

ETHYLGLUCURONIDE IN HAIR by LC-MS/MS – Code LC95010

INTRODUCTION

Ethylglucuronide is a non-volatile, polar, relatively stable, metabolite of ethanol formed by conjugation with glucuronic acid through the mediation of the UDP-glucuronyl transferase (UGT), a superfamily of highly polymorphic enzymes. 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.

Individuals belonging to different ethnic groups, in all probability, synthesize the same percentage of the compound following doses of alcohol. In general, it was estimated that it took 0,02 – 0,06% for ethanol to be converted into Ethylglucuronide.

The dose of Ethylglucuronide in hair allows you to monitor acute abuse of alcohol. With the following protocol the concentration of Ethylglucuronide in hair can be determined by LC-MS/MS



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° 005	Ethylglucuronide in in hair by LC-MS/MS	06/2024
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TECHNICAL FEATURES

Principle of the Method :

This method enables the Ethylglucuronide after hydrolysis of hair, in liquid phase and reading extraction LC-MS.

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

Recovery : 109,5 %

Sensitivity (LLOD) : 0,2 pg/mg

Minimum concentration analyzable (LLOQ) : 2 pg/mg

Linearity : 2 – 2000 pg/mg

Accuracy intra serie (relative error %) :

Ci	Cs
10 pg/mg	100 pg/mg
6,4%	2,1%

Accuracy inter serie (relative error %) :

Ci	Cs
10 pg/mg	100 pg/mg
8,0%	3,2%

Reproducibility intra serie (relative error %) :

Cl	Cm	Ch
2 pg/mg	40 pg/mg	200 pg/mg
6,9%	2,7%	2,3%

Reproducibility inter serie (coefficient of variation %):

Cl	Cm	Ch
2 pg/mg	40 pg/mg	200 pg/mg
9,7%	4,5%	3,8%

Coefficient of Correlation R2 + Dev Std : 0,9973 ± 0,0012

Cut off : < 7 pg/mg abstinence evaluation
<30 pg/mg chronic consumption

Bibliography

1- J Mass Spectrom. 2006 Jan;41(1):34-42. Determination of ethyl glucuronide in hair samples by liquid chromatography/electrospray tandem mass spectrometry. Morini L, Politi L, Groppi A, Stramesi C, Poletini A.
2- Ethylglucuronide Determination in Urine and Hair from Alcohol Withdrawal Patients. Journal of Analytical Toxicology, Vol. 33, April 2009. Marta Concheiro, Angelines Cruz, Marisol Mon, Ana de Castro, Oscar Quintela, Angeles Lorenzo and Manuel López-Rivadulla. Forensic Toxicology Service, Institute of Legal Medicine, University of Santiago de Compostela, C/ San Francisco s/n, 15782. Santiago de Compostela, Spain and Alcoholism Treatment Unit (UTA), Department of Psychiatry, Hospital Clínico Universitario de Santiago de Compostela, Santiago de Compostela, Spain

Components of the kit (50 test) :

All the Reagents are ready to use and stable for 3 years at Room Temperature, except **Reagent B** and **Reagents D** stored at -20 °C.

Reagent A – Extraction Solution, **1 x 35 ml**

Reagent B – Internal Standard Solution, **1 x 150 µl**

See Warnings
Store at -20 °C

Reagent C – Hair Wash Solution, **1 x 250 ml**

Reagent D0 – Chemical Std Solution – Level 0, **1 x 250 µl**

Code LC95016
(Packed separately – see data sheet)

Reagent D1 – Chemical Std Solution – Level 1, **1 x 250 µl**

Code LC95016
(Packed separately – see data sheet)

Reagent D2 – Chemical Std Solution – Level 2 **1 x 250 µl**

Code LC95016
(Packed separately – see data sheet)

Reagent D3 – Chemical Std Solution – Level 3, **1 x 250 µl**

Code LC95016
(Packed separately – see data sheet)

Reagent D4 – Chemical Std Solution – Level 4, **1 x 250 µl**

Code LC95016
(Packed separately – see data sheet)

Reagent D5 – Chemical Std Solution – Level 5, **1 x 250 µl**

Code LC95016
(Packed separately – see data sheet)

Reagent S – Blank hair, **1 x 4000 mg**

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

Reagent M2 – Mobile Phase M2, **1 x 500 ml**

Minimum Instrumental equipment required:

Instrument LC-MS triple quadrupole
MRM modality
Interface ESI (-)
Binary Pump
Ultrasonic bath (sonicator)

Hair collection procedure:

Recommended length: 5 cm, starting from the scalp. A strand is cut, not torn (the bulb has no purpose at the end of these investigations) in the region of the vertex back of the head, of at least 200 mg which is divided into 2 aliquots of similar weight (A and B) each of which is fixed to the proximal end. These aliquots are placed in separate non-transparent containers with airtight caps, sealed with irremovable tape and stored at room temperature. The hair must be cut with scissors that have been cleaned with a small amount of alcohol and well dried.

Hair collection procedure :

And it is necessary to cut 200 mg of hair from the pubic region; the hair thus collected is divided into two aliquots (A and B).

ANALYTICAL PROCEDURE :

A) PREPARATION of unknown samples

STEP 1 : Wash hair intact inside a glass tube of 10 ml with 2 ml of **Reagent C – Hair Wash Solution**

Seal the tube with the cap

Vortex for 1 minute

Centrifuge at 3.500 rpm for 5 minutes

Open glass tube

STEP 2 : Evaporate to dryness the solvent with the aid of a light stream of helium or nitrogen paying attention to keep the hair / fur on the bottom of the tube. Alternatively remove the hair / fur from the tube with forceps cleaned with Acetone (not supplied in the kit), and place on paper towels until completely dry. In this last case the blotter paper should be changed for each sample.

Repeat steps 1 and 2 for two consecutive

STEP 3 : Cut your hair, washed and dried, in segments of 1-2 cm with scissors cleaned with Acetone (not supplied in the kit)

STEP 4 : Weigh an aliquot of sample, 100 mg (or depending on the availability of the sample), in a Pyrex glass tube with screw cap clean and previously named.

PREPARATION OF INTERNAL STANDARDS

STEP 5 : Reagent B - Internal Standards Sol supplied in the kit should be diluted 1:20 with water before each use, in the amount necessary for the entire assay.

Eg. for an analytic session with 10 unknown samples and 6 calibrators 320 µl is required of each Standard Internal dilution (**diluted Reagent B**). Therefore take 16 µl of **Reagent B - Internal standard Sol** and dilute with 304 µl of H₂O in two glass tubes. Vortex for at least 10 sec. The solutions are now ready for use.

Reagent B - Internal Standard Sol which is undiluted and unused should be closed tightly and kept the residual quantity in a freezer at -20°C.

A) PREPARATION of CALIBRATORS ON 6 LEVELS

STEP 6: Prepare 6 glass tubes of 10 ml call them C0-C1-C2-C3-C4-C5.

Add at each tube C0-C1-C2-C3-C4-C5 100 mg of **Reagent S – Blank Hair** (supplied in kit).

STEP 7: Prepare a number of tubes equal to the number of samples to which the unknown hair were added as from Step 4.

STEP 8: Add in each tubes prepared in Step 6 and 7 (calibrators, controls and samples) 700 µl of **Reagent A** – Extraction Sol.

STEP 9: Add in each tubes prepared in Step 6 and 7 (calibrators, controls and samples) 20 µl of **Reagent B diluted** (as in STEP B)

STEP 10: Add in each tubes of calibrators (C0-C1-C2-C3-C4-C5) the chemical standards **Reagent D** as below:

Calibrator C0	Calibrator C1	Calibrator C2	Calibrator C3	Calibrator C4	Calibrator C5
20 µl of Reagent D0	20 µl of Reagent D1	20 µl of Reagent D2	20 µl of Reagent D3	20 µl of Reagent D4	20 µl of Reagent D5

At this point Calibrators of 6 points are ready and the concentrations to be used for the calibration curve will be:

Analyte	Calibrator C0 (pg/mg)	Calibrator C1 (pg/mg)	Calibrator C2 (pg/mg)	Calibrator C3 (pg/mg)	Calibrator C4 (pg/mg)	Calibrator C5 (pg/mg)
EtG	0	2	10	20	40	200

Seal the tubes with the cap - Centrifuge for 3 minutes at 3500 rpm so as to submerge the hair in liquid

STEP 11: Incubate at 25 °C overnight

STEP 12 : Put in ultrasonic bath (sonicator) for 2 hours

Open the glass tubes

STEP 13 : Withdraw all the liquid being careful not to remove the hair and transfer in Eppendorf.

Centrifuge for 10 minutes at 14000 rpm

STEP 14 : Take the supernatant and put it in a glass vial.

E) INJECTION :

STEP 15 : Inject 12 µl of solution in LC system

ETHYLGLUCURONIDE in hair - Warnings

REAGENT B: INTERNAL STANDARD SOLUTION

Ethylglucuronide-D5	10.000 ng/ml
Storage: store at – 20 °C	

COLUMN CONDITIONING

Install a new analytical column Polaris 3u C18 (100 x 2 mm, 3 um) thermostated at 40 °C. Disconnect the detector and filter a solution of H₂O: Acetonitrile (10 : 90 v/v), set flow at 300 ul / min for 10 min. **Don't recycle the washing solutions.** Condition the column with a solution of Mobile phase M1: Mobile Phase M2 (99 : 1 v/v) set flow at 600 ul/min for 10 min.
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 : Acetonitrile (5 : 95 v/v) for 10 min at 600 ul/min and discharge. Store in a solution of H₂O : Acetonitrile (5 : 95 v/v).

INJECTION NEEDLE WASH

Wash the syringe before each injection with Water.

Interface ESI Sciex 4500 Parameters

Curtain Gas (CUR): 40
Collision Gas (CAD): 8
Ion Spray Voltage: (IS): -4500 V
Temperature (TEM): 650°C
Ion Source Gas 1 (GS1): 60
Ion Source Gas 2 (GS2): 60

Fragmentations (optimized on TRIPLE QUADRUPOLE Sciex 4500)

Analyte	Q1	Q3	COLLISION ENERGY
Ethylglucuronide	221.1	75/85	-19/-21
Ethylglucuronide-D5	226.1	75/85	-20/-21

FLOW SET

GRADIENT			
TIME (min)	% M1 (PUMP A)	% M2 (PUMP B)	FLOW (µl/min)
0.00	99	1	600
2.00	99	1	600
2.01	10	90	600
4.50	10	90	600
4.51	99	1	600
7.00	99	1	600

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
LC95016	Calibrator in hair for Ethylglucuronide	6 x 250 ul x 4.000 mg
LC95019	Control in hair for Ethylglucuronide – Levels 1 and 2	2 x 250 ul x 1500 mg
SK95010	Starter kit for Ethylglucuronide	1 Pc
L-30-210	Eppendorf with caps	1 x 100 Pcs
Z-1636/26	Pyrex Tubes of 10 ml	1 x 40 Pcs
ZA2001100X020	Polaris C18 A100 (100 x 2,0 mm, 3 um) Analytical Column	1 Pc
Z51843550	Clear glass vials with reduced volume from 1,5 ml to 15 ul	1 x 100 Pcs
Z51820717	Caps for Clear glass vials with reduced volume from 1,5 ml to 15 ul	1 x 100 Pcs

ETHYLGLUCURONIDE IN HAIR

(Reference Chromatograms)

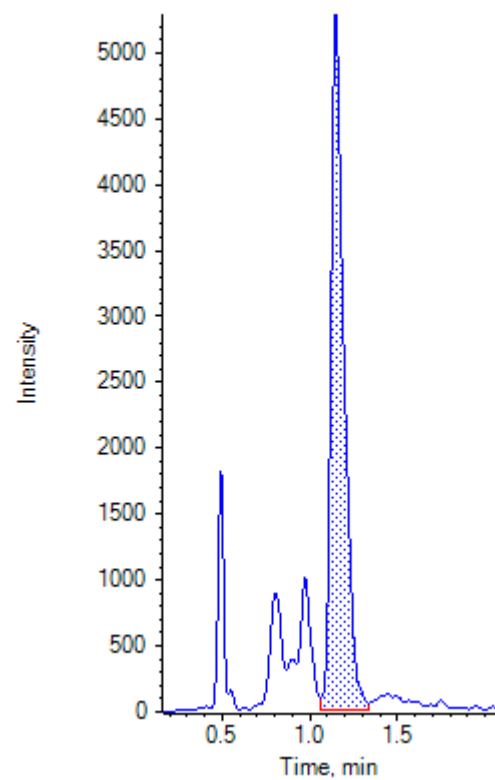


Fig. 1 :	Calibrator 20 pg/mg
	R.T. 1.15 Ethylglucuronide

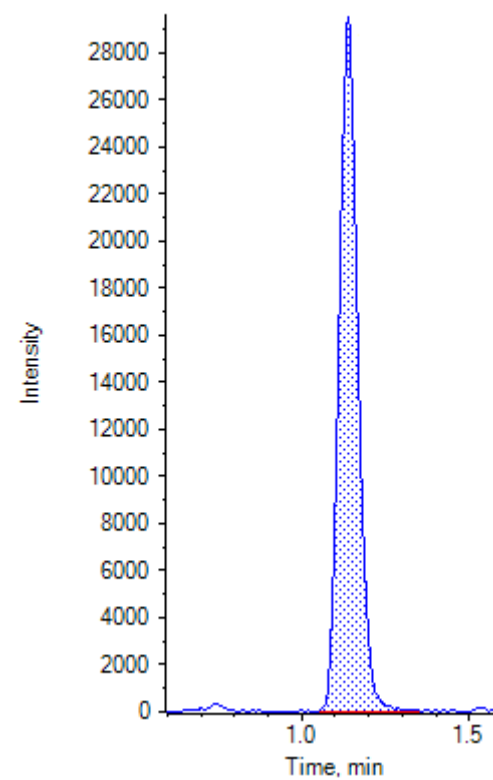


Fig. 2 :	Calibrator 20 pg/mg
	R.T. 1.15 Ethylglucuronide-D5