WA20714

Waters OASIS®
SAMPLE EXTRACTION PRODUCTS

The Development of LC/MS Methods for Determination of MDMA (Ecstasy) and Metabolites in Biological Samples

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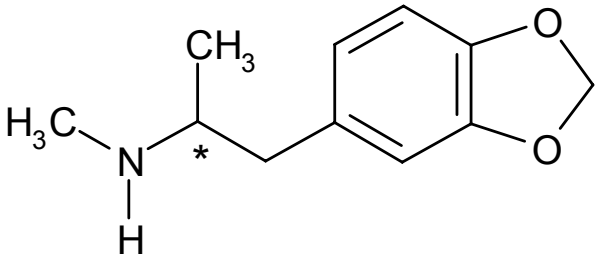
Introduction

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 is 3,4-methylenedioxy-methamphetamine (MDMA, Ecstasy). The most common methods of analysis for this compound and its metabolites utilize GC or GC/MS analysis after cumbersome derivatization steps. In this presentation, we will demonstrate LC-MS methods for a much more rapid and straightforward determination of GHB and MDMA and metabolites in biological fluids.

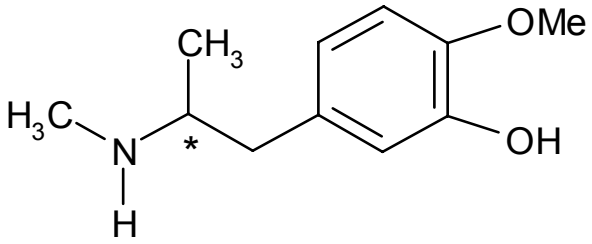
MDMA (Ecstasy) and Metabolites

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.

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.

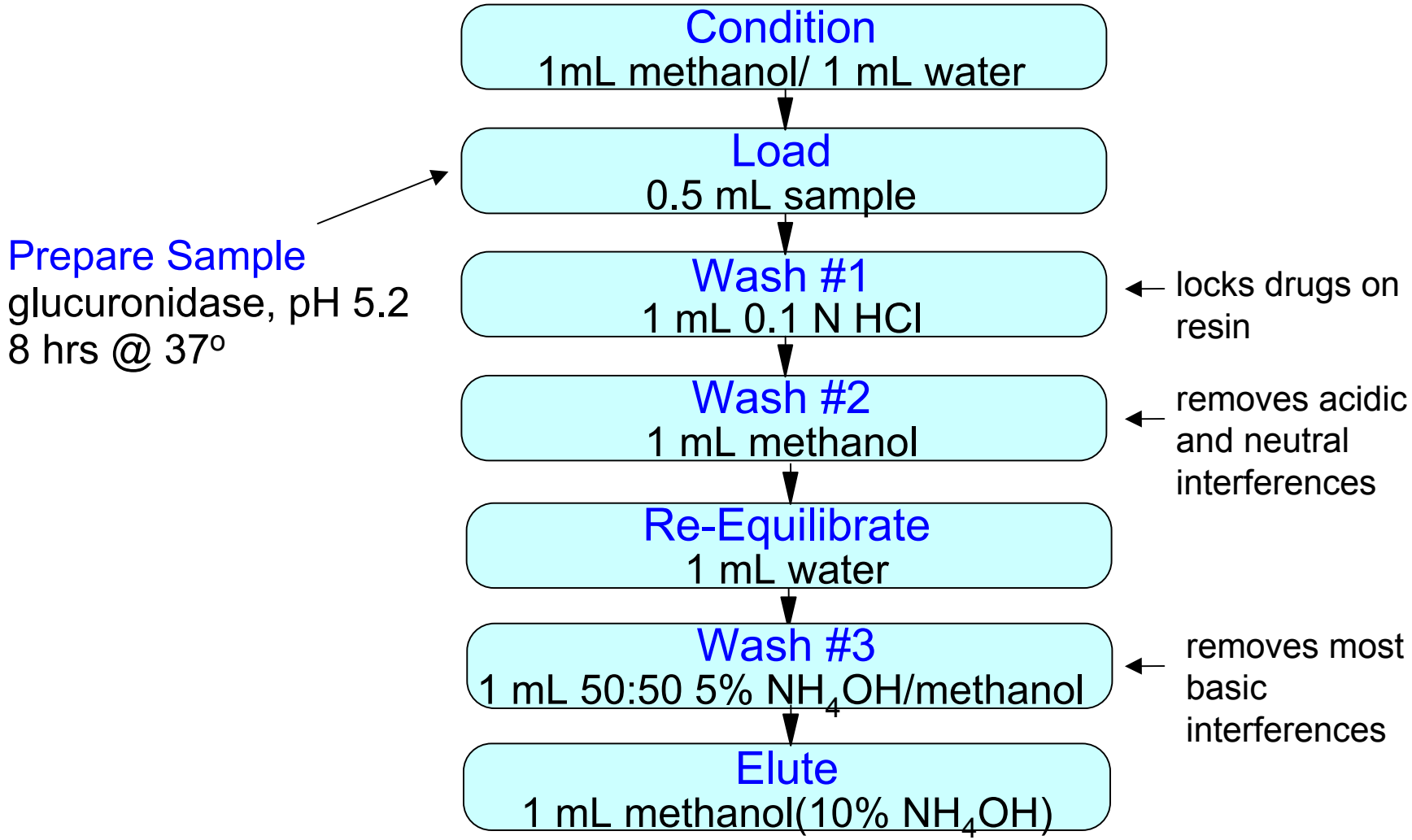
Its chemical structure (3-4 methylenedioxymethamphetamine) is similar to methamphetamine, methylenedioxyamphetamine (MDA), and mescaline.

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.

SPE Procedure for MDMA and Metabolites

Oasis® MCX (30 mg Cartridge)

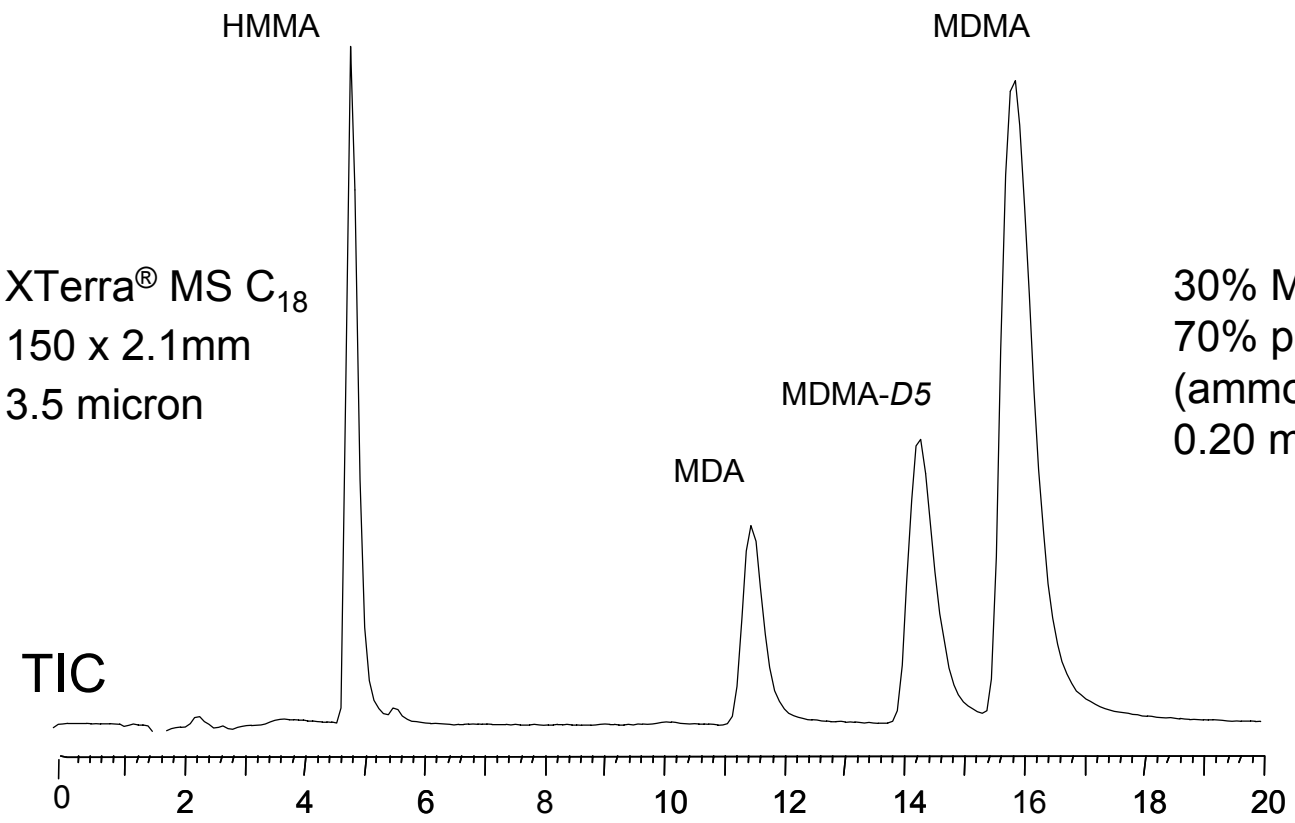
Mixed-Mode Cation Exchange



Dilute the extract with 2 mL of 20 mM ammonium bicarbonate buffer (pH 9.0) and inject 15 µL

Waters OASIS® SAMPLE EXTRACTION PRODUCTS

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!

XTerra™ COLUMNS

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

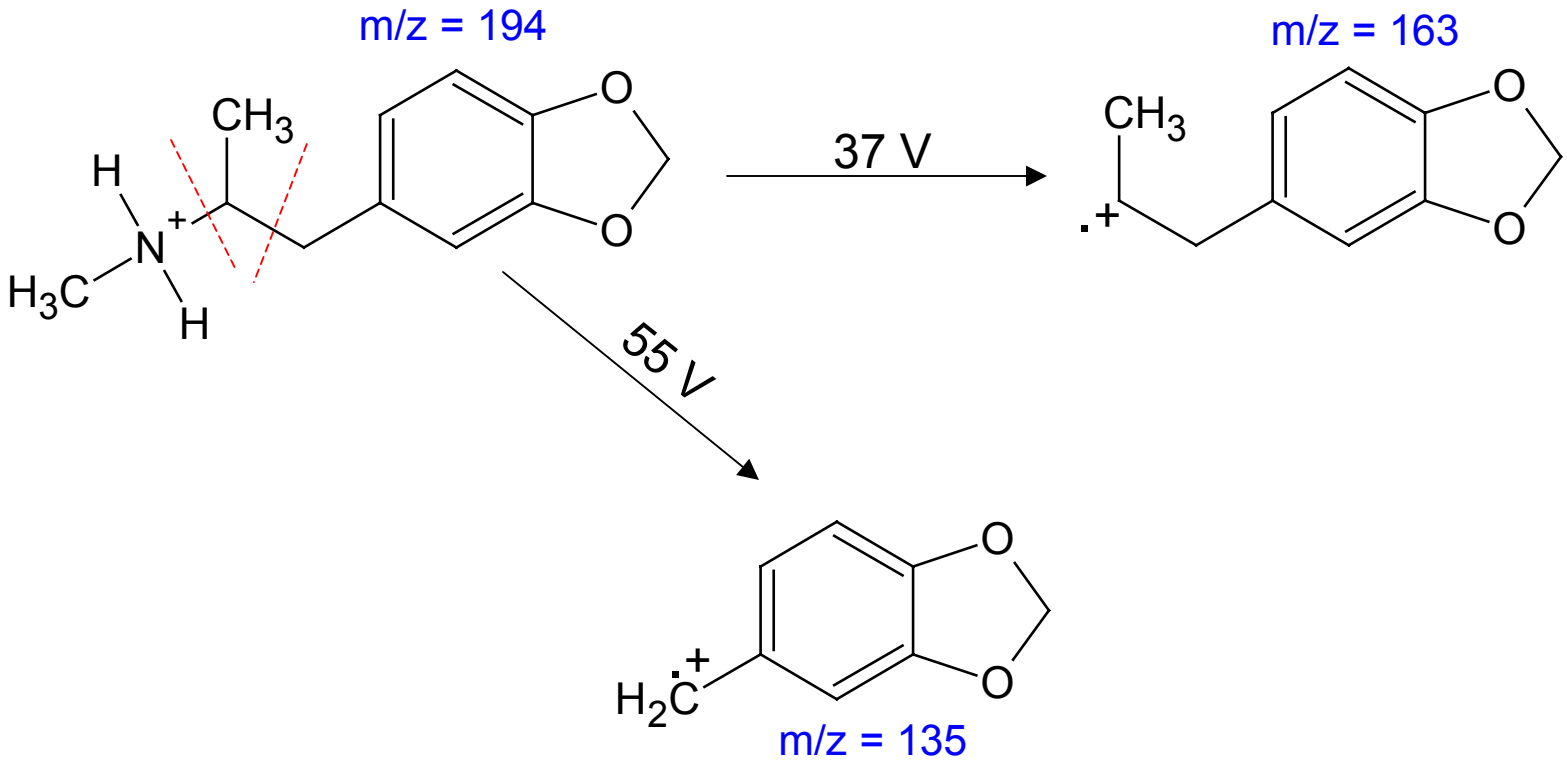
Ion (m/z)	Cone (V)	Delay (s)
180.10	20.0	0.05
163.08	37.5	0.05
135.00	55.0	0.05

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

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.



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)

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

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.

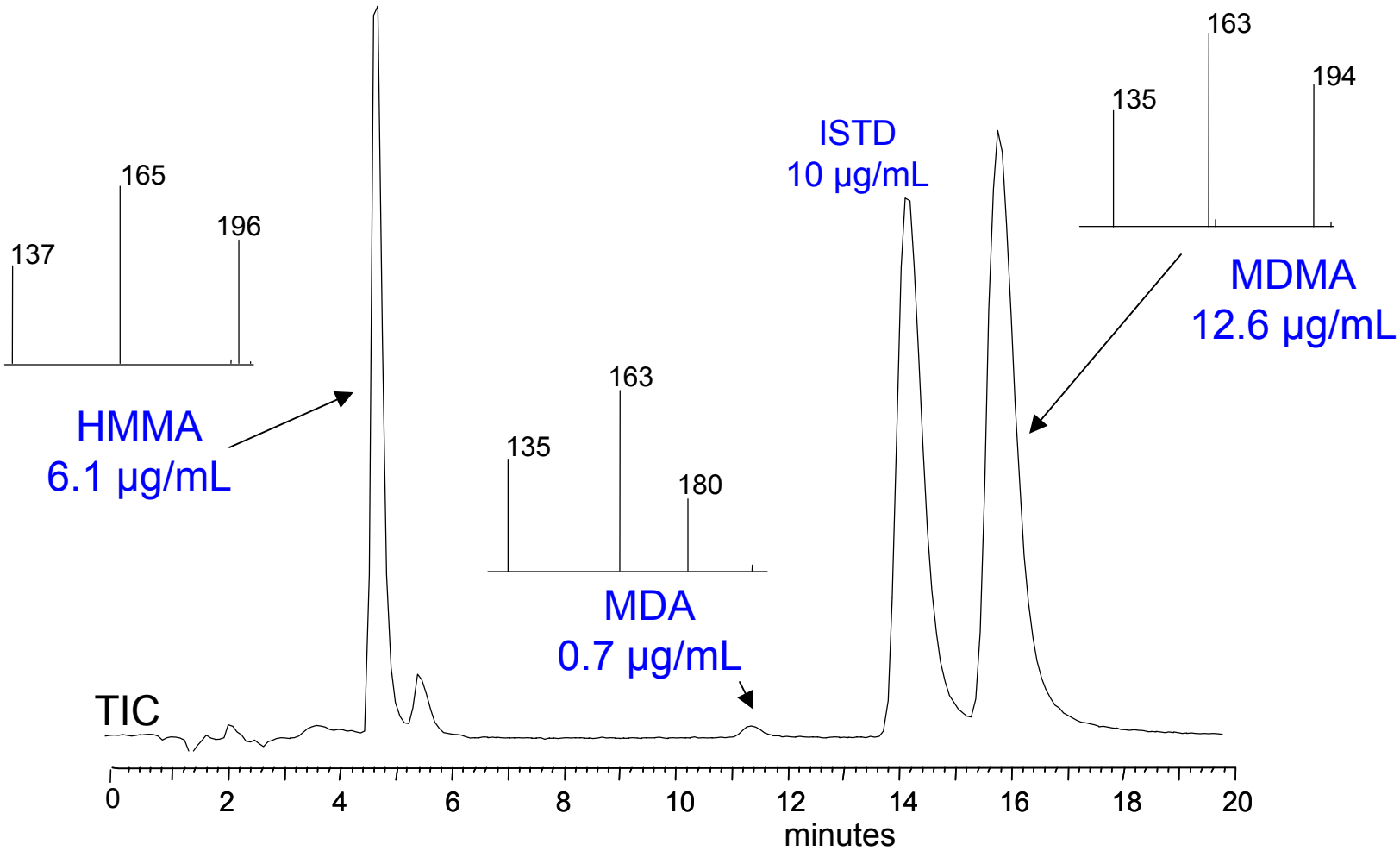
A plastic bag containing approximately 5 g ecstasy was found under the driver's seat.

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.

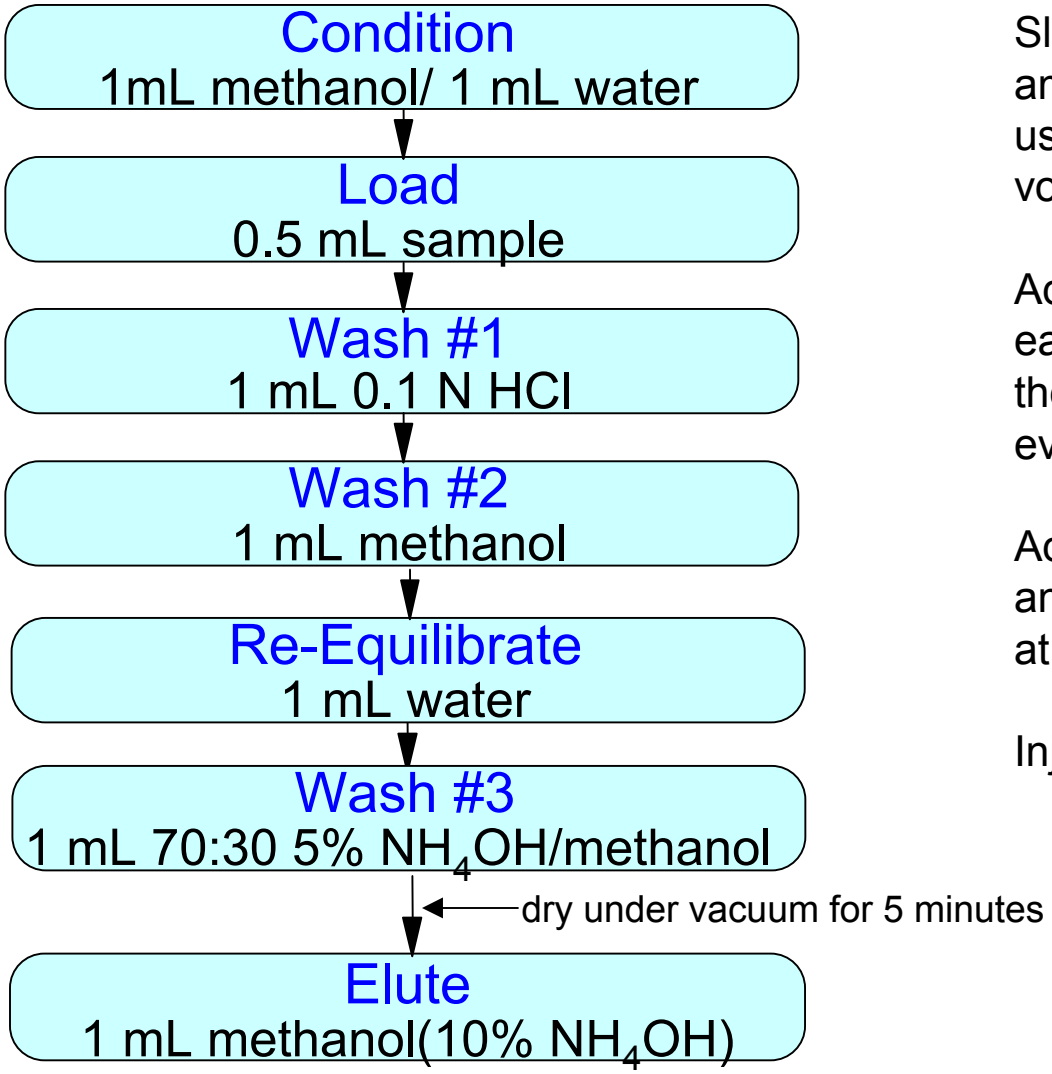
Real Forensic Sample
LC-MS Analysis



Can the SPE Method be Used for GC Analysis?

Yes, with minor modifications.

SPE



Derivatization

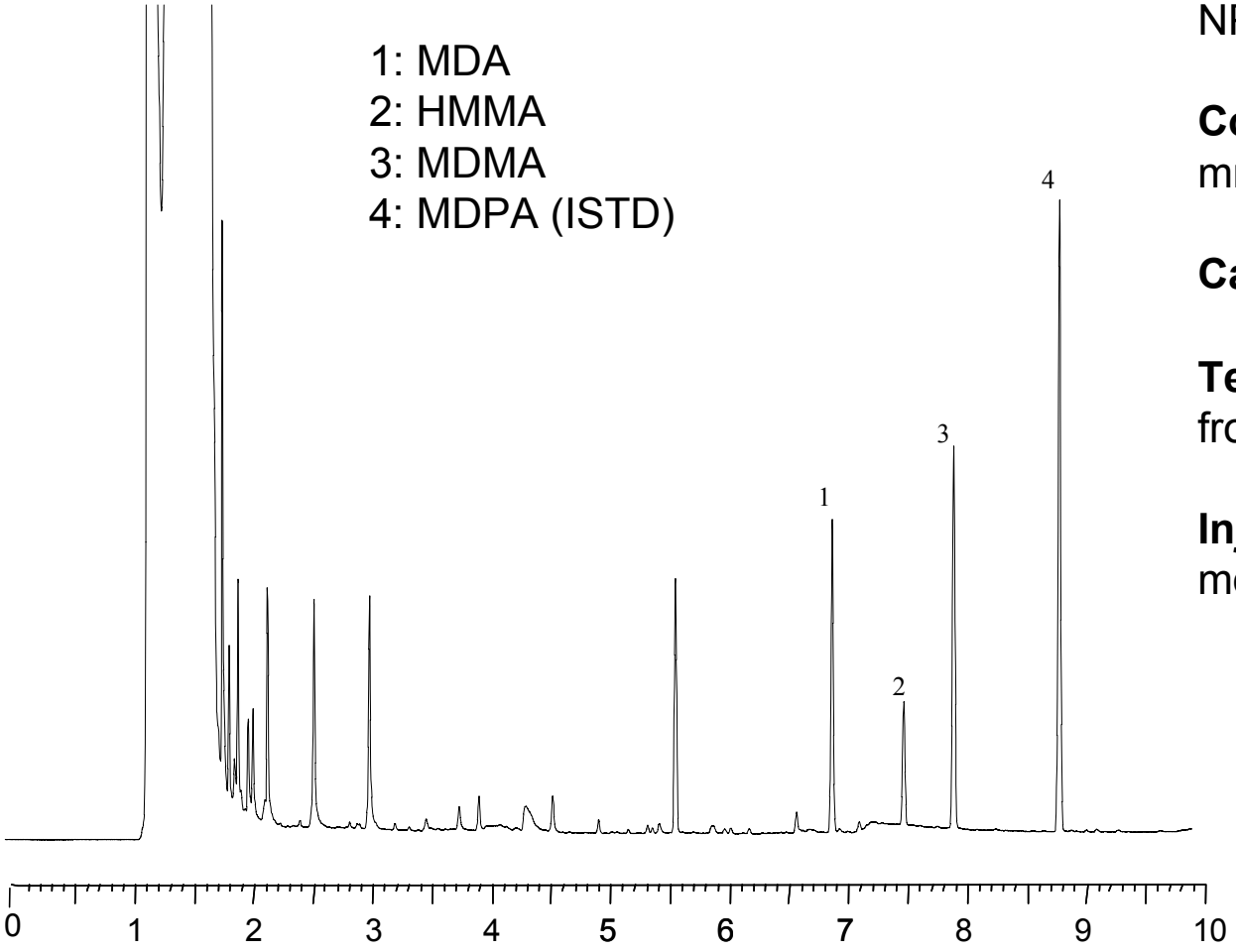
Slowly evaporate the methanolic ammonia extract at room temperature using a nitrogen evaporator to a volume of 200-300 µL

Add 100 µL of 1% HCl in methanol to each sample and completely remove the solvent using the nitrogen evaporator set at 50°C

Add 150 µL of MBTFA, cap the vial, and mix well (derivatize for 45 minutes at 70°C)

Inject 1 µL onto the GC/NPD or GC/MS

GC/NPD Analysis
5 µg/mL Spiked Urine



Instrument: HP5890 Series II with NPD

Column: Restek, RTX 30 m, 0.32 mm I.D., 0.25 µm film thickness

Carrier Gas: Helium @ 0.8 mL/min.

Temperature Program: 15°C/min from 100°C to 280°

Injection: 1 µL using a split-splitless mode

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
- An effective, reproducable SPE procedure has been developed that is suitable for LC or GC based analytical methods
- The LC/MS method has been demonstrated to give comparable results to the standard GC/MS method