SHIMADZU

WP158

Determination of Ethyl Glucuronide in plasma by ATLAS-LEXT NHD combined with LC-MS/MS

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1. Introduction

Ethyl glucuronide (EtG) is the iconic metabolite of alcohol in human body, and it is an important evidence to judge whether drivers drink alcohol within 12 hours.

EtG was generally detected by LC-MS/MS or GC-MS/MS. Since derivation is not required, the former method is more convenient and faster than the latter.

ATLAS-LEXT NHD automatic pre-treatment device can automatically perform liquid-liquid extraction and protein precipitation on biological samples such as blood and urine. With a high degree of automation, it can save a lot of time, improve efficiency, reduce human errors, and reduce the physical harm caused by the use of large amount of organic solvents.

In this paper, ATLAS-LEXT NHD combined with LC-MS/MS was used to determine EtG in plasma.

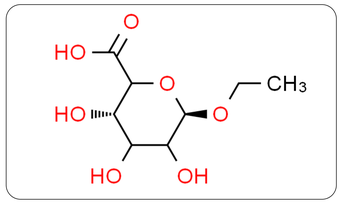

Figure 1 Structure of EtG

2. Instruments

1. ATLAS-LEXT NHD automatic pre-treatment device (Shimadzu Corporation, Kyoto, Japan) ;

2. The LC-MS/MS system include Nexera X3 system and triple quadrupole mass spectrometry (Shimadzu Corporation, Kyoto, Japan). Nexera XR system consist of a CBM-40 system controller, a LC-40BX3 pump, a SIL-40CX3 autosampler, a CTO-40C column oven, and a DGU-405 online degasser. MS/MS detection was performed by LCMS-8050. Data acquisition and processing were performed with Labsolution software Version 5.99. Electrospray ionization was operated in multiple-reaction-monitoring (MRM) mode .

Figure 2 ATLAS-LEXT NHD and LCMS-8050

3. Results

3-1. Method development for EtG

ATLAS-LEXT NHD conditions

Sample volume: 0.2 mL
Protein precipitation solvent: acetonitrile, 1.8 mL
Mixing intensity: Level 9
Centrifugal speed: 5750 rpm
Mixing time: 90 s
Centrifugal time: 180 s

HPLC conditions

Column: Shim-pack GIST C18□ 2.1 mm I.D.× 150 mm L., 3 μm□

Mobile phase A: 0.01% formic acid aqueous solution

B: Acetonitrile

Elution Mode: Gradient Elute, the initial concentration of MP B was 5%,

Table 1. LC Time Programme

Time	Module	Command	Value
1.20	Pumps	Pump B Conc.	10
1.80	Pumps	Pump B Conc.	90
2.80	Pumps	Pump B Conc.	90
2.90	Pumps	Pump B Conc.	5
6.00	Controller	Stop	

Injection vol.: 1 uL

Column temperature: 40° C

MS conditions (LCMS-8050)

Ionization: ESI, Negative MRM mode
Nebulizer Flow: 3.0 L/min
Heating Gas Flow: 10.0 L/min
Interface Temperature: 300 ° C
DL Temperature: 250 ° C
Heat block Temperature: 400 ° C
Dry Gas: 10.0 L/min

Table 2. MRM transition

Compound	MRM transition	Q1 Pre Bias (V)	CE	Q3 Pre Bias (V)
EtG	221.10>75.10*	11	16	14
	221.10>85.10	11	18	15

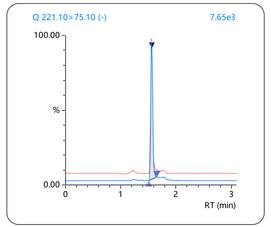

Figure 3 MRM chromatograms of standard solution of EtG(1 μg/mL □

3-2. Analytical Performance

3-2-1. Linearity

The determination of EtG was verified using an external standard method. The external calibration was performed by plotting peak area versus concentration of EtG(As seen in Figure 4). The sample solutions were spiked with stock solution to get final concentrations of EtG at 0.1, 0.5, 1, 5, 10 μg/mL.

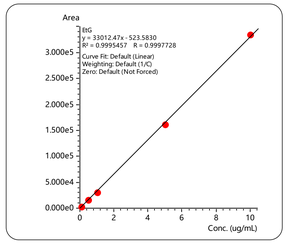

Figure 4 Calibration curve of EtG

3-2-2. Repeatability and Recovery

Preparation of blank plasma samples spiked at 1 μg/mL and 10 μg/mL. According to the mentioned method before, each sample was measured six times in parallel. The repeatability and recovery results were shown in table 3.

Table 3. Repatability and Recovery results

No.	Concentration	R.T. RSD (%)	Area RSD (%)	Recovery (%)
1	1 μg/mL	0.16	4.96	80.3
2	10 μg/mL	0.11	2.88	78.1

4. Conclusions

In this paper, ATLAS-LEXT NHD combined with LC-MS/MS was used to determine EtG in plasma. This method has the characteristics of high automation, high throughput, good recovery rate and repeatability. It utilizes ATLAS-LEXT NHD custom programming function for protein precipitation, which can reduce errors caused by human operations and exposure risks to chemical reagents. It can be used for the determination of EtG in blood samples.