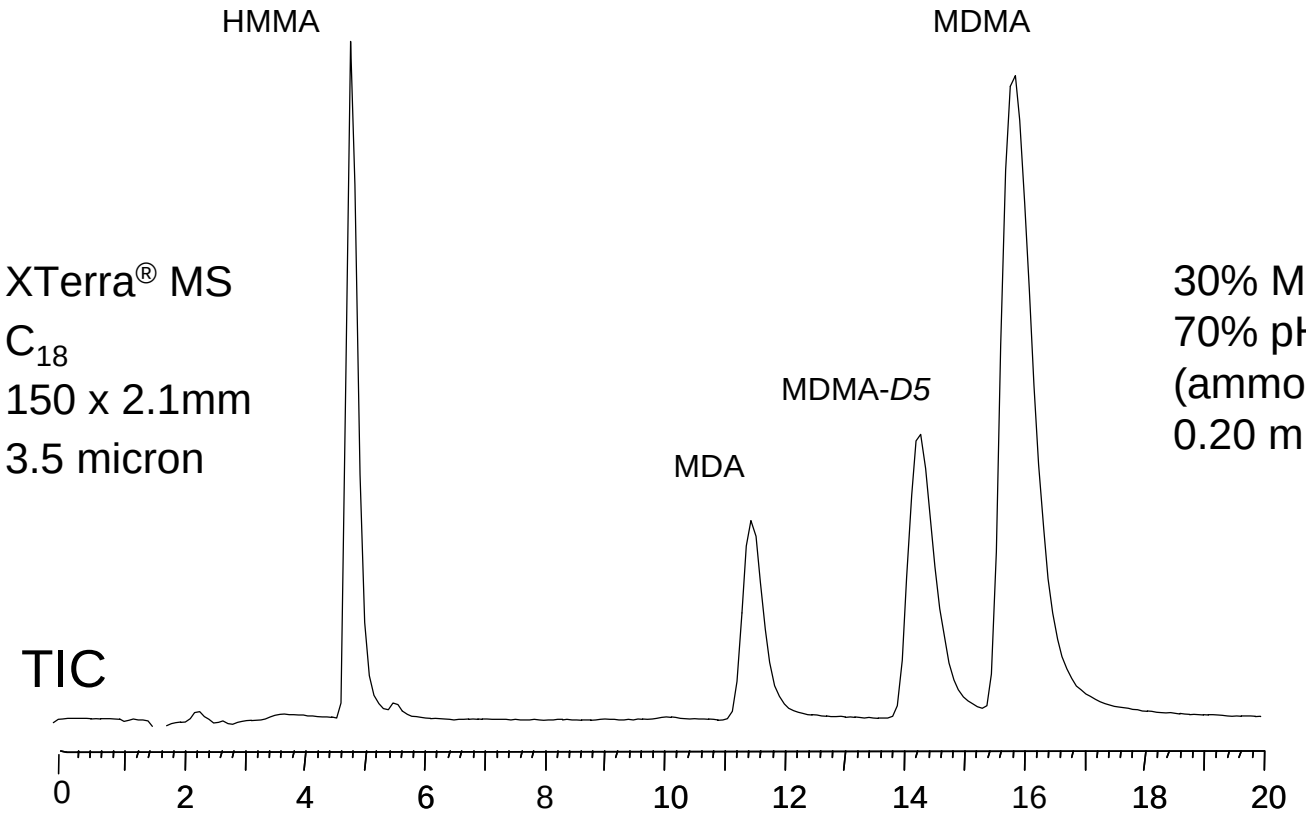
SPE Procedure for MDMA and Metabolites

Oasis® MCX Mixed-Mode Cation Exchange



© 2002 Waters Corp.

LC-MS for Ecstasy and Metabolites
HPLC Conditions



XTerra® MS
C_{18}
150 x 2.1mm
3.5 micron

30% Methanol
70% pH 9 buffer
(ammonium bicarbonate, 20mM))
0.20 mL/min

Analysis at pH 9 allows good peak shape and maximum retention for basic compounds with no modifiers that can interfere with LC-MS analysis.

Do not try this with traditional silica based columns!

© 2002 Waters Corp.

LC-MS for Ecstasy and Metabolites
MS Conditions

LC/MS System

Waters 2690 Separations Module interfaced to a Waters/Micromass ZQ™ mass spectometer

Acquisition parameters.

Capillary (kV)	3.00
Extractor (V)	3.00
RF Lens (V)	0.1
Source Temp (°C)	150
Desolvation Temp (°C)	350
Cone gas Flow (L/hr)	50
Delsovation Gas Flow	500
High Mass resolution	15.0
Low Mass Resolution	15.0
Ion Energy	0.1
Multiplier (V)	650
Interchannel delay:	0.1 s
Span:	0.1 Da
Dwell	0.3 s

MDMA

Ion (m/z)	Cone (V)	Delay (s)
194.11	25.0	0.05
163.08	37.5	0.05
135.00	55.0	0.05

HMMA

Ion (m/z)	Cone (V)	Delay (s)
196.16	20.0	0.05
165.08	37.5	0.05
137.00	55.0	0.05

MDA

Ion (m/z)	Cone (V)	Delay (s)
180.10	20.0	0.05
163.08	37.5	0.05
135.00	55.0	0.05

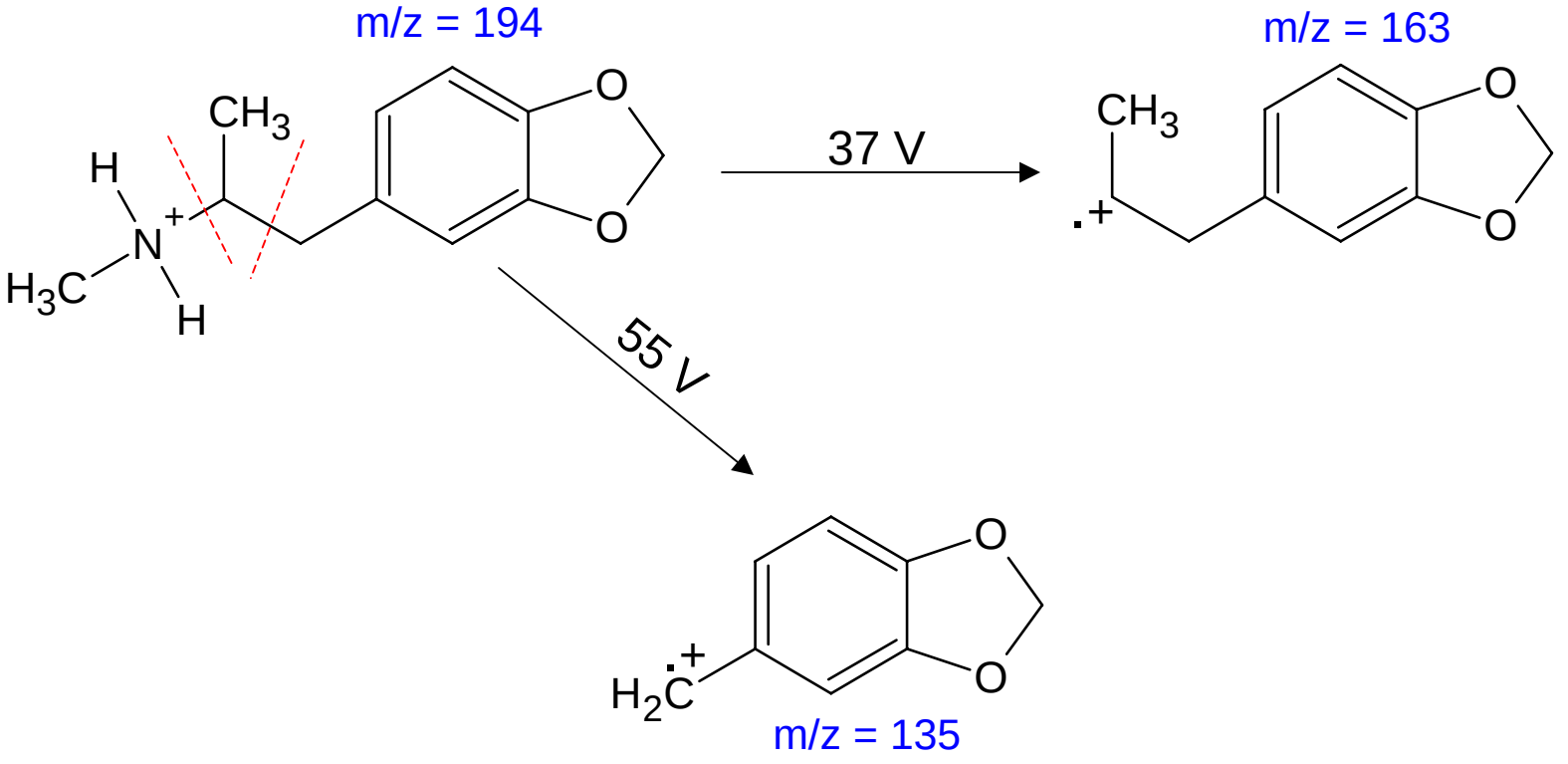
MDMA-D_5 (Internal Standard)

Ion (m/z)	Cone (V)	Delay (s)
199.20	25.0	0.10
165.10	35.0	0.10
137.00	55.0	0.10

© 2002 Waters Corp.

Mass Spectrometry
Fragmentation of MDMA (ESI+)

The following fragmentation was observed from in-source collision induced dissociation (CID) of MDMA. Similar fragmentation patterns were observed for the metabolites.



© 2002 Waters Corp.

Why is LC-MS attractive?
Compare Methodologies

- **GC-MS Method** (recovery 50 – 75%, 2 hours)
  - perform SPE
  - evaporate to dryness
  - derivatize with heptafluorobutyric anhydride
  - evaporate again to remove excess derivatizing agent
  - take up residue with solvent
  - inject

- **LC-MS Method** (recovery 85 – 95%, 20 minutes)
  - perform SPE
  - dilute with mobile phase
  - inject

Enforcement methods require mass-spectral confirmation of identity (two fragment ions recommended)

© 2002 Waters Corp.

LC-MS for Ecstasy and Metabolites

Results (n = 12 for each level)

Analyte	Recovery (%)	Concentration (µg/mL)	RSD (%)
MDMA	108.0	0.10	9.8
	89.3	0.50	4.9
	88.1	1.25	4.6
	98.8	2.50	3.7
	99.9	5.00	5.7
MDA	103.0	0.10	8.8
	84.2	0.50	13.9
	83.8	1.25	9.8
	95.4	2.50	9.0
	104.5	5.00	13.4
	93.7	20.00	13.1
HMMA	90.5	0.04	8.2
	88.1	0.25	4.5
	84.8	0.50	5.4
	94.8	1.00	4.0
	100.0	2.00	5.3
	97.9	8.00	11.4

© 2002 Waters Corp.

Real Forensic Sample

DUI Case:

A 21-year-old male was arrested for driving under the influence (DUI) of drugs after being involved in an accident.

A plastic bag containing approximately 5 g ecstasy was found under the driver's seat.

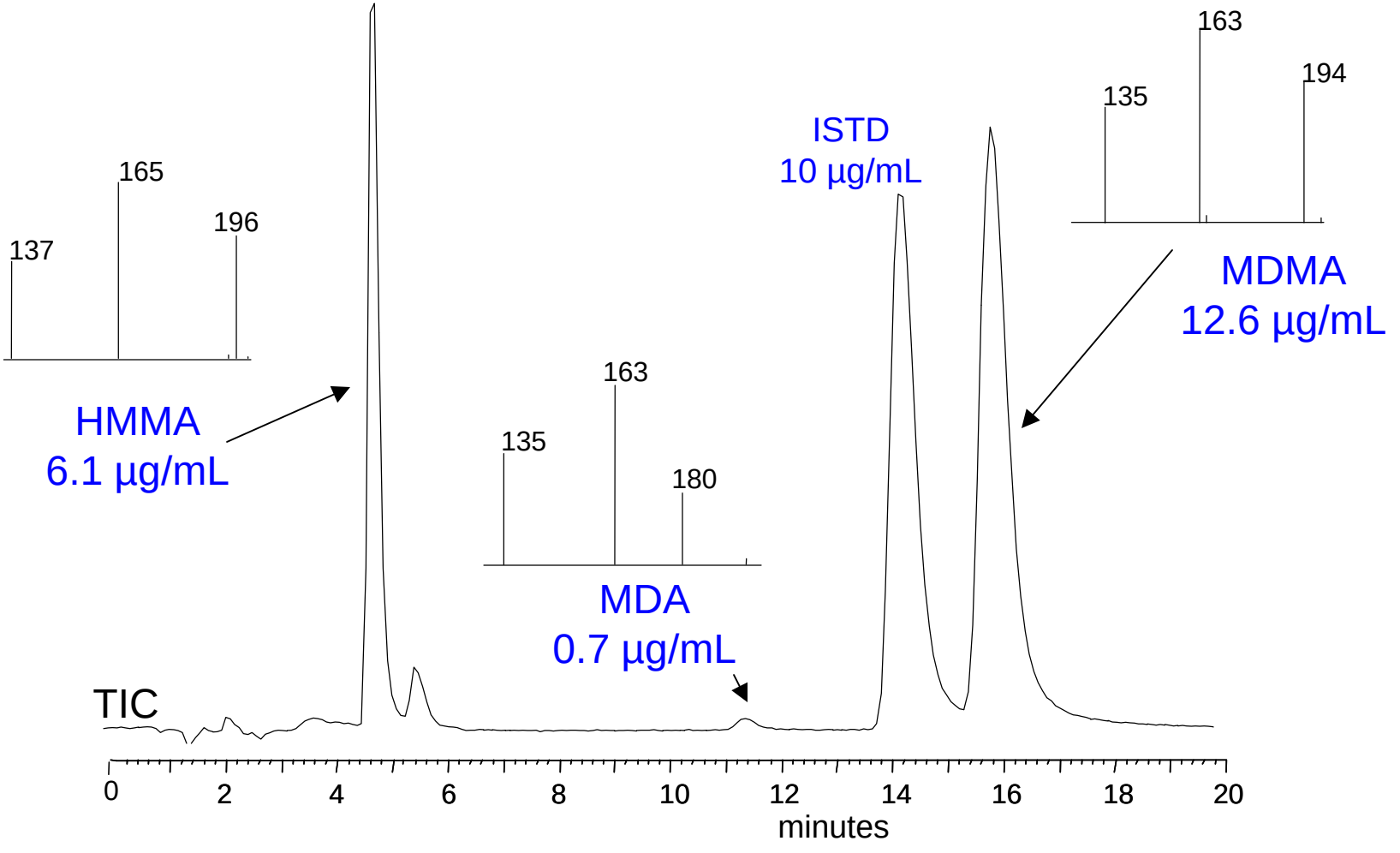
The subject's urine tested positive for benzoylecgonine (cocaine), 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (cannabis), and MDMA (ecstasy).

The Police Lab found 11.3 μg/mL of MDMA by GC-MS.

At Waters, we found 12.6 μg/mL of MDMA by LC-MS.

© 2002 Waters Corp.

Real Forensic Sample
LC-MS Analysis



© 2002 Waters Corp.

Gamma-Hydroxybutyric Acid (GHB) Gamma-Butyrolactone (GBL)

Because of its intoxicating effects such as euphoria, enhancement of sensory perceptions, and reduction of inhibitions, the use of gamma-hydroxybutyric acid (GHB) as a recreational drug has been increasing worldwide. Gamma-butyrolactone (GBL), a product of the internal esterification of GHB, may also be utilized for such purposes as an alternative source of GHB.

Prior analytical methods that employ GC-MS techniques for determination of GHB and GBL require cumbersome derivitization steps and do not provide rapid determination of both species in one analysis.

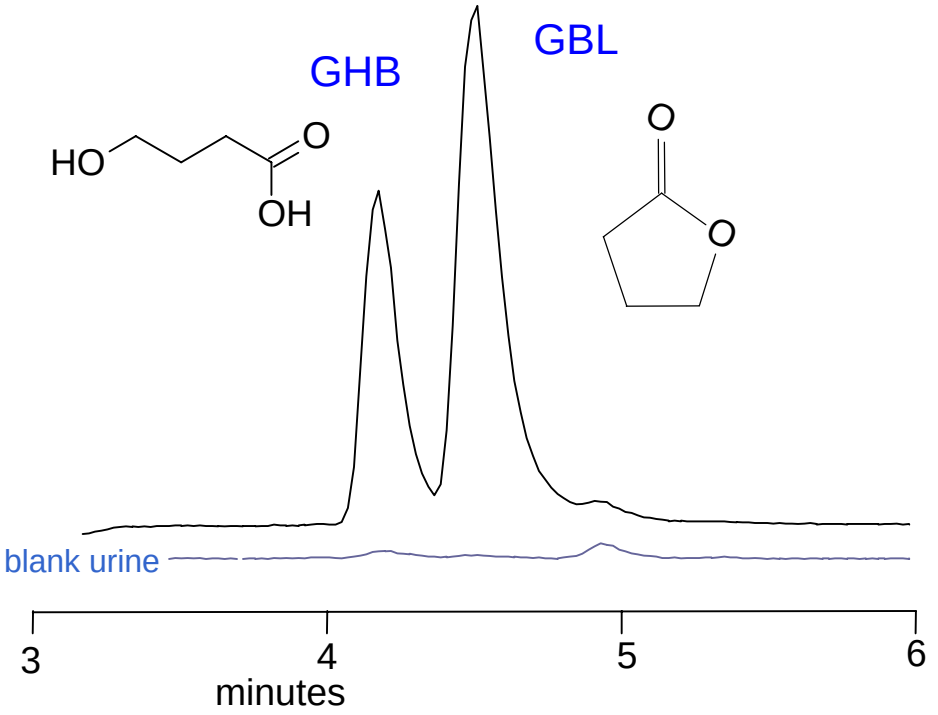
In this work, GHB and GBL are determined simultaneously in human urine by LC-MS after cleanup on an Oasis® MCX cartridge. A 0.15 mL aliquot of the urine sample is simply loaded onto the preconditioned cartridge and is then eluted with 1 mL of mobile phase.

© 2002 Waters Corp.

LC/MS Determination of GHB and GBL in Human Urine (25 ppm)

HPLC Method

Column:	XTerra® MS C_{18}, 2.1 x 250 mm
Mobile Phase:	A: 0.1% formic acid; 90%
	B: methanol 10%
Flow Rate:	0.2 mL/min
Detection:	MS (ESI+)
Injection:	10 µL
Temperature	30 °C



Oasis® MCX SPE Method

3 cc 60 mg

Condition/Equilibrate
1 mL methanol/ 1 mL water

↓

Load
0.15 mL of urine

↓

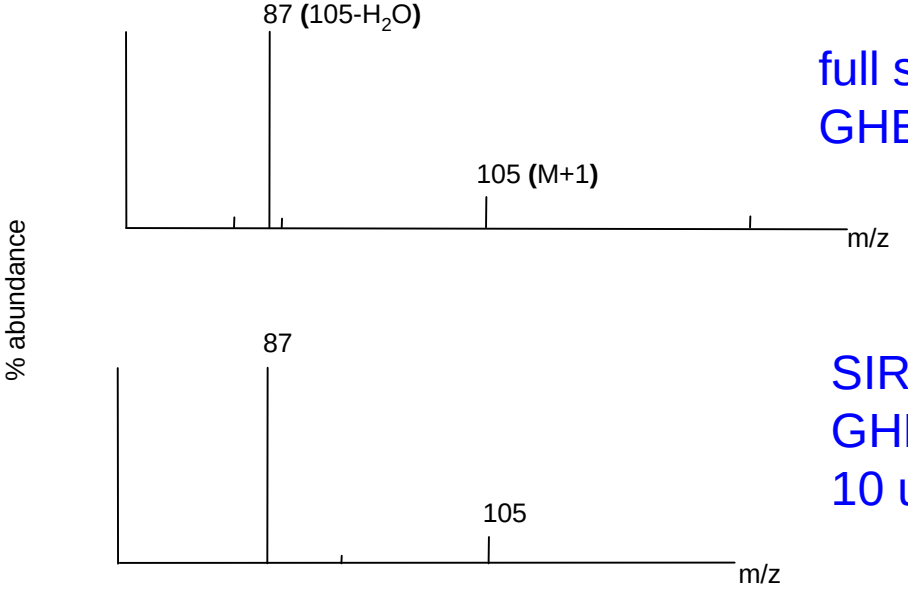
Elute
1 ml mobile phase

© 2002 Waters Corp.

Mass Spectrometry of GHB in Human Urine (10 ppm)

- The LC/MS system was a Waters 2690 Separations Module interfaced to a Waters/Micromass ZQ™ mass spectometer operated in the positive electrospray mode (ESI+)
- Urine analyses were performed using selected-ion recording (SIR)
- The ions monitored for GHB were:
  (m/z) 105 (M + 1) at cone voltage of 10 V
  (m/z) 87 (M + 1 - H_2O) at cone voltage of 25 V

- The ion monitored for GBL was m/z 87 (M + 1) at cone voltage of 25 V



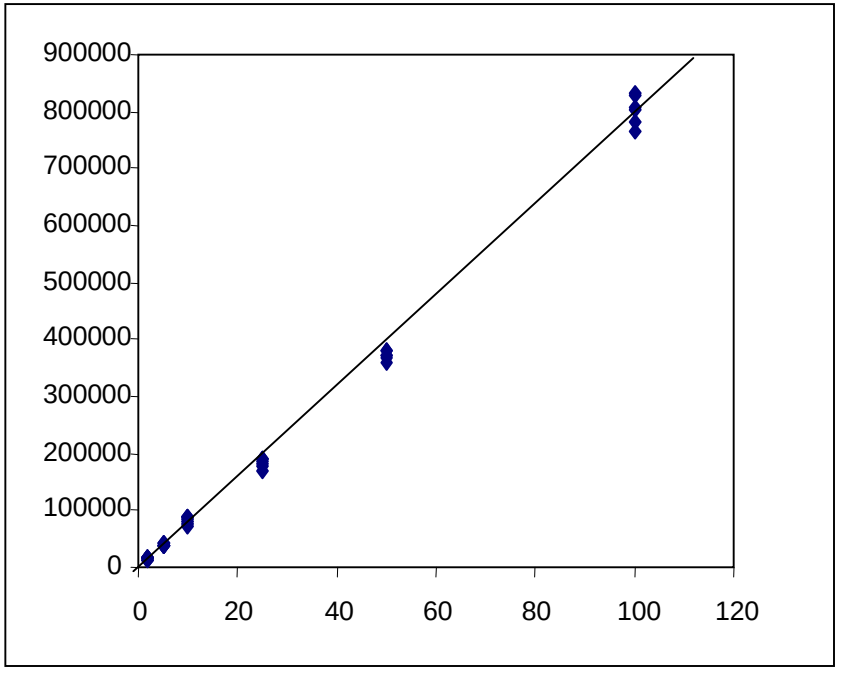
full scan mass spectrum of GHB from 50 ug/mL std

SIR mass spectrum of GHB from urine spiked at 10 ug/mL

© 2002 Waters Corp.

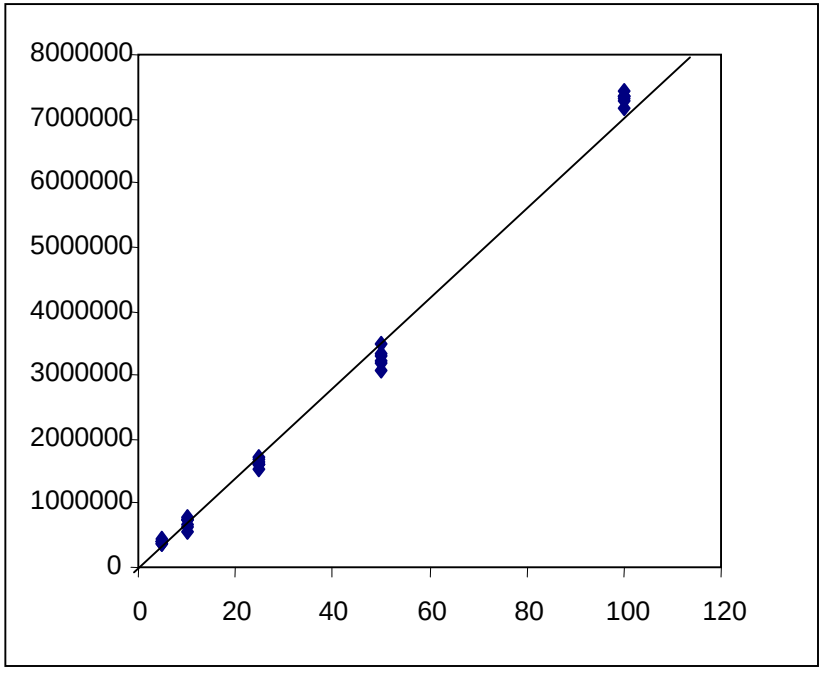
LC/MS Determination of GHB and GBL in Human Urine - Calibration

GHB



spike level (µg/mL)	calculated	% RSD (n=6)
2	2.0 (99%)	7.1
5	5.1 (102%)	4.7
10	10.6 (106%)	8.8
25	23.6 (94.5%)	4.4
50	48.3 (96.5%)	2.0
100	104 (104%)	3.3

GBL



spike level (µg/mL)	calculated[2]	% RSD (n=6)
5	4.9 (97.2%)	7.0
10	9.8 (98.3%)	11
25	22.5 (89.9%)	4.2
50	46.0 (91.9%)	4.3
100	104 (104%)	1.1

© 2002 Waters Corp.

Conclusions

- LC/MS is an attractive alternative to GC based methods for ecstasy and metabolites
  - low quantitation limits
  - high sample throughput
  - fast sample preparation
    - no derivatization required

- LC/MS is an attractive alternative to GC based methods for GHB and GBL
  - fast sample preparation (1 minute)
    - no derivatization required
    - no filtration required
    - GHB and GBL are determined in one analysis
  - high sample throughput

© 2002 Waters Corp.