

AMPHETAMINES in urine by GC/MS – Code GC43010
(Amphetamine, Methamphetamine, 3,4-MDMA, 3,4-MDA, 3,4-MDE and MBDB)
Method of Confirmation by GC-MS

INTRODUZIONE

Amphetamines are alkaloid drugs with a long history of use and abuse. Their screening in biological liquids (principally in urine) is generally carried out using immunometric techniques with the utilization of monoclonal antibodies, following marking with various reagents.

As these techniques can't exclude false positives, the positive results of screening must be confirmed by an alternative technique. This is generally done by chromatographic technique both Gas (GC) and Liquid (LC), coupled with a mass detector (MS). This method determinates the Amphetamine, Methamphetamine, 3,4-MDMA, 3,4-MDA, 3,4-MDE and MBDB levels after solid-liquid extraction and reading by GC-MS.



EUREKA S.R.L. LAB DIVISION
Via M. D'Antona 28
60033 Chiaravalle (AN) - Italy
Tel. +39 071 7450790
eureka-support@sentinel.it
www.eurekakit.com



This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD). The declaration of conformity is available upon request.

N° 010	Amphetamines in urine by GC/MS	08/2024
--------	--------------------------------	---------

TECHNICAL FEATURES

Principle of the Method :

This method allows to determinate the **Amphetamine**, the **Methamphetamine**, the **3,4-MDMA (Ecstasy)**, the **3,4-MDA**, the **3,4-MDE** and the **MBDB** in urine after SPE extraction, derivatization and reading in GC-MS.

Recovery :

Amphetamine	98%
Methamphetamine	98%
3,4-MDMA	98%
3,4-MDA	98%
3,4-MDE	90%
MBDB	87%

Sensitivity :

50,0 ng/ml

Dynamic Range of the Method :

50 – 10.000 ng/ml

Cut-off:

Amphetamine	250 ng/ml
Methamphetamine	250 ng/ml
3,4-MDMA	250 ng/ml
3,4-MDA	250 ng/ml
3,4-MDE	250 ng/ml
MBDB	250 ng/ml

Ref. Procedure applicative del procedimento n.99/cu 30 ottobre 2007

LOD:

12 ng/ml

LOQ:

40 ng/ml

Components of the kit :

All the components are ready-to-use.
Stability: 3 years at 2 – 8 °C.

Reagent A – Buffer Solution, **1 x 50 ml**

Reagent B – Conditioning Solution N°1, **1 x 50 ml**

Reagent C – Conditioning Solution N°2, **1 x 50 ml**

Reagent D – Wash Solution N°1, **1 x 50 ml**

Reagent E – Wash Solution N°2, **1 x 50 ml**

Reagent F – Wash Solution N°3, **1 x 50 ml**

Reagent G – Eluting Solution, **1 x 50 ml**

Reagent H – Derivatization Solution N° 1, **5 x 1 ml**

Reagent I – Derivatization Solution N° 2, **2 x 1 ml**

Reagent J – Test Solution, **1 x 0,5 ml**

See Warnings

Reagent K – Internal Standard Solution, **1 x 300 µl**

Urine Calibrator lyoph. – Level 1, 2 x 5 ml

Code CC43016 (packed separately – see data sheets)

Urine Calibrator lyoph. – Level 2, 2 x 5 ml

Code CC43016 (packed separately – see data sheets)

Urine Calibrator lyoph. – Level 3, 2 x 5 ml

Code CC43016 (packed separately – see data sheets)

Urine Calibrator lyoph. – Level 4, 2 x 5 ml

Code CC43016 (packed separately – see data sheets)

Urine Calibrator lyoph. – Level 5, 2 x 5 ml

Code CC43016 (packed separately – see data sheets)

Clean up Columns, 50 pcs

Minimum Instrumental equipment required:

GC-MS Instrument
Operational Computer

Optional Equipment:

Autosampler

Urine Collection Procedure:

Take 10 ml of urine
If stored at -20 °C, the sample is stable
about 3 months.

PREANALYTICAL PROCEDURE:

STEP 1 : In suitable glass test tubes of 10 ml, dispense in sequence:

- 100 µl of **Reagent J – Test Solution**
- 10 µl of **Reagent K – Internal Standard Solution**

Dry the eluate by the help of helium or nitrogen flow (at this step the work temperature must be < 40 °C)

Vortex for 20 sec.

STEP 2 : Add 100 µl of **Reagent H – Derivatization Solution N°1**

Vortex for 20 sec.

STEP 3: Incubate at 80 °C for 5 minutes

Cooling at Room Temperature

STEP 4 : Add 30 µl of **Reagent I – Derivatization Solution N°2**

Vortex for 20 sec.

STEP 5: Incubate at 80 °C for 5 minutes

STEP 6 : : Transfer the whole sample in vials

NOTE : Before starting the analytical session it would be better to inject 2 µl of this solution in GC to identify the time of retention of Amphetamines, that must be similar to that shown in Fig. below. If the Test has given positive result, proceed with the analytical session. If it wouldn't be so, verify the functionality of the analytical system.

ANALYTICAL PROCEDURE:

Dilute the **Reagent K – Internal Standard Solution** 1:10 with Water HPLC grade, preparing the sufficient sample size to cover the assay.

N.B.: this dilution is prepared fresh for each run

STEP 1 : Clean-up Columns Conditioning

- Condition clean up columns by fluxing 1 ml of **Reagent B – Conditioning Solution N° 1**
Discharge the percolated liquid
- Condition clean up columns by fluxing 1 ml of **Reagent C – Conditioning Solution N° 2**
Discharge the percolated liquid

Vortex for 20 sec.

STEP 2 : Samples Preparation

Make a Calibration Curve on 5 points :

	Calibrator – Level 1	Calibrator – Level 2	Calibrator – Level 3	Calibrator – Level 4	Calibrator – Level 5	Sample	Controls
Urine Calibrator – Level 1	1,0 ml						
Urine Calibrator – Level 2		1,0 ml					
Urine Calibrator – Level 3			1,0 ml				
Urine Calibrator – Level 4				1,0 ml			
Urine Calibrator – Level 5					1,0 ml		
Sample						1,0 ml	
Controls							1,0 ml
Reagent A – Buffer Solution	1,0 ml	1,0 ml	1,0 ml	1,0 ml	1,0 ml	1,0 ml	1,0 ml
Reagent K – Sol. Internal Std (diluted 1:10 with Water HPLC grade)	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl	10 µl

Vortex for 20 sec.

STEP 3 : Dispense all the samples, previously prepared, into clean up columns. Discharge the percolated liquid, if needed a vacuum system could be used for percolation, at speed of 1 ml / min (drop by drop).

STEP 4 : Clean-up Column Wash:

- Wash with 1 ml of **Reagent D – Wash Solution 1**
Discharge the percolated liquid and dry with a light flux of air
- Wash with 1 ml of **Reagent E – Wash Solution 2**
Discharge the percolated liquid and dry with a light flux of air
- Wash with 1 ml of **Reagent F – Wash Solution 3**
Discharge the percolated liquid and dry with a light flux of air

STEP 5 : Add 1,0 ml of **Reagent G – Eluting Solution** **strictly in glass tubes capped Pyrex**

Dry the eluate by the help of helium or nitrogen flow (at this step the work temperature must be < 40 °C)

STEP 6 : Add 100 µl of **Reagent H – Derivatization Solution N°1**

Vortex for 20 sec.

STEP 7: Incubate at 80 °C for 5 minutes

Cooling at Room Temperature

STEP 8 : Add 30 µl of **Reagent I – Derivatization Solution N°2**

Vortex for 20 sec.

STEP 9: Incubate at 80 °C for 5 minutes

STEP 10 : : Transfer the whole sample in vials

INJECTION :

- Inject 1 µl of the solution in GC

N.B.: samples should be injected immediately, can not be frozen for deferred analysis.

AMPHETAMINES in urine - Warnings

REAGENT J: TEST SOLUTION

Amphetamine Methamphetamine 3,4-MDMA 3,4-MDA 3,4-MDE MBDB	about 5.000 ng/ml about 5.000 ng/ml about 5.000 ng/ml about 5.000 ng/ml about 5.000 ng/ml about 5.000 ng/ml
--	--

SET OF GAS-CROMATOGRAPH:

• VF-5ms Column 30 m x 0,25 mm, 0,25 µm (conditioned) • Injector's Temperature 250 °C • Split Report Start: ON split 20 0 min: OFF 1 min: ON split 100 3 min: ON split 20 • Temperature 70 °C x 3.5 min + 40 °C/min up to 190 °C + 10 °C/min up to 250 °C x 10 min (run 22.5 min) • Column Flow: 1 ml/min
--

SET OF MASSA DETECTOR (4000) :

• Range of Massa 50 – 350 • Temperature of Massa: 180 °C; • Temperature Transfer Line: 270 °C; • Temperature Manifold: 80 °C • Filament on: 5 min

CONDITIONING OF VF-5ms COLUMN AND SYRINGE

Before use wash the syringe with ACETONE.
Follow the manufacturer prescription.
Do not condition column/s if connected to the Massa Detector.

CLEANING OF THE COLUMN

ACETONE injected before and after the assay. Wash the syringe before injection with acetone. At the end of each run to clean the liner of the injector and run the backout of the ion trap. At the end of each run to bring the maximum column temperature gradient (recommended by the manufacturer) with a speed of 30 ° C / minute.
(See the manufacturer 's instructions)

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
CC43016	Calibrator in urine for Amphetamines	2 x 5 x 5 ml
CC43019	Multiparametric Control for Drugs of Abuse – Levels 1 and 2	2 x 6 x 5 ml
ZRE13623	Rxi-Sil 5MS (30 m x 0,25 mm) ID, DF=0,25 Capillary Column	1 Pc
SCP8944	VF-5ms (30 m x 0,25 mm) ID DF=0,25 Column	1 Pc
S29057U	Standard glass vial of 2 ml with screw cap	1 x 100 Pcs
S29046U	Caps for Standard glass vials of 2 ml with screw cap	1 x 100 Pcs
S24717	Insert 100 ul for vials of 2 ml	1 x 100 Pcs

Amphetamines in urine
(Reference Chromatograms/Spectrum GC-MS)

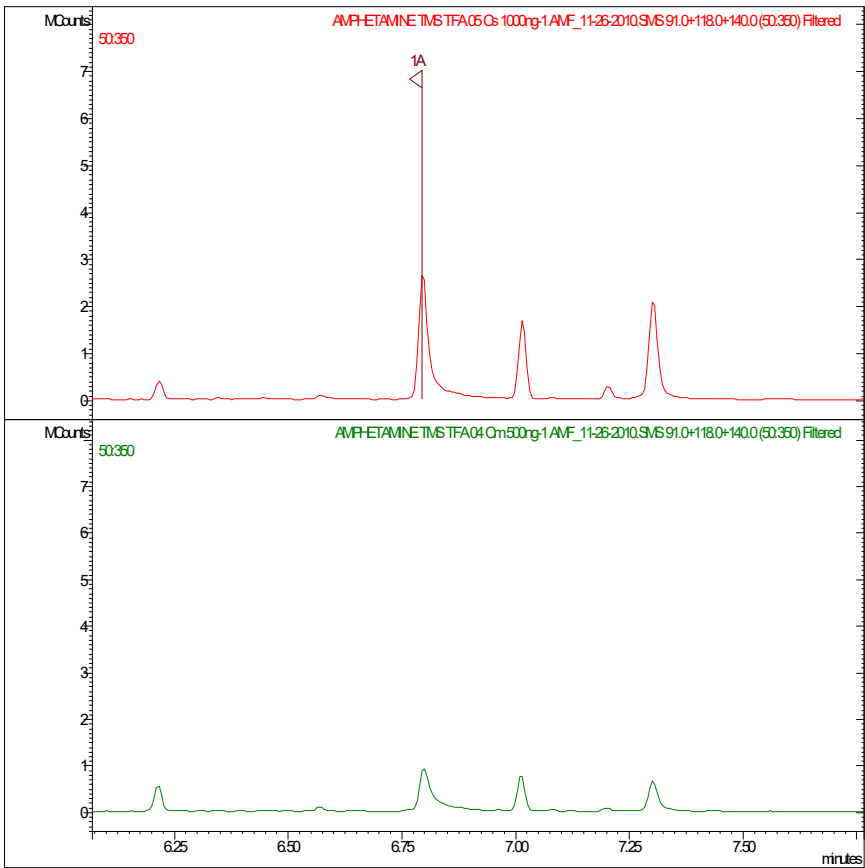


Fig. 1 :	Urine Sample Amphetamine
	R.T. 6.79 AMPHETAMINE

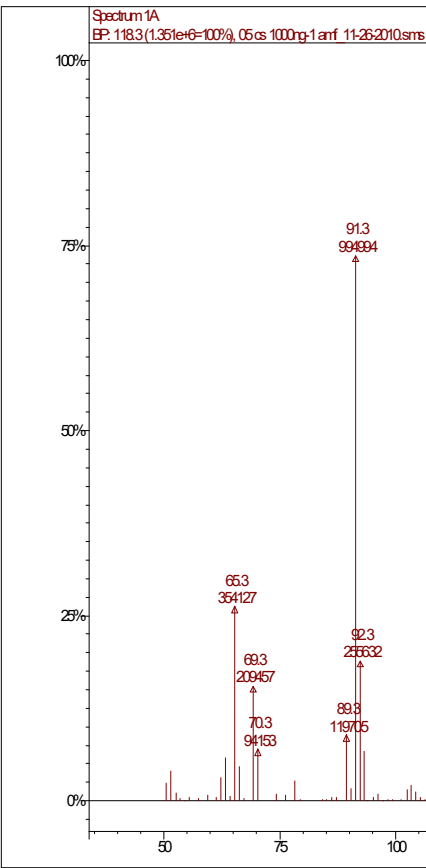


Fig. 2 :	Massa Spectrum of A
	MOLECULAR IONS: 9

Amphetamines in urine

(Reference Chromatograms/Spectrum GC-MS)

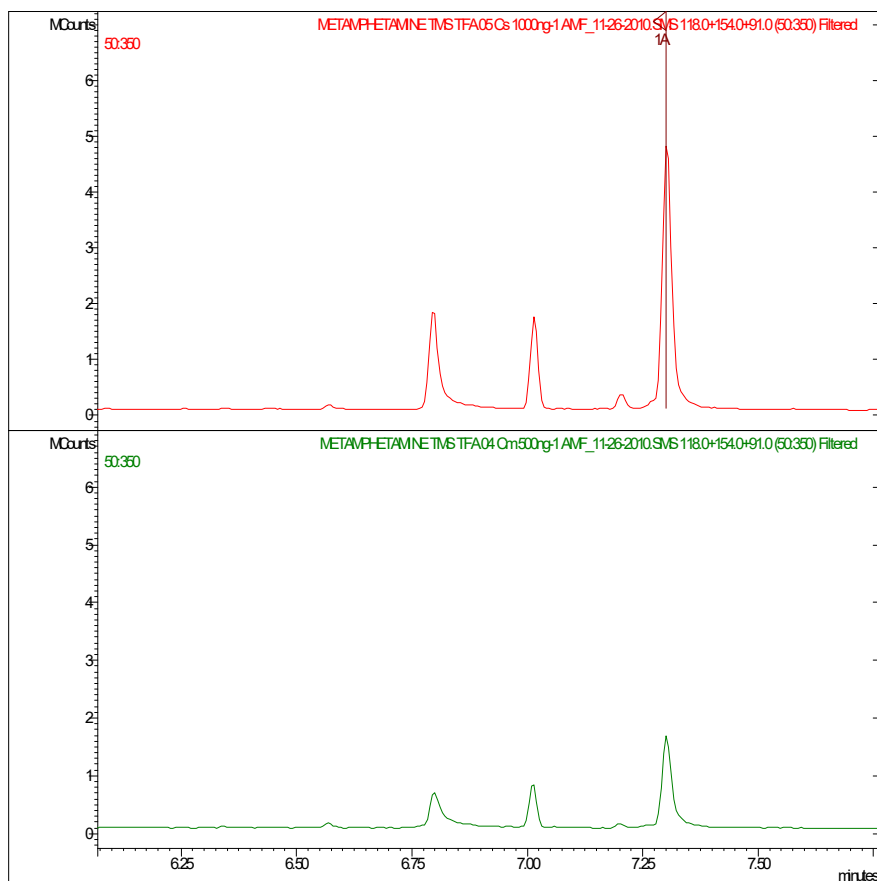


Fig. 3 :	Urine Sample Methamphetamine
	R.T. 7.30 METAMPHETAMINE

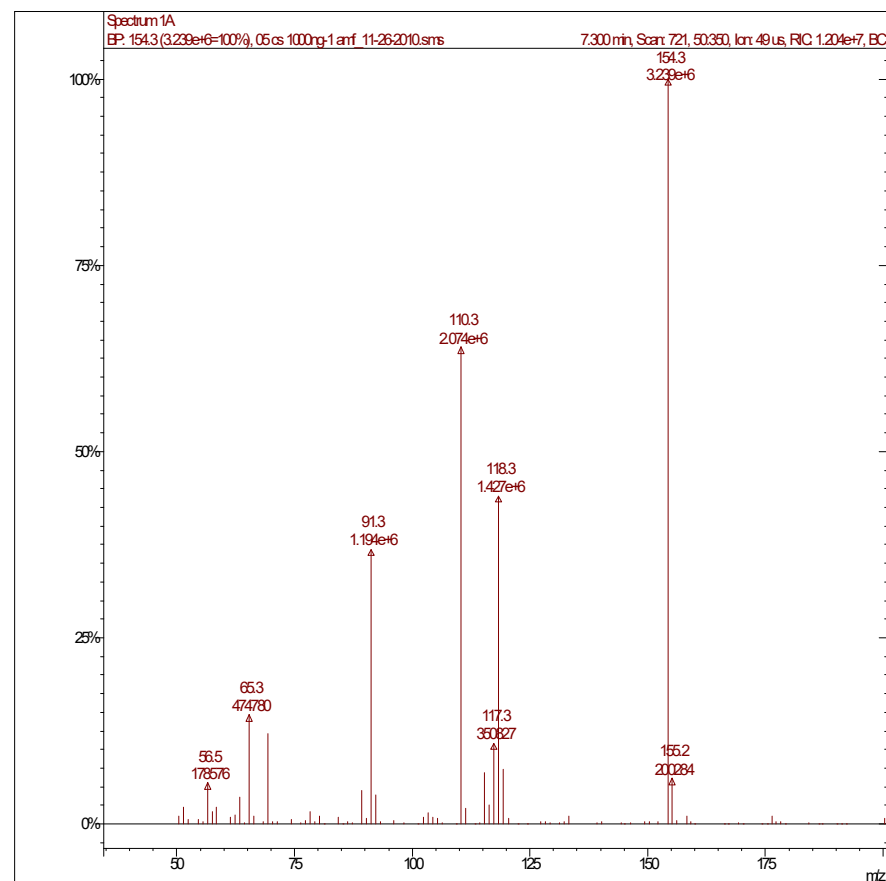


Fig. 4 :	Massa Spectrum of Methamphetamine
	MOLECULAR IONS: 91-110-118-154

Amphetamines in urine

(Reference Chromatograms/Spectrum GC-MS)

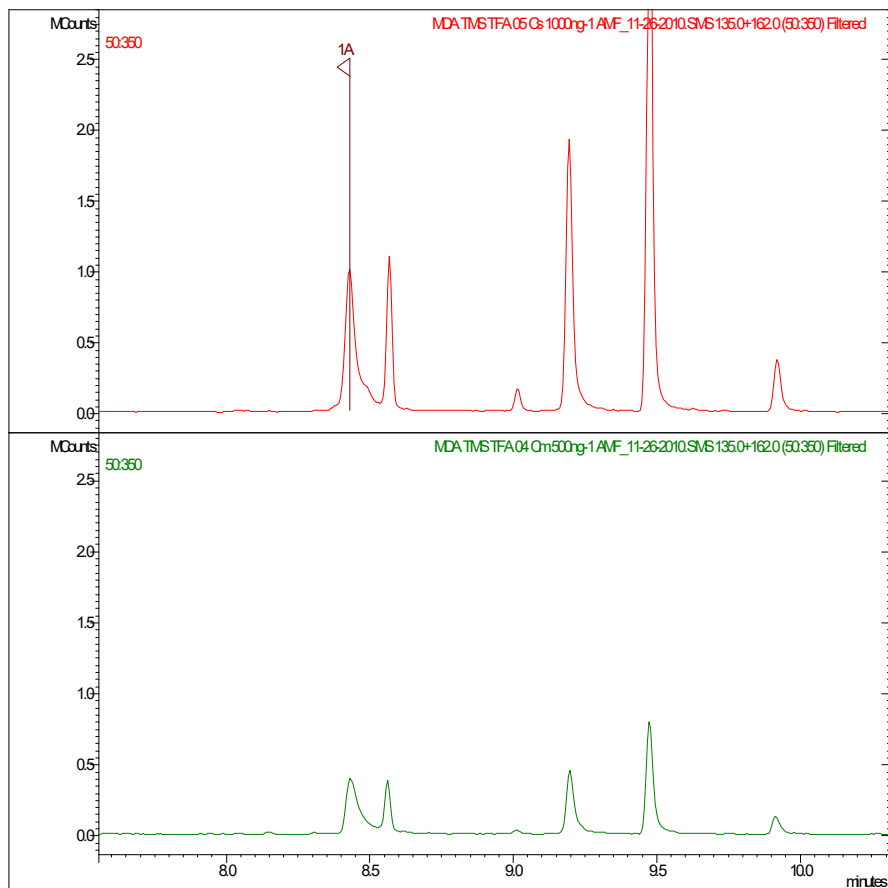


Fig. 11 :	Urine Sample 3,4-MDA
	R.T. 8.40 3,4-MDA

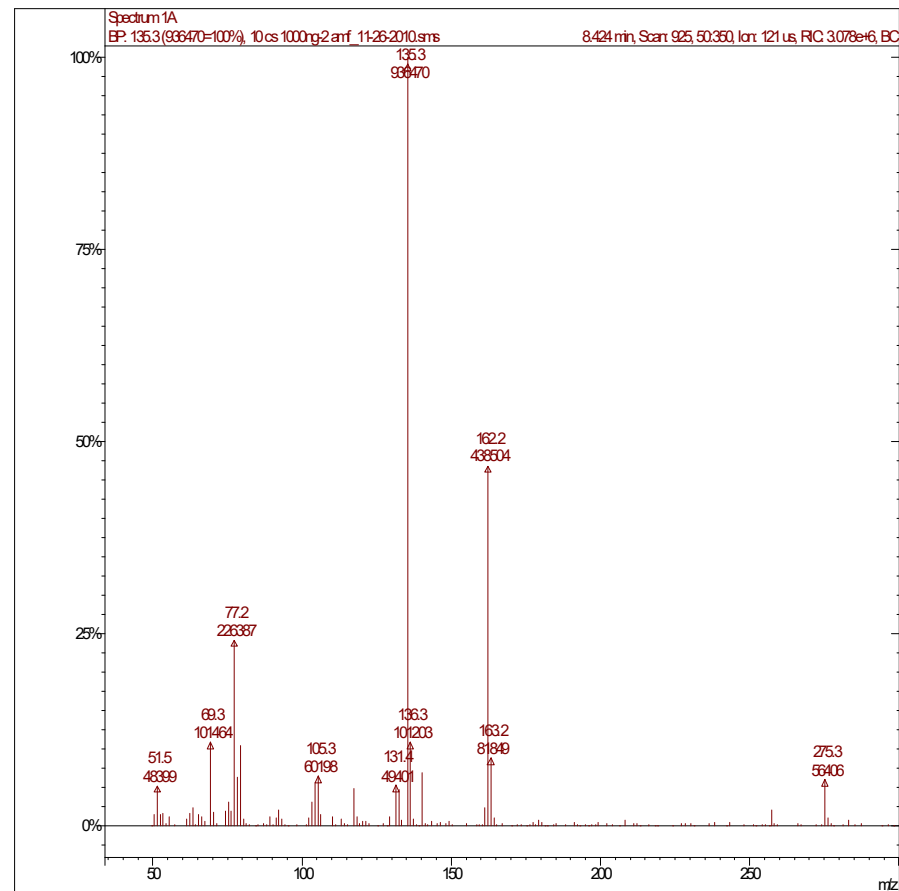


Fig. 12 :	Massa Spectrum 3,4-MDA
	MOLECULAR IONS: 135-162-275

Amphetamines in urine

(Reference Chromatograms/Spectrum GC-MS)

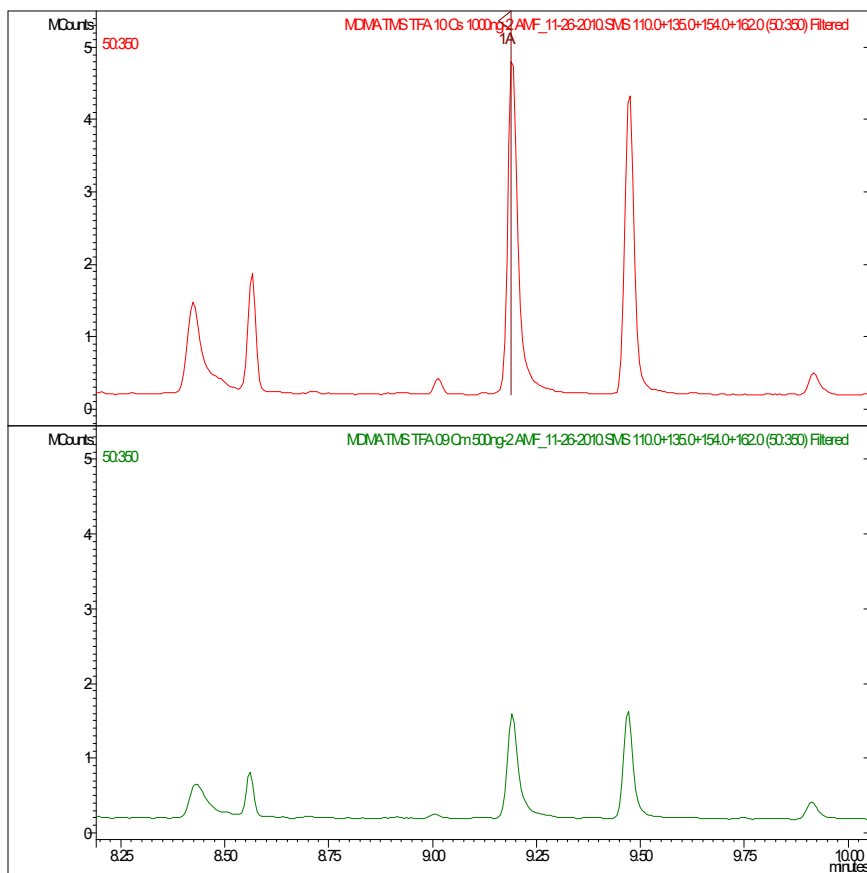


Fig. 13 :	Urine Sample 3,4-MDMA
	R.T. 9.20 3,4-MDMA

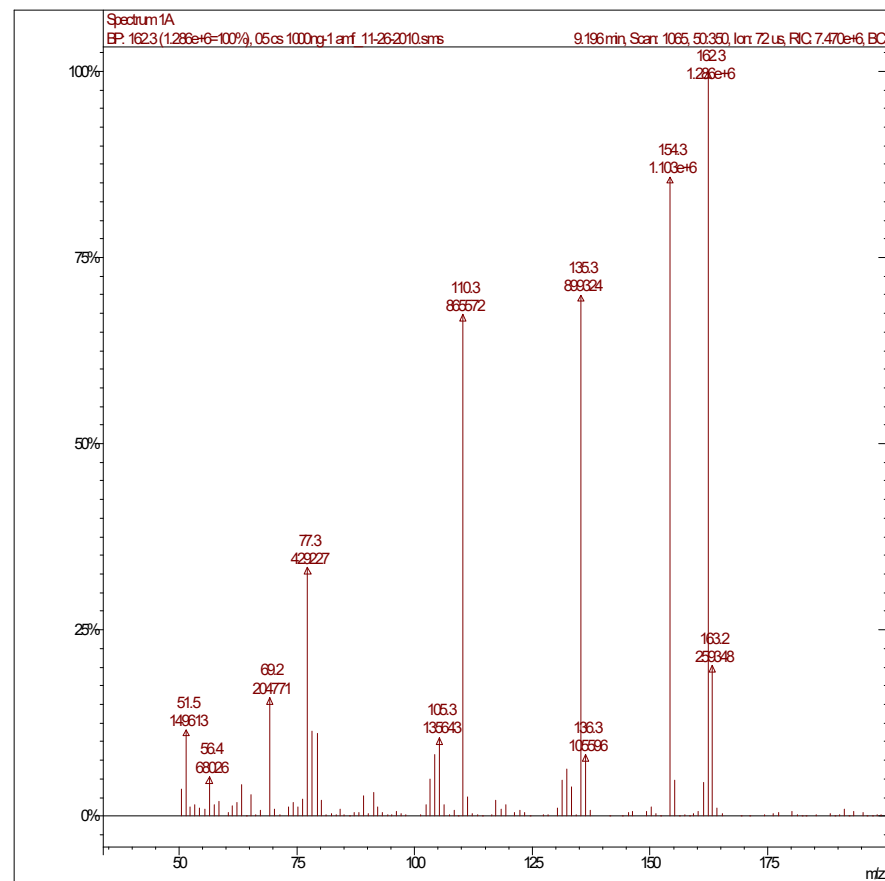


Fig. 14 :	Massa Spectrum 3,4-MDMA
	MOLECULAR IONS: 110-135-154-162

Amphetamines in urine

(Reference Chromatograms/Spectrum GC-MS)

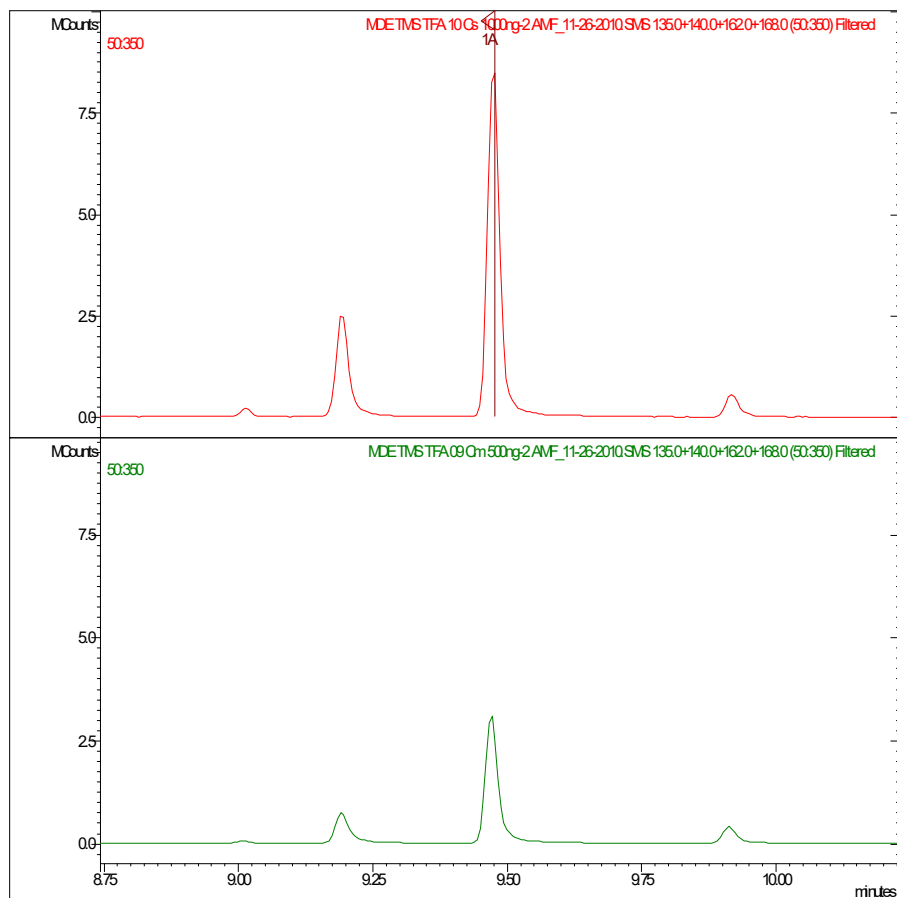


Fig. 15 :	Urine Sample 3,4-MDE
	R.T. 9.50 3,4-MDE

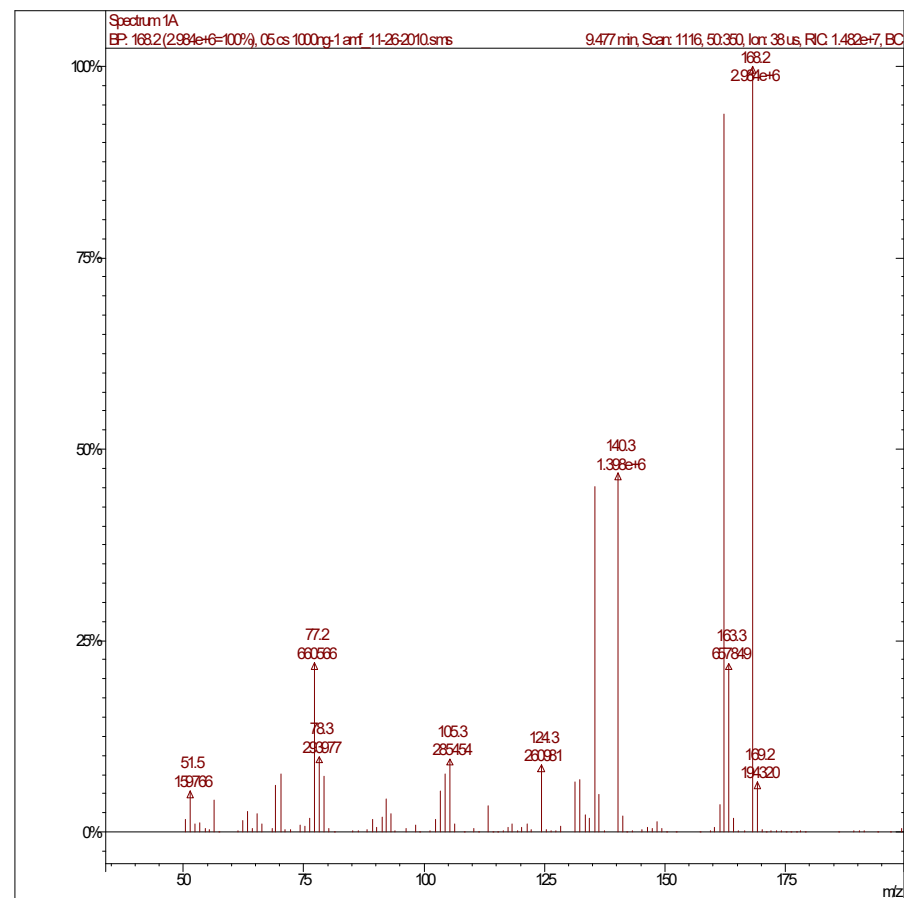


Fig. 16 :	Massa Spectrum 3,4-MDE
	MOLECULAR IONS: 135-140-162-168

Amphetamines in urine

(Reference Chromatograms/Spectrum GC-MS)

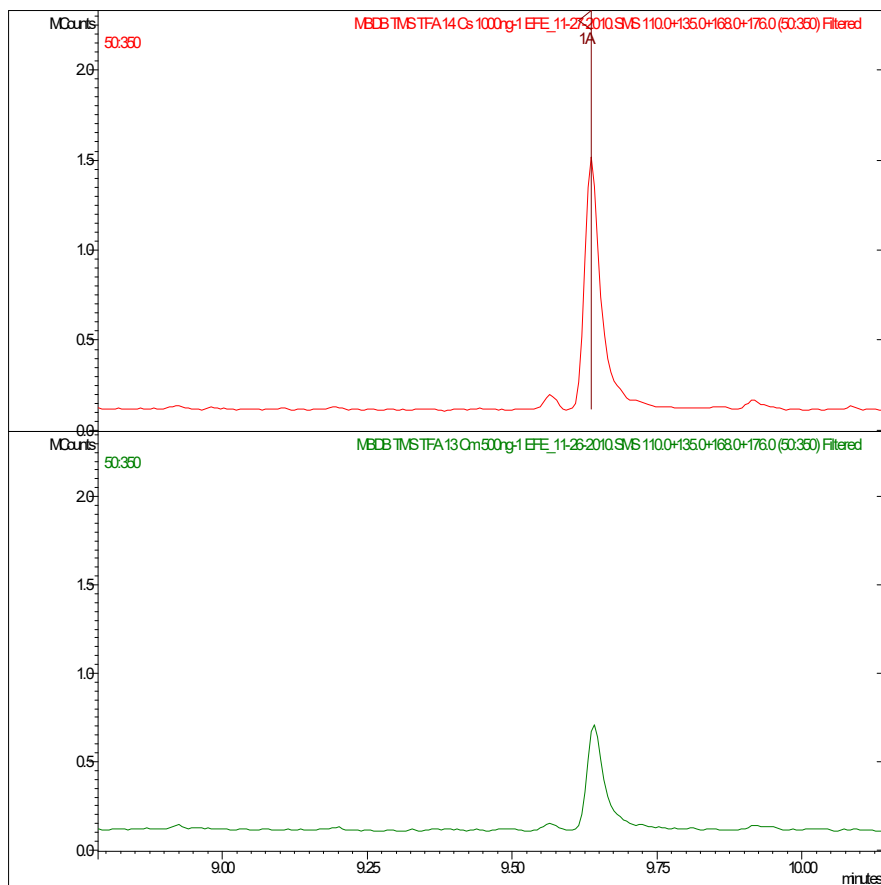


Fig. 17 :	Urine Sample MBDB
	R.T. 9.65 MBDB

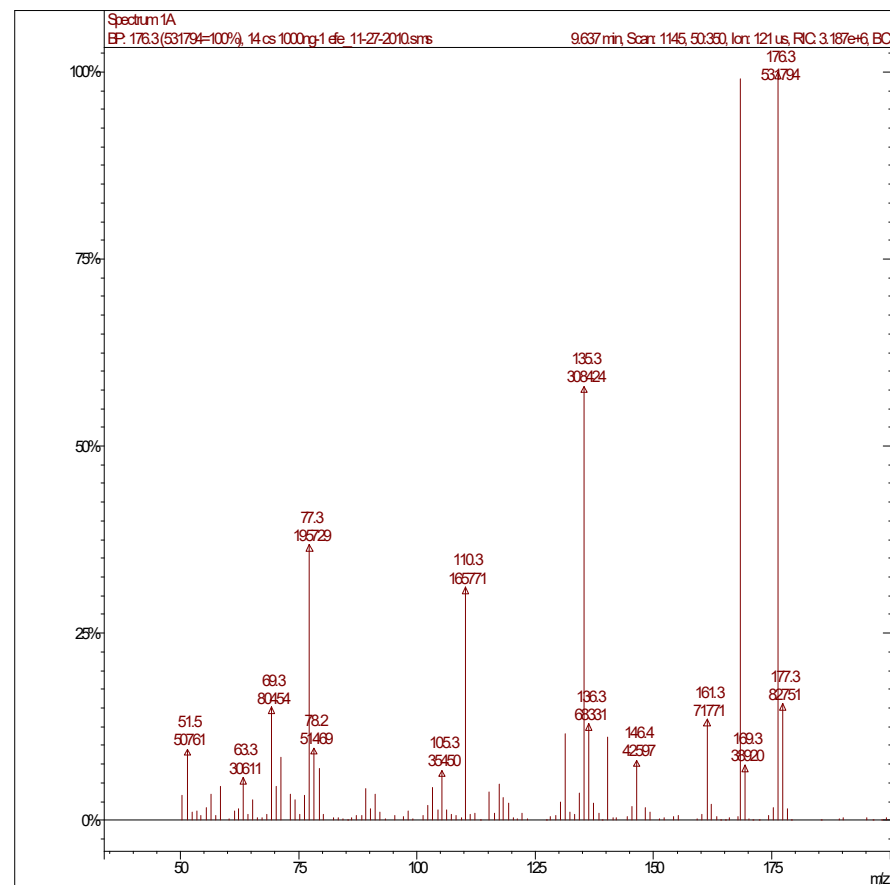


Fig. 18 :	Massa Spectrum MBDB
	MOLECULAR IONS: 110-135-168-176

Amphetamines in urine

(Reference Chromatograms/Spectrum GC-MS)

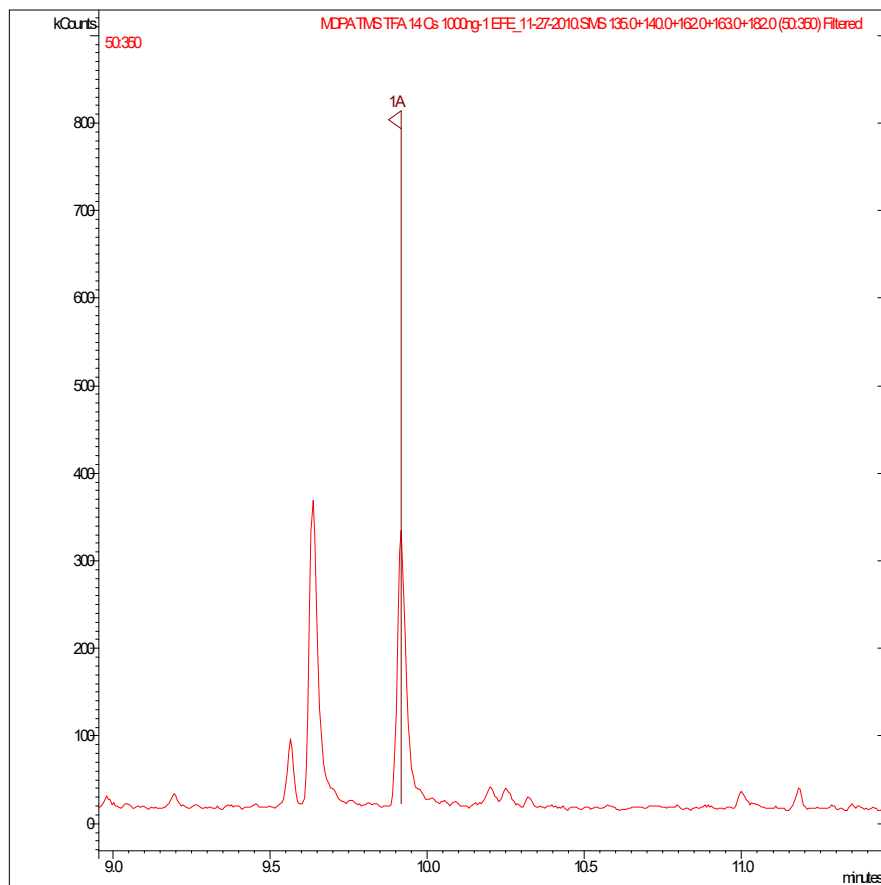


Fig. 19 :	Urine Sample with Internal Standard
	R.T. 9.94 INTERNAL STANDARD

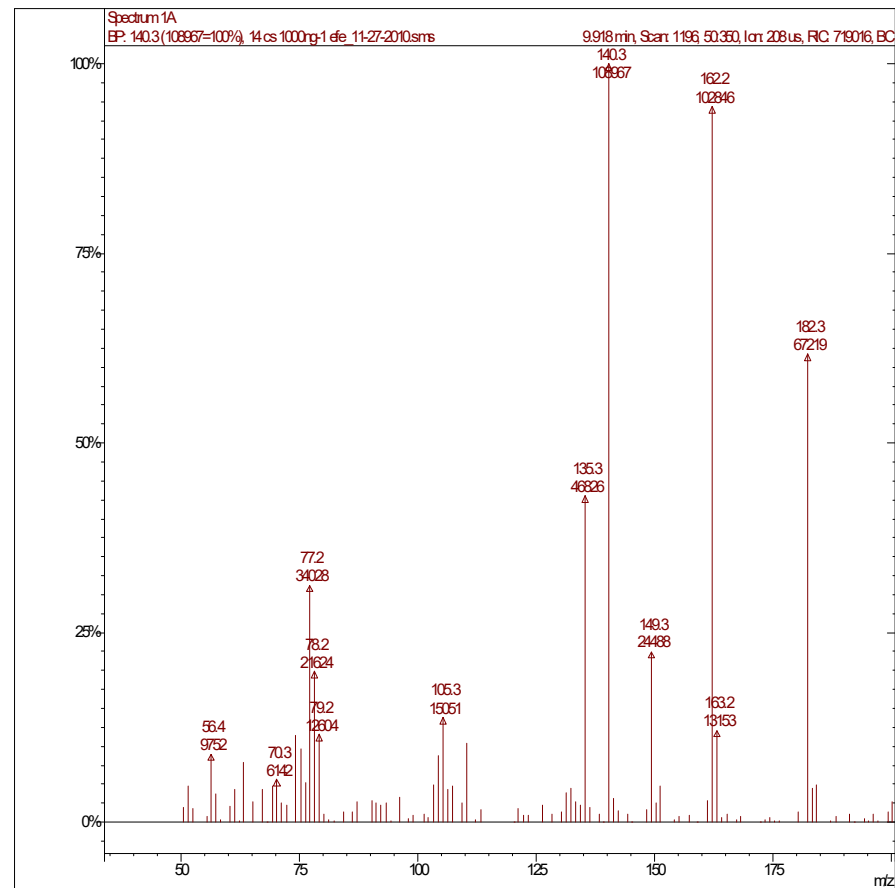


Fig. 20 :	Massa Spectrum of Internal Standard
	MOLECULAR IONS: 135-140-162-182