

BENZOYLECGONINE (Cocaine's metabolite) – Cocaethylene - Cocaine in urine by GC-MS - Code GC46010

Method of Confirmation by GC-MS

INTRODUCTION

Cocaine, a psychostimulant drugs of abuse, is an alkaloid that is obtained from the leaves of coca (*Erythroxylum Coca*), a plant native to South America. It is extracted using calcium hydroxide or ammonia. Inside the body, cocaine is rapidly hydrolyzed into benzoylecgonine. This metabolite is excreted in urine and its determination in this biological fluid, by way of an antigen-antibody reaction, is evidence of cocaine use. If a screening shows positive (cut off = 300ng/ml), analysis is confirmed, usually with gaseous liquid chromatography coupled with a mass spectrometry detector. This method allows the determination of urinary Benzoylecgonine after extraction of the substance from the urine matrix on micro columns of solid phase extraction and subsequent reading in 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° 019	Benzoylecgonine – Cocaethylene - Cocaine in urine by GC/MS	08/2024
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TECHNICAL FEATURES

Principle of the Method :

This method allows to determinate the **Benzoylecgonine** in urine, the main metabolite of **Cocaine**, the **Cocaethylene** and the **Cocaine** after SPE extraction and reading in GC-MS.

The method was validated according to international criteria established by the FDA and by the ICH.

ANALYTE	Correlation Coefficient $\pm$ SD	LOD (ng/ml)	LOQ (ng/ml)
BEG	0,9979 $\pm$ 0,0014	5	20
COCAETHYLENE	0,9977 $\pm$ 0,0026	8	20
COCAINE	0,9986 $\pm$ 0,0024	6	20

ANALYTE	Intra-Day Accuracy			Inter-Day-Precision			Inter-Day-Accuracy		
	(Error %)			(CV%)			(Error %)		
	Calibrator			Calibrator			Calibrator		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
BEG									
COCAETHYLENE	8,6	11,66	6,75	20,21	15,07	13,05	15	12,46	10,87
COCAINE	10	8,28	7,11	15,95	9,35	9,56	11,5	10,09	10,58
	17,4	5,73	5,9	25,91	11,6	13,19	21,2	10,49	12,87

ANALYTE	Recovery (%)		
	Calibrator		
	Low	Medium	High
BEG			
COCAETHYLENE	78,22	84,29	95,07
COCAINE	65,14	91,19	105,17
	83,46	92,99	104,05

The table below shows for each analyte concentrations corresponding to the calibrators whose values Low, Medium and High reference cited above.

ANALYTE	Concentrations ng/ml		
	Calibrator		
	Low	Medium	High
BEG			
COCAETHYLENE	75,00	200,00	500,00
COCAINE	75,00	200,00	500,00
	75,00	200,00	500,00

Recovery of the Method Benzoilecgonine, Cocaethylene, Cocaine:

20 - 1000 ng/ml

Benzoilecgonine, Cocaethylene, Cocaine Cut-off:

100 ng/ml

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

Components of the kit :

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

Reagent A – Conditioning Solution 1 and Wash Solution 2, **1 x 200 ml**

Reagent B – Conditioning Solution 2, **1 x 100 ml**

Reagent C – Buffer Solution, **1 x 100 ml**

Reagent D – Wash Solution 1, **1 x 100 ml**

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

Reagent F – Diluting Solution, **1 x 5 ml**

Reagent H – Test Solution Benzoilecgonine, **1 x 0,5 ml**

See Warnings

Reagent I – Test Solution Cocaethylene, **1 x 0,5 ml**

See Warnings

Reagent K – Test Solution Cocaine, **1 x 0,5 ml**

See Warnings

Reagent J – Internal Standard Solution, **1 x 200 µl**

See Warnings

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

Code CC46016 (packed separately – see data sheets)

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

Code CC46016 (packed separately – see data sheets)

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

Code CC46016 (packed separately – see data sheets)

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

Code CC46016 (packed separately – see data sheets)

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

Code CC46016 (packed separately – see data sheets)

Reagent M – Derivatization Solution, **3 x 1 ml (3 ampullas of 1 ml)**

See Warnings. For the sampling of Reagent M, the cap sealing requires the use of 100 ul Hamilton syringe Cod. ZRE24563

Clean-up Columns, 50 pcs

Minimum Instrumental equipment required:

GC-MS Instrument
Operative Computer

Optional Equipment:

Autosampler

Urine Collection Procedure:

Take 10 ml of urine

NB: If the analysis is not performed immediately, urine, previously aliquoted in respect of the chain of custody must be preserved at -20 ° C. The time of storage at -20 ° C to be verified by the laboratory with appropriate stability tests on different aliquots of the same urine sample enriched with the substance of abuse. The international literature reports that the major substances of abuse do not undergo a degradation statistically significant over the 3-6months, if maintained under controlled conditions at -20 ° C.

PREANALYTICAL PROCEDURE:

STEP 1 : Pipette in a glass tube with teflon cap :

- 350 µl of **Reagent F – Diluting Solution**
- 50 µl of **Reagent H – Test Solution Benzoylecgonine**
- 50 µl of **Reagent I – Test Solution Cocaethylene**
- 50 µl of **Reagent K – Test Solution Cocaine**

Vortex for 20 seconds

STEP 2 : Dry the eluate by the help of an helium or nitrogen flow (at this step the work temperature must be < 40 °C).

STEP 3 : Put in 50 µl of **Reagent M – Derivatization Solution using the syringe Hamilton of 50 ul code Z80500**

Vortex for 20 seconds

STEP 4: Incubate at 70 °C for 20 minutes

Cooling at Room Temperature

STEP 5 : Transfer the whole sample in vials goblet form

NOTE : Before starting the analytical session it would be better to inject 1 µl of this solution in GC to identify the time of retention of Benzoylecgonine and Cocaethylene, 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:

STEP 1 : Conditioning of the Clean-up columns

- Pour out in column 1,5 ml of **Reagent A – Conditioning Solution 1 and Wash Solution 2**
Discharge the percolated liquid
- Pour out in column 2 ml of **Reagent B – Conditioning Solution 2**
Discharge the percolated liquid

STEP 2 : Preparation of the sample, Calibrator and Controls

The reagent J consists of 200 µl of Internal Standard and must be diluted 1:100 in H₂O before use. Because need 20 µl for each sample analyzed for each sample of the validation test, we must calculate the volume required for each run and at the same time dilute the reagent.

Ei. for an analytic session with 20 unknown samples and 5 calibrators are needed 500 µl of diluted Reagent J. So you pick up 5 µl of Reagent J and are diluted with 495 µl of H₂O. Vortex for 10 sec. The solution is now ready for use.

The reagent J undiluted and not used will hermetically seal and is preserved the residual amount in a freezer at -20 ° C.

Place in a test tube rack a number of glass tubes corresponding to the 5 calibrators, 2 controls and n unknown samples. In each tube insert the volume of calibrators, controls and unknown samples indicated in the table below.

	Unkown Sample	Calibrator – Level 1	Calibrator – Level 2	Calibrator – Level 3	Calibrator – Level 4	Calibrator – Level 5	Control
Sample	1000 µl						
Urine Calibrator lyophil. – Level 1		1000 µl					
Urine Calibrator lyophil. – Level 2			1000 µl				
Urine Calibrator lyophil. – Level 3				1000 µl			
Urine Calibrator lyophil. – Level 4					1000 µl		
Urine Calibrator lyophil. – Level 5						1000 µl	
Reagent J – Internal Standard (diluted as above)	20µl	20µl	20µl	20µl	20µl	20µl	20µl
Control							1000 µl
Reagent C – Buffer Solution	2000 µl	2000 µl	2000 µl	2000 µl	2000 µl	2000 µl	2000 µl

Control the pH which should be ≈ 6 , with pH indicator strips (dip the tip of a pasteur pipette or a tip in the tube; use a separate pipette for each tube).

In the case (<1% of unknown samples) that the pH is not between 5.5 and 6.5, you can make corrections with few ul of 0.1 M HCl or NaOH 0.1 M by mixing and rechecking the pH after each addition.

NOTE: If you would also result in the cocaine, it is necessary to prepare the calibration curve of 5 points using the proper liophyl (code CC46116) that are not included in the kit but are available separately. For their preparative simply use the same mode of reagent P1, P2, P3, P4, P5 in the above table.

Vortex for 20 seconds

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 columns Wash:

- Wash with 2 ml of **H₂O HPLC grade**
Discharge the percolated liquid
- Wash with 2 ml of **Reagent D – Wash Solution 1**
Discharge the percolated liquid
- Wash with 2 ml of **Reagent A – Conditioning Solution 1 e Wash Solution 2**
Discharge the percolated liquid and dry with a light flux of air

STEP 5 : Elution in **glass tubes with teflon caps only**

Dry with a light flux of air

- Elute with 1 ml of **Reagent E – Eluting Solution**

*Gather the eluate **in glass test tubes** and vortex*

STEP 6 : Dry the eluate by the help of an helium or nitrogen flow (at this step the work temperature must be < 40 °C).

STEP 7 : Take again 50 µl of **Reagent M - Derivatization Solution** using the syringe Hamilton of 50 µl code Z80500)

Close hermetically

Vortex for 20 seconds

STEP 8: Incubate at 70 °C for 20 minutes

Cooling at Room Temperature

STEP 9 : Transfer the whole sample in vials goblet form

N.B.: the eluate is stable 24 hours at 2-8 °C

INJECTION :

- Inject 1 µl of the solution in the GC

BENZOYLECGONINE and COCAETHYLENE in urine - Warnings

REAGENT H: TEST SOLUTION BENZOYLECGONINE

Benzoylecgonine	about 10.000 ng/ml
Storage: After opening the vial, withdrawn the volume needed, close it with the reagent used and stored at -20 °C.	

REAGENT I: TEST SOLUTION COCAETHYLENE

Cocaethylene	about 10.000 ng/ml
Storage: After opening the vial, withdrawn the volume needed, close it with the reagent used and stored at -20 °C.	

REAGENT K: TEST SOLUTION COCAINE

Cocaine	about 10.000 ng/ml
Storage: After opening the vial, withdrawn the volume needed, close it with the reagent used and stored at -20 °C.	

REAGENTE J: INTERNAL STANDARD SOLUTION

Atropine	1 mg/ml
Storage: stored at – 20 °C	

REAGENT M: DERIVATIZATION SOLUTION

Once opened an ampulla, transfer the whole content, 1 ml, in a glass vial. Close carefully the cap and store at Room Temperature.

SET OF MASSA DETECTOR (4000) :

- Range of Massa 78 – 400 m/z
 - Temperature of Massa: 180 °C;
 - Temperature Transfer Line: 270 °C;
 - Temperature Manifold: 80 °C
- Filament on: 3 minutes

SET OF GAS-CROMATOGRAPH:

- VF-5ms Column 30 m x 0,25 mm, 0,25 µm (conditioned following manufacturer prescription)
- Injector's Temperature 280 °C
- Split Report Start: ON split 20

0 min: OFF
1 min: ON split 100
3 min: ON split 20

NB: The splitting ratio can be varied depending on the instrument used

- Temperature Programmed:
120 °C x 3,25 min
120-250°C a 20 °C/min
250°C x 1 min, 250-300°C a 40 °C/min
300 °C x 3 min (run 15 minutes)
- Helium Gas 1 ml/min

CONDITIONING OF VF-5 MS COLUMN

Follow the manufacturer prescription.

CLEANING OF THE COLUMN

If the analytical performances undergo significant changes (eg, low sensitivity or the presence of interfering in the chromatograms), it is necessary to clean the column as reported in accordance with the instructions of the supplier.

AUTOSAMPLER WASH

Wash the syringe before each injection with ACETONE.

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
CC46016	Calibrator in urine for Benzoylecgonine and Cocaethylene	2 x 5 x 5 ml
CC46116	Calibrator in urine for Cocaine	2 x 5 x 5 ml
CC43019	Multiparametric Control for Drugs of Abuse – Levels 1 and 2	2 x 6 x 5 ml
CC48019	Control in urine for EDDP, 6-MAM and Cocaine – 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

Benzoylecgonine (Cocaine's metabolite) – Cocaethylene – Cocaine in urine
(Reference Chromatograms/Spectra GC-MS)

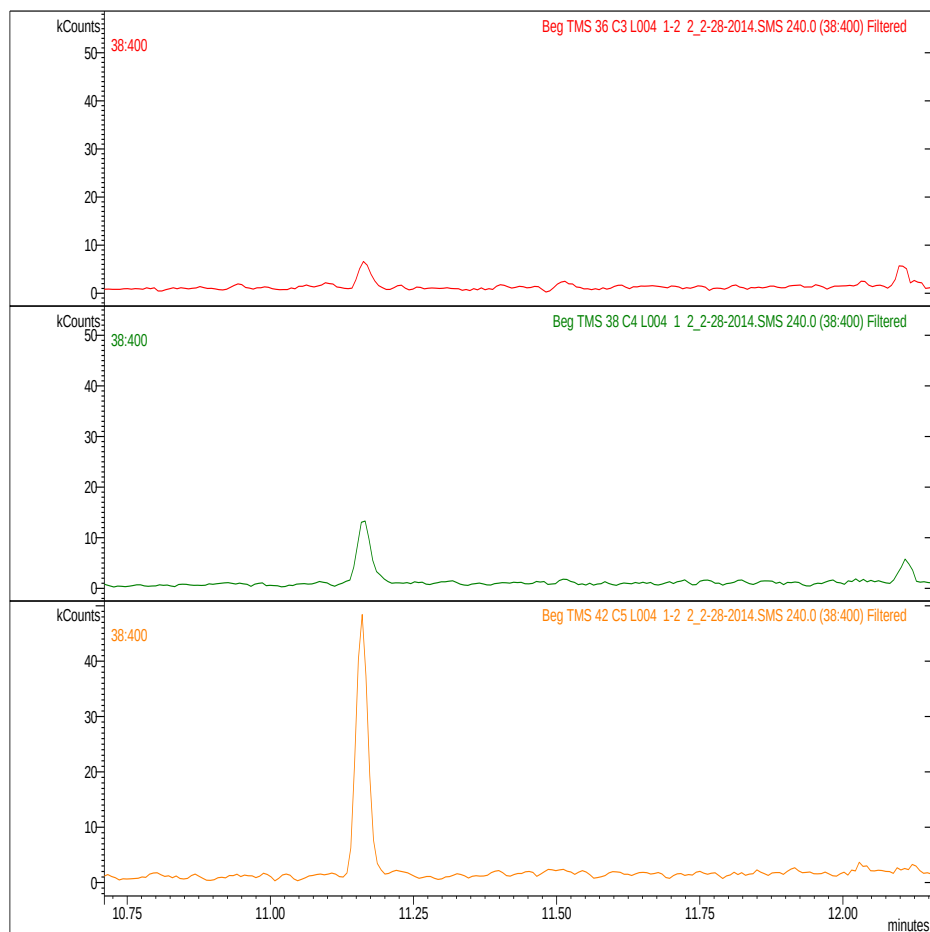


Fig. 1 :	Urine Samples Beg
	R.T. 11.17 Benzoylecgonine 50 –100 – 200 ng/ml

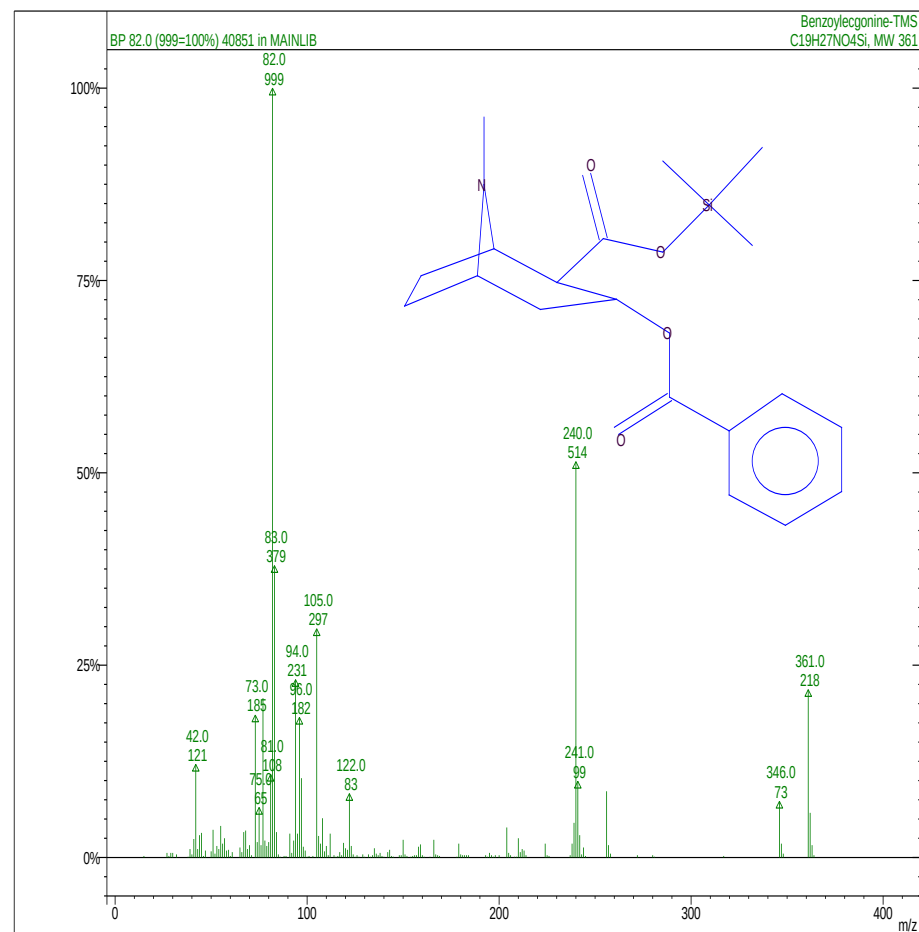


Fig. 2 :	Urine Sample Beg Massa Spectrum
	MOLECULAR IONS : 82 - 240 - 361

Benzoylecgonine (Cocaine's metabolite) – Cocaethylene – Cocaine in urine
(Reference Chromatograms/Spectra GC-MS)

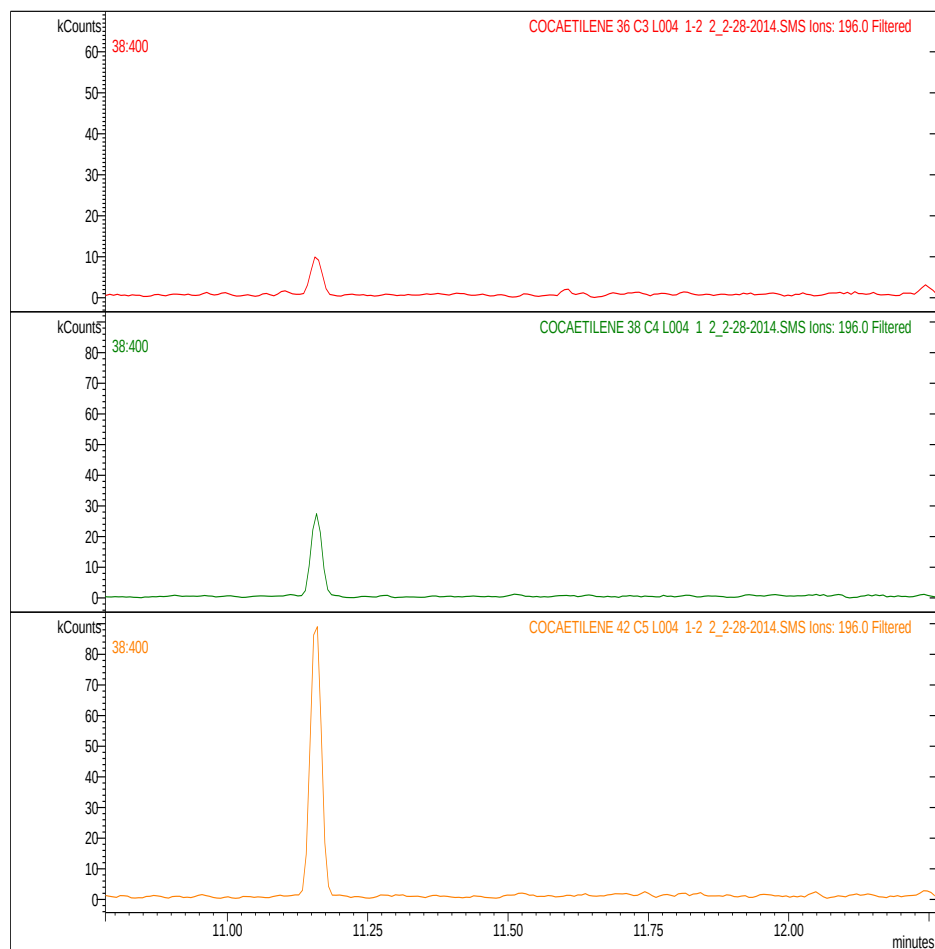


Fig. 3 :	Urine Samples Cocaethylene
	R.T. 11.16 Cocaethylene 50 –100 – 200 ng/ml

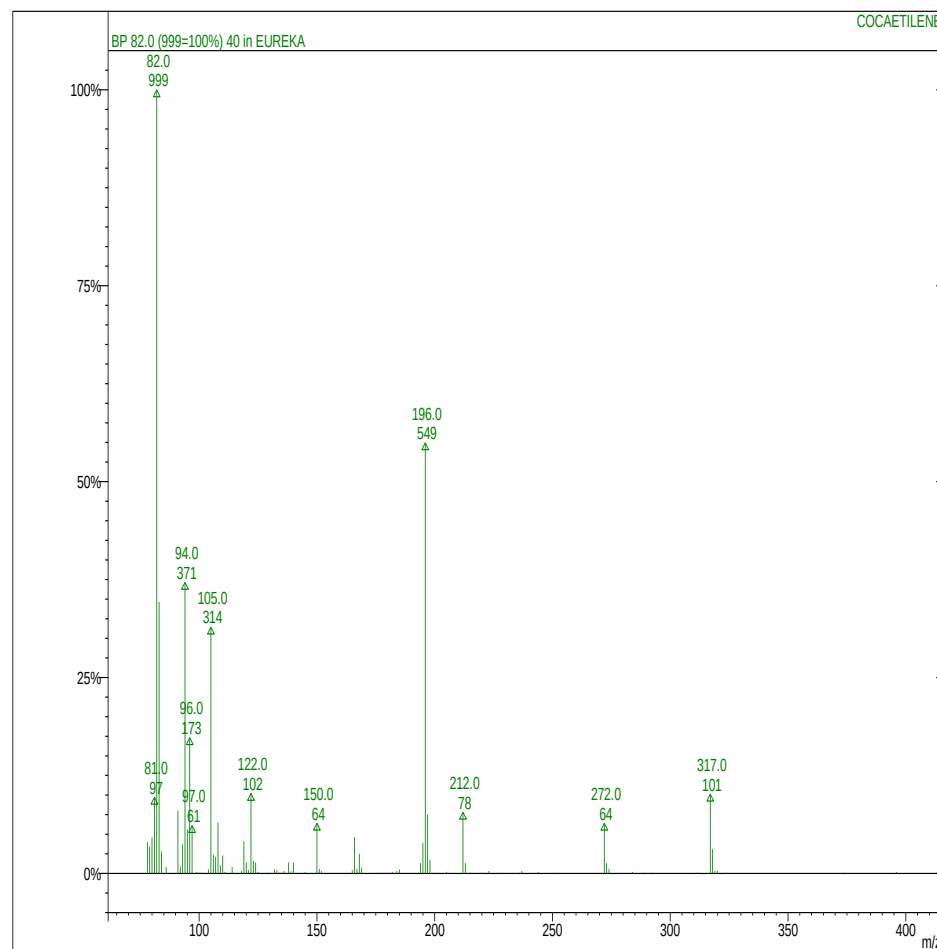


Fig. 4 :	Urine Sample Cocaethylene Massa Spectrum
	MOLECULAR IONS : 82 - 94 - 196

Benzoylecgonine (Cocaine's metabolite) – Cocaethylene – Cocaine in urine
(Reference Chromatograms/Spectra GC-MS)

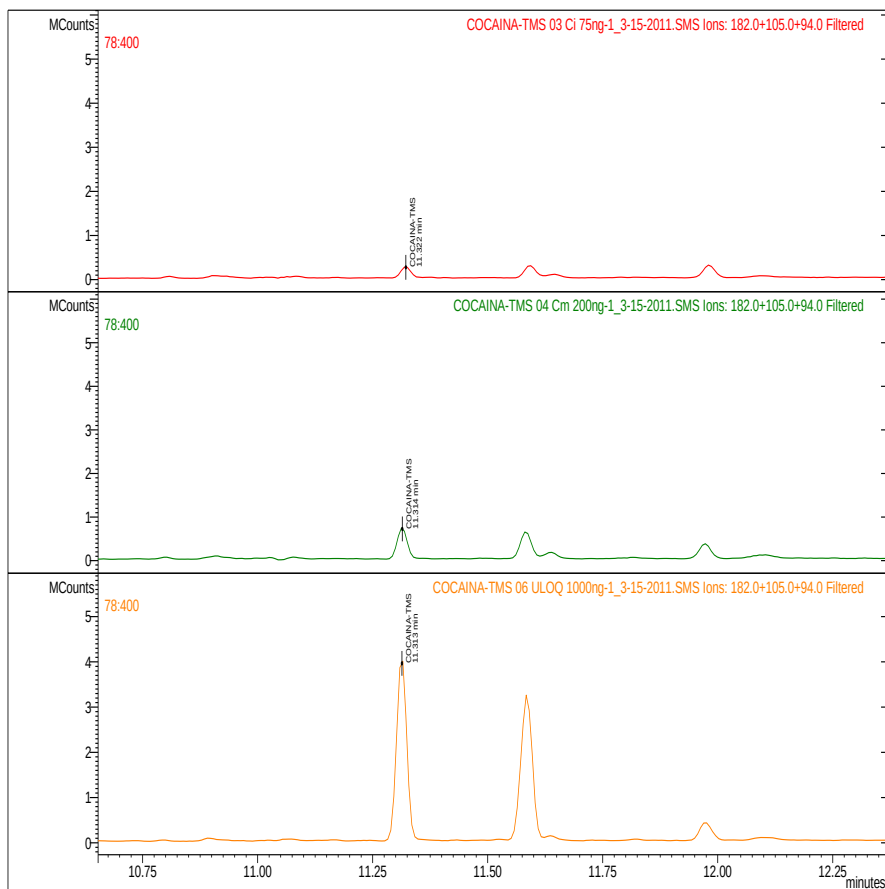


Fig. 5 :	Urine Samples Cocaine
	R.T. 11.30 Cocaine 75 –200 – 1000 ng/ml

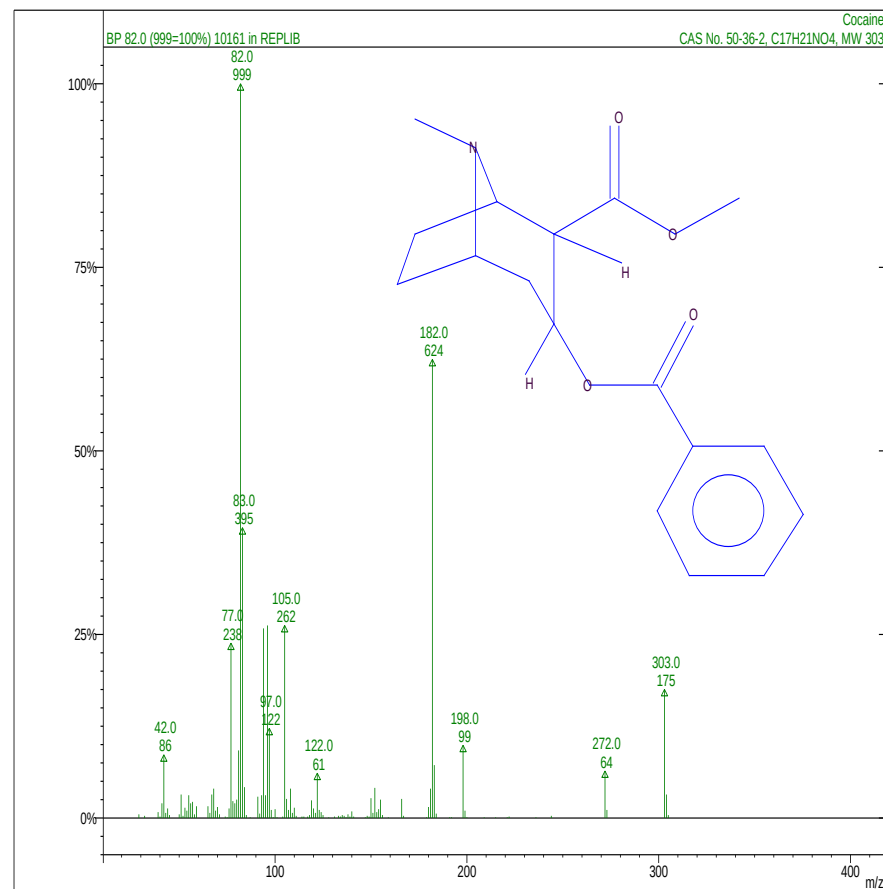


Fig. 6 :	Urine Sample Cocaine Massa Spectrum
	MOLECULAR IONS : 82 - 182 - 94

Benzoylecgonine (Cocaine's metabolite) – Cocaethylene – Cocaine in urine
(Reference Chromatograms/Spectra GC-MS)

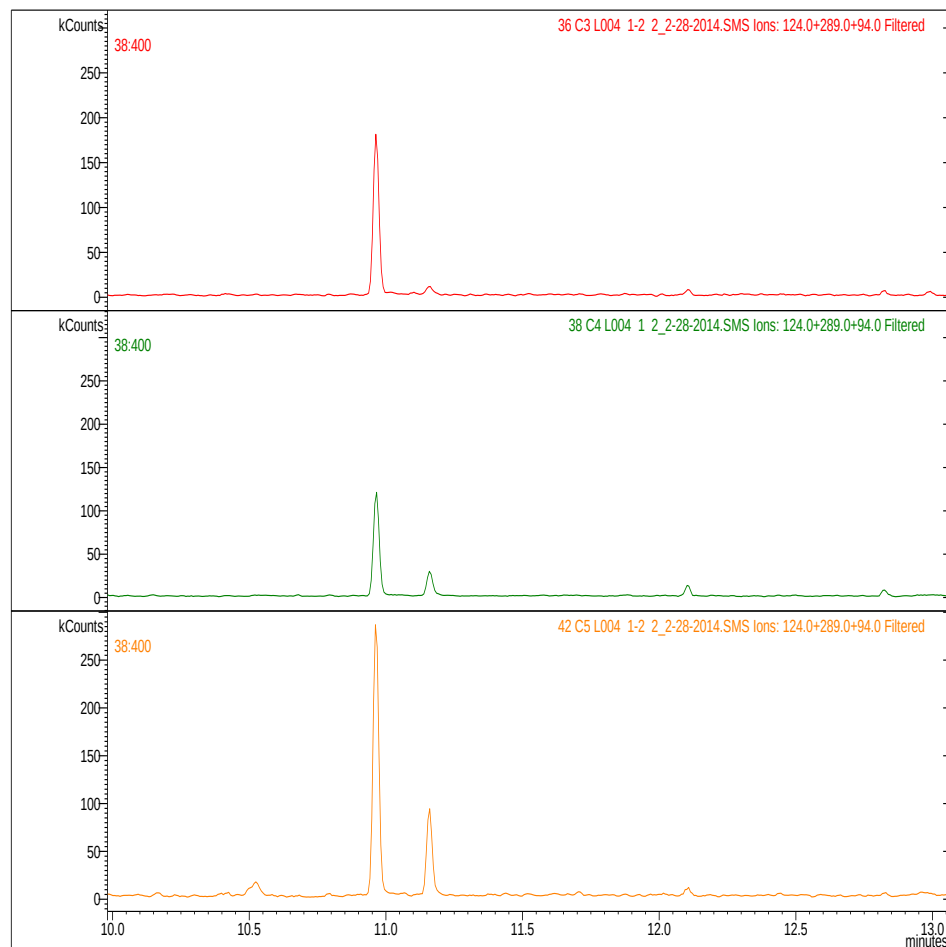


Fig. 7 :	Urine Samples with Internal Standard
	R.T. 10.90 INTERNAL STANDARD

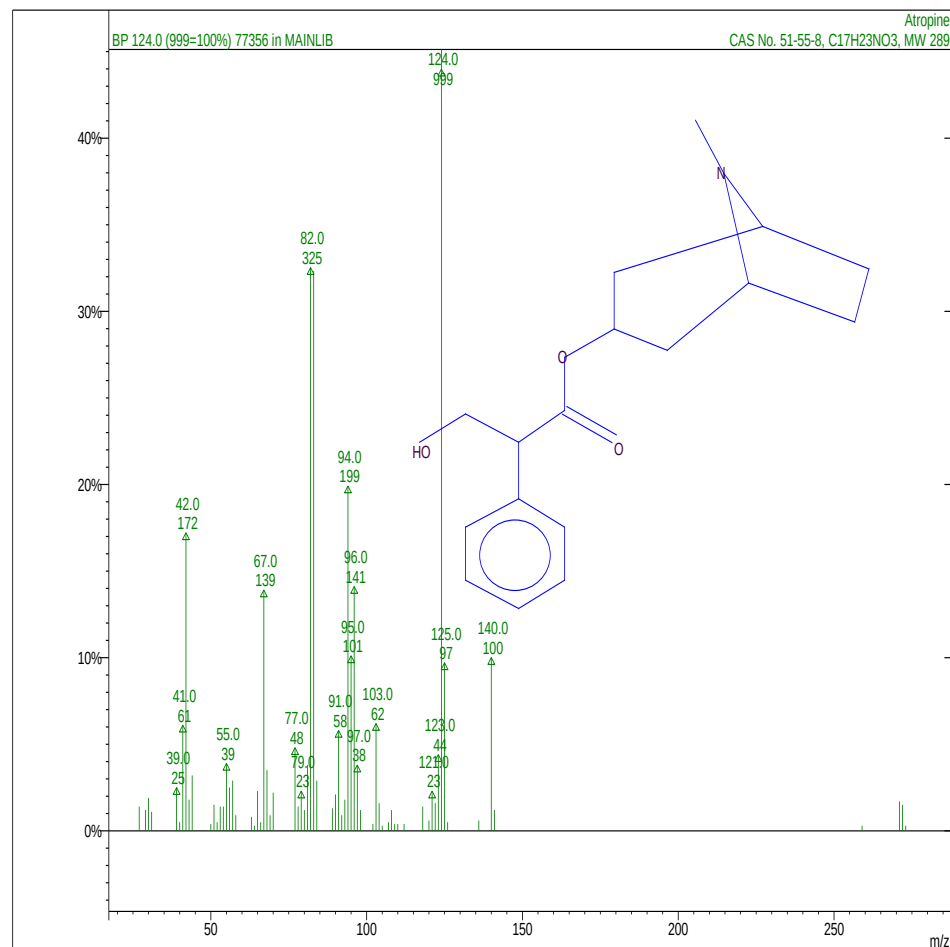


Fig. 6 :	Massa Spectrum of Internal Spectrum
	MOLECULAR IONS : 124 – 94 - 289