

ETHYLGLUCURONIDE AND ETHYLSULFATE IN URINE by LC-MS – Code LC78010

INTRODUCTION

The Ethylglucuronide is a molecule non-volatile, polar, relatively stable, ethanol formed by conjugation with glucuronic acid through the mediation of the UDP-glucuronyl transferase (UGT), a superfamily of enzymes highly polymorphic. The Ethylglucuronide seems to be synthesized by various isoforms of UGT function so that individual differences should not result in a distortion of the analytical data. Consequently, the lack in an individual of one isoform of the enzyme does not assume significant changes in the formation of Ethylglucuronide, and then individuals belonging to different ethnic groups synthesize all probability the same percentage of the compound following doses of alcohol. In general, it was estimated that it took 0,02 – 0,06% ethanol is converted into Ethylglucuronide.

The dose in the urine of Ethylglucuronide allows you to monitor the acute abuse of alcohol.

With the following protocol can be determined by LC / MS as well as the concentration of Ethylglucuronide also the urinary Ethylsulfate.



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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° 007	Ethylglucuronide and Ethylsulfate in urine by LC/MS	06/2024
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TECHNICAL FEATURES

Principle of the Method :

This method allows the determination of urinary Ethylglucuronide and Ethylsulfate after a simple dilution of the sample and reading in LC-MS.

Recovery :

100 %

Sensitivity (LLOD) :

0,05 mg/L

Minimum Concentration analyzable (LLOQ) :

0,1 mg/L

Dynamic Range of Method :

0,1 - 5 mg/L

Accuracy intra serie (relative error %)

Ethylglucuronide:

C LLOQ	Cm	Cs
0,1 mg/l	0,5 mg/l	1 mg/l
12,3%	5,1%	5,1%

Accuracy intra serie (relative error %) Ethylsulfate:

C LLOQ	Cm	Cs
0,1 mg/l	0,5 mg/l	1 mg/l
9,0%	8,3%	2,8%

Reproducibility intra serie (relative error %)

Ethylglucuronide:

C LLOQ	Cm	Cs
0,1 mg/l	0,5 mg/l	1 mg/l
4,3%	3,5%	5,4%

Reproducibility intra serie (relative error %)

Ethylsulfate:

C LLOQ	Cm	Cs
0,1 mg/l	0,5 mg/l	1 mg/l
7,9%	3,0%	5,4%

Cut-off:

0,5 mg/L

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

Components of the kit :

All the reagents are ready to use and stable 3 years at 2 – 8 °C except Internal Standards.

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

Reagent B – Internal Standard Solution for Ethylglucuronide, **1 x 2,5 ml**

Store at – 20 °C

Reagent C – Internal Standard Solution for Ethylsulfate, **1 x 2,5 ml**

Store at – 20 °C

Urine Calibrator – Level 1, 2 x 1 ml

Code LC78016 (packed separately – see data sheet)

Urine Calibrator – Level 2, 2 x 1 ml

Code LC78016 (packed separately – see data sheet)

Urine Calibrator – Level 3, 2 x 1 ml

Code LC78016 (packed separately – see data sheet)

Urine Calibrator – Level 4, 2 x 1 ml

Code LC78016 (packed separately – see data sheet)

Urine Calibrator – Level 5, 2 x 1 ml

Code LC78016 (packed separately – see data sheet)

Reagent M1 – Mobile Phase M1, **1 x 500 ml**

Minimum Instrumental equipment required:

LC-MS Instrument triple quadrupole
Work mode MRM, ESI negative
Operational Computer

Urine Collect Procedure :

Urine samples stored at 4 o -20° C from sampling to analysis. Stable 1 months.

Test tubes containing fluoride ions to inactivate the β -glucuronidase (limiting false negatives).

Tubes containing salts of azide to prevent bacterial growth (limitation of false positives).

SampleCheck assay: allows to identify the urine with bacterial growth (results between 110 and 150%).

ANALYTICAL PROCEDURE

STEP 1 :

In 10 ml test tubes dispense:

	Calibrator	Sample	Control
Reagent A – Diluting Sol.	475 µl	475 µl	475 µl
Calibrator	25 µl		
Sample		25 µl	
Control			25 µl
Reagent B – Internal Std Sol. for Ethylglucuronide	25 µl	25 µl	25 µl
Reagent C – Internal Std Sol. for Ethylsulfate	25 µl	25 µl	25 µl

Vortex for 20 sec.

INJECTION :

- Inject 20 µl of solution in LC system

Ethylglucuronide and Ethylsulfate in urine - Warnings

COLUMN CONDITIONING

Install a new analytical column Polaris 3 μ C18 A (100 x 2 mm, 3 μ m). Thermostated the column at 40°C Disconnect the detector and flow for 10 minutes a solution of H₂O: Mobile Phase M1 (5 : 95 v/v), set flow at 300 μ l / min then a solution of H₂O: Mobile Phase M2 (5 : 95 v/v) set flow at 300 μ l / min. **Don't recycle the washing solutions.** Condition the column with a solution of H₂O: Mobile Phase M1 (5 : 95 v/v), set flow at 300 μ l / min for 5 minutes.

It ISN'T possible to make analysis at recycling phase

If room temperature is > 20 °C store the Mobile Phase at 2-8 °C between an analytical session and another.

COLUMN CLEANING

Disconnect the detector. Flux a solution made of H₂O : Mobile Phase M2 (5 : 95 v/v) for 10 minutes at flow of 300 μ l/minute and discharge. The column is stored in a solution of H₂O : Mobile Phase M2 (5 : 95 v/v).

AUTOSAMPLER SYRINGE WASH

Before use wash the syringe with Methanol. Wash of autosampler needle intra - series with Methanol.

PARENT ION WITH TRIPLE QUADRUPOLE VARIAN 320 ESI NEGATIVE

ANALYTE	PARENT ION
Ethylglucuronide	221.0
Ethylglucuronide – D5	226.0
Ethylsulfate	125.8
Ethylsulfate – D5	130.8

FLOW SETTING

GRADIENT			
Time (min)	% M1 (PUMP A)	% M2 (PUMP B)	Flow (μl/min)
0 – 0:50	95	5	300
0:50 – 2:30	95	5	300
2:30 – 2:60	5	95	300
2:60 – 2:61	5	95	300
2:61 – 3:50	95	5	300

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
LC78016	Calibrator in urine for Ethylglucuronide and Ethylsulfate	5 x 2 x 1 ml
LC78019	Control in urine for Ethylglucuronide and Ethylsulfate – Levels 1 and 2	2 x 5 x 1 ml
SK78010	Starter kit for Ethylglucuronide and Ethylsulfate	4 x 1 ml
ZA2001100X020	Analytical Column Polaris 3u C 18 A (100 x 2 mm, 3 um)	1 Pc
S51843550	Clear glass vials with reduced volume from 1,5 ml to 15 ul	1 x 100 Pcs
S51820717	Caps for Clear glass vials with reduced volume from 1,5 ml to 15 ul	1 x 100 Pcs



Ethylglucuronide and Ethylsulfate in urine

(Reference Chromatograms/Spectra LC-MS)

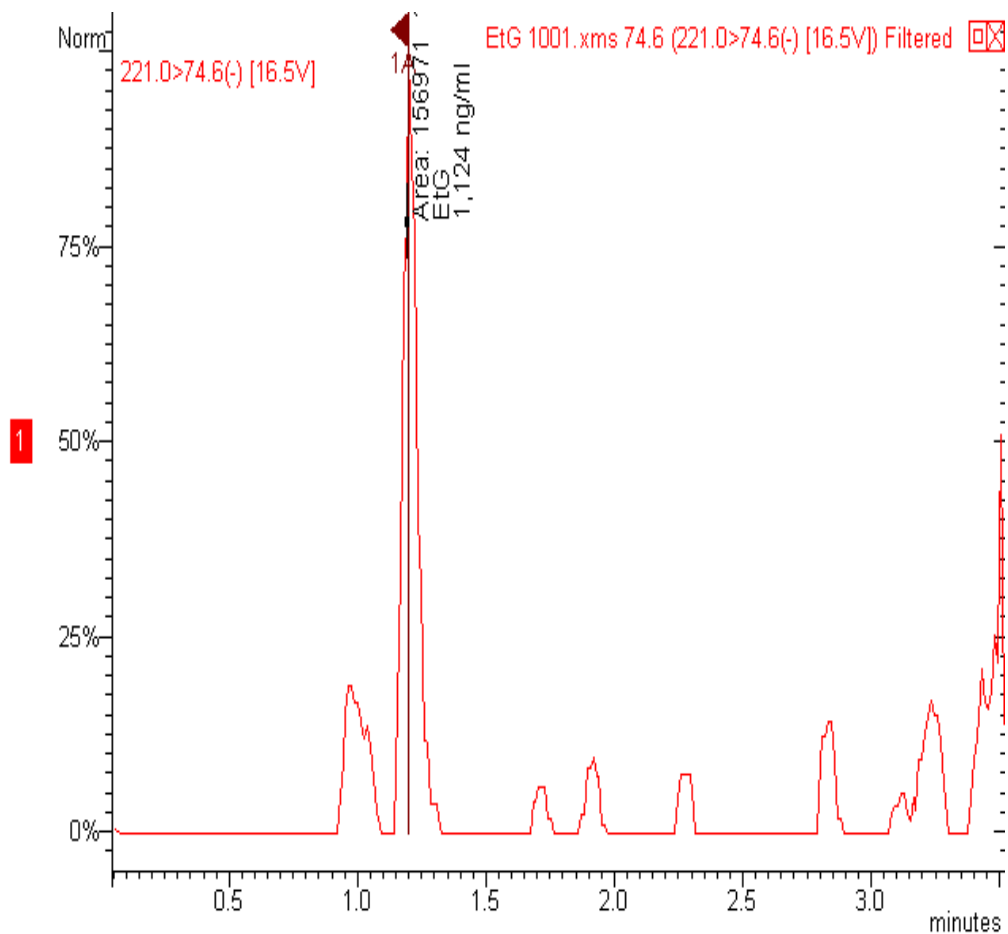


Fig. 1 : **Urine Calibrator**

R.T. 1.25	Ethylglucuronide	1 mg/l
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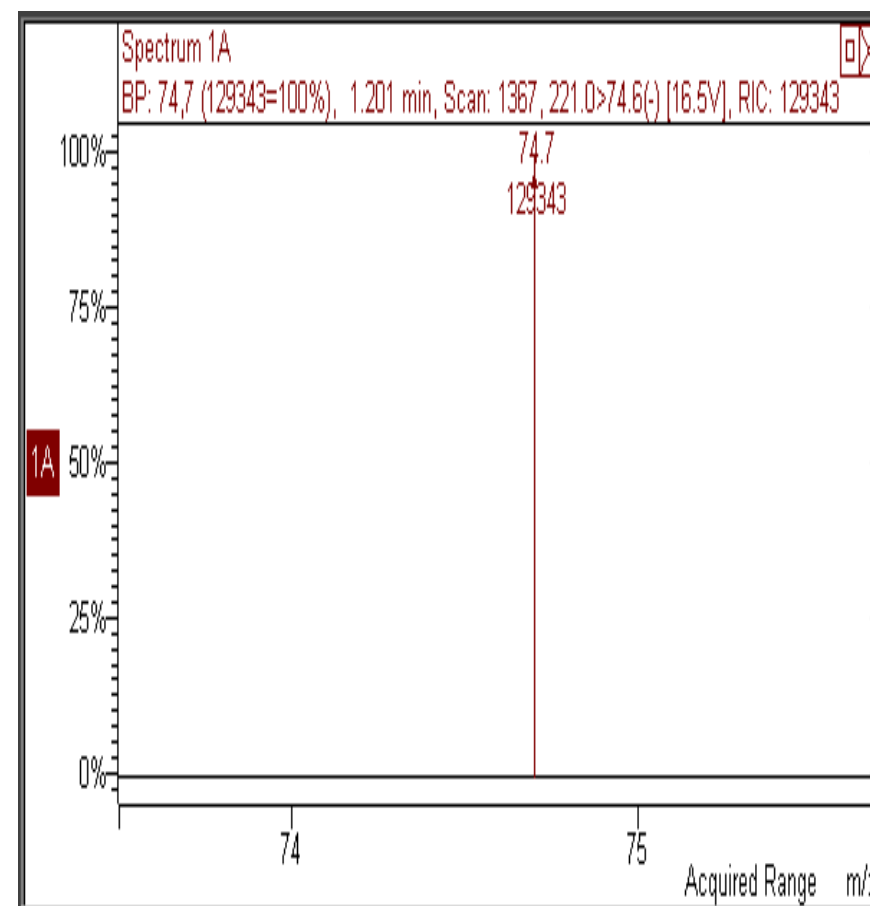


Fig. 2 : **Massa Spectrum of Ethylglucuronide**

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Ethylglucuronide and Ethylsulfate in urine

(Reference Chromatograms/Spectra LC-MS)

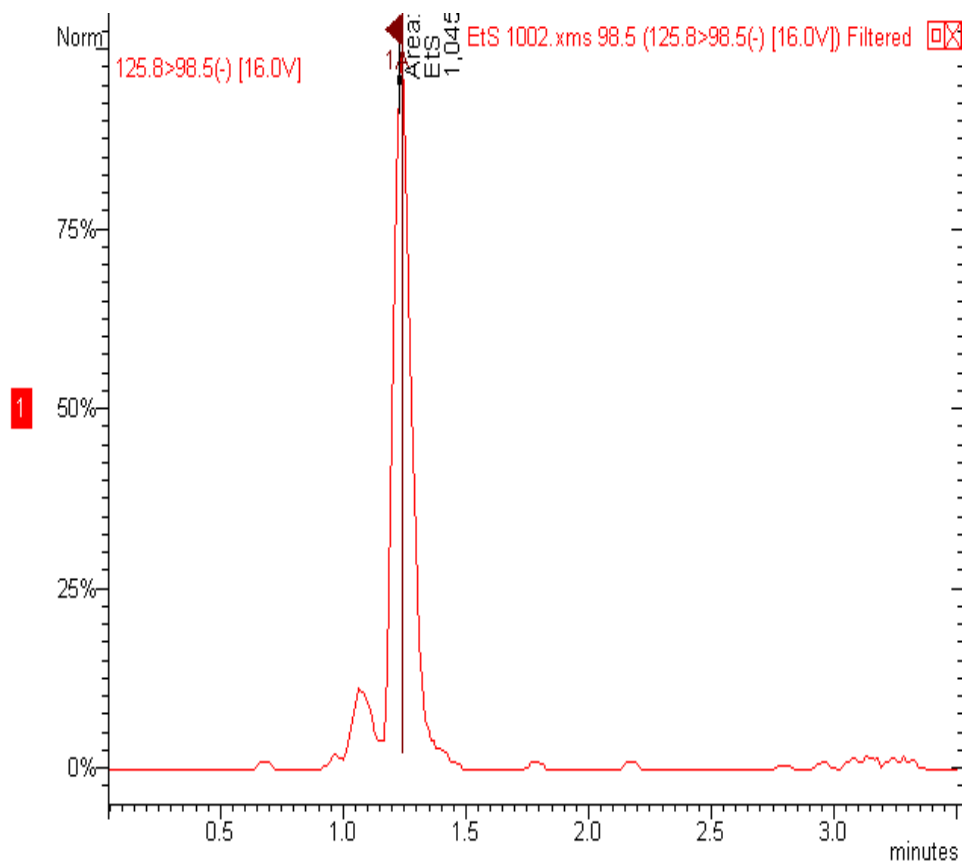


Fig. 3 :	Urine Calibrator
	R.T. 1.25 Ethylsulfate 1 mg/l

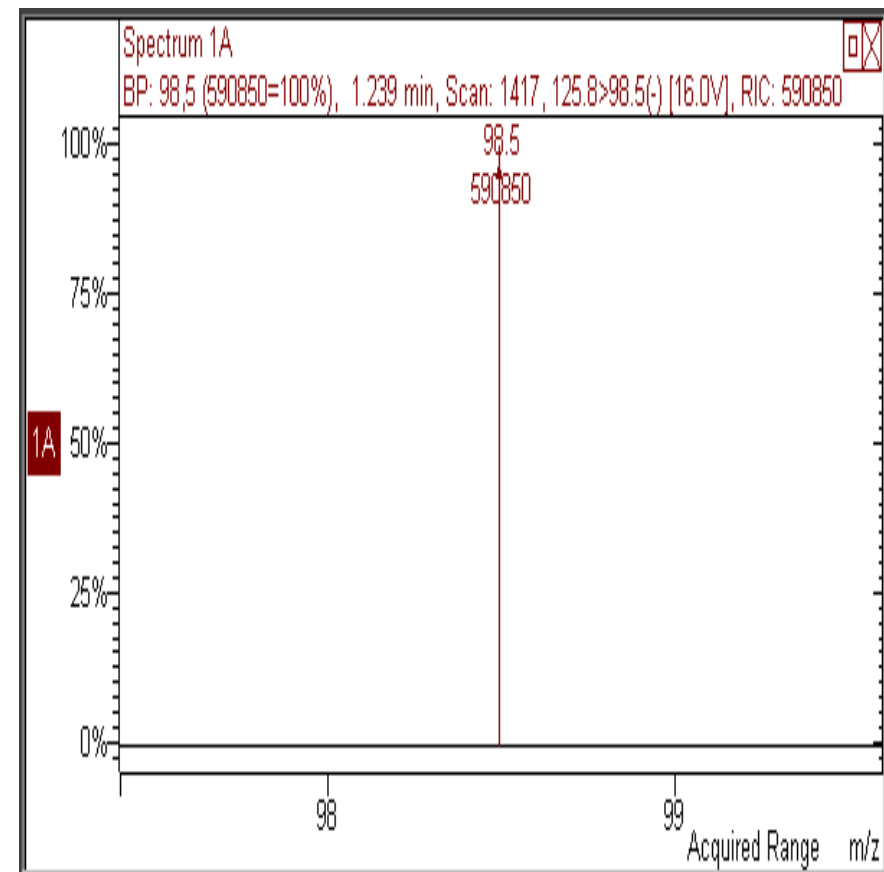


Fig. 4 :	Massa Spectrum of Ethylsulfate

Ethylglucuronide and Ethylsulfate in urine

(Reference Chromatograms/Spectra LC-MS)

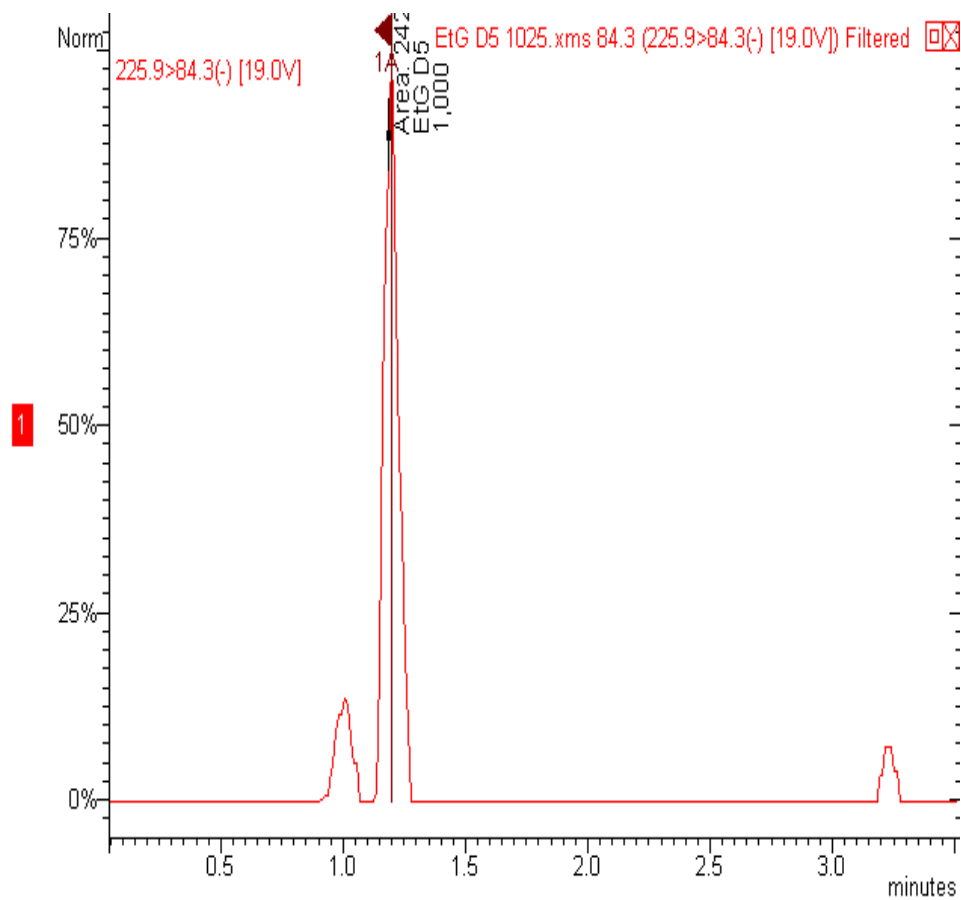


Fig. 5 :	Urine Calibrator
	R.T. 1.15 Ethylglucuronide-D5

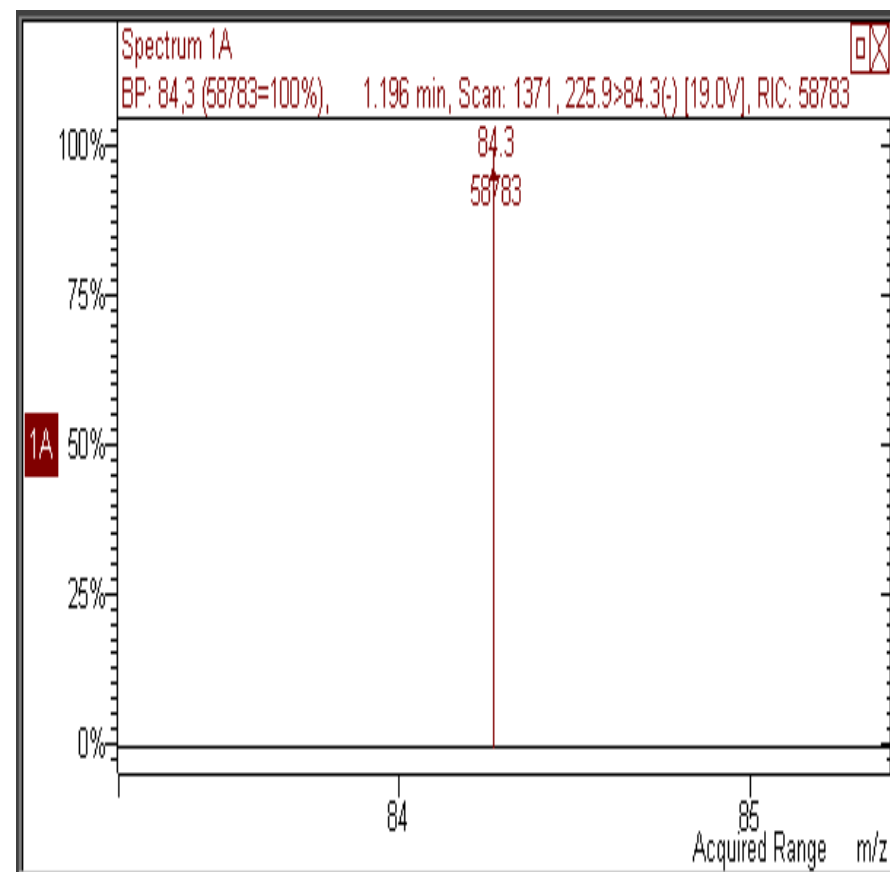


Fig. 6 :	Massa Spectrum of Ethylglucuronide-D5

Ethylglucuronide and Ethylsulfate in urine

(Reference Chromatograms/Spectra LC-MS)

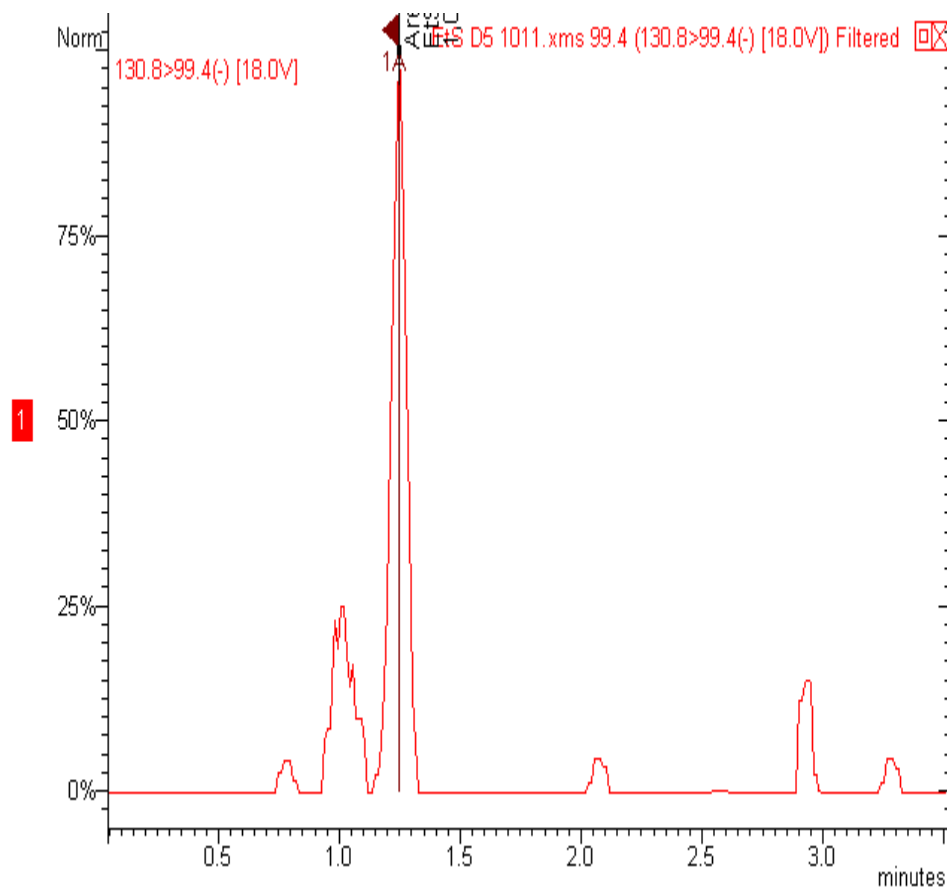


Fig. 7 :	Urine Calibrator
	R.T. 1.25 Ethylsulfate-D5

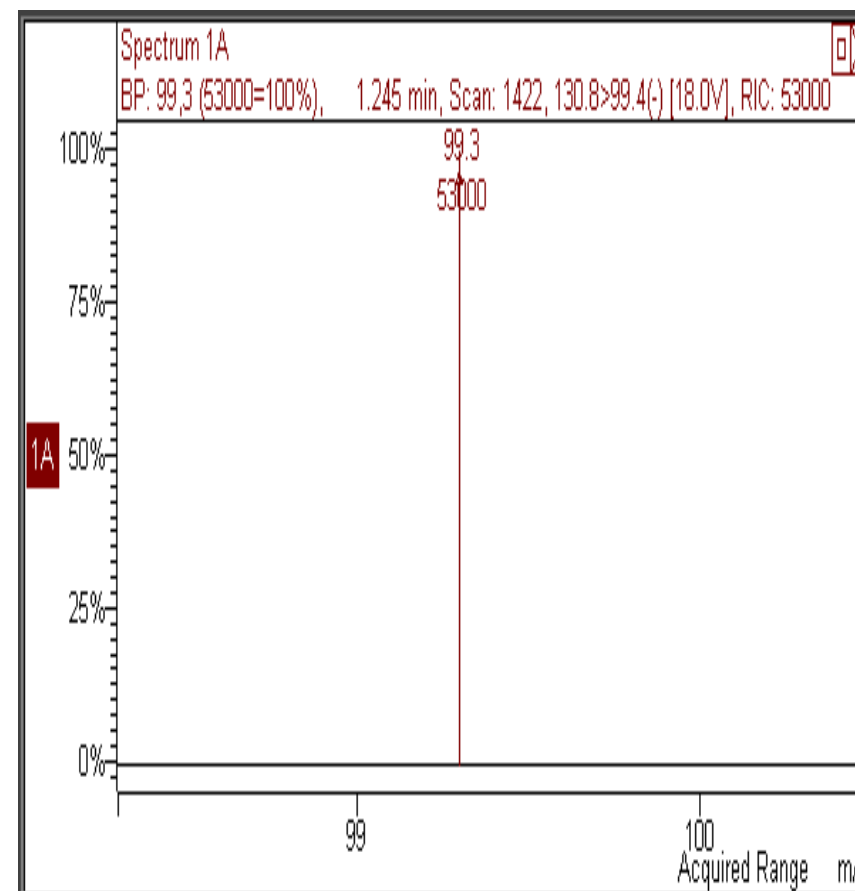


Fig. 8 :	Massa Spectrum of Ethylsulfate-D5