Determination of ethyl glucuronide in human urine by solid phase extraction-gas chromatography-mass spectrometry

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Abstract: A solid phase extraction (SPE) and gas chromatography-mass spectrometry (GC-MS) method for determination of ethyl glucuronide (EtG) in human urine was established. One mL urine sample was deproteinized by 100 μL 3 mol/L hydrochloric acid and cleaned up through a solid phase extraction column. The target analytes were eluted from an NH2-column with 4% ammonia solution and then treated with BSTFA + trimethylchlorosilane (TMCS) 99 : 1 for derivatization. The derivatized samples were analyzed by GC-MS. Data were acquired in the selected ion monitoring (SIM) mode and the quantitation of EtG was done through internal standard method. Good linearity was obtained at the mass concentration range of 0.1 - 3.2 mg/L with a correlation coefficient r² of 0.992 1. The limit of detection (LOD) was 28.4 μg/L. The range of recoveries was 92.5% – 108.7% and the relative standard deviations (RSDs) of intra-day and inter-day were all less than 5%. This method is sensitive, specific, accurate and can be applied to the determination of EtG for medicolegal identification and clinical laboratory.

Key words: solid phase extraction, SPE, gas chromatography-mass spectrometry, GC-MS, derivatization, ethyl glucuronide, EtG

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1.2.1 SPE

- 1 mL [ ] 100 µL [ ] 3 mol/L HCl [ ]
- 5 000 r/min [ ] 5 min [ ]
- [ ] 3 mL [ ]

1.2.2 BSTFA + TMCS

100 µL BSTFA + TMCS [ ]

1.3 HP-5MS 30 m × 0.25 mm × 0.25 µm [ ]

1.4 LOD [ ] 1/10 [ ]

2.1 SPE

EtG [ ]

SPE [ ]

EtG [ ]

4% [ ]

SPE [ ]

EtG [ ]

LOD [ ] 1/10 [ ]

2.2 BSTFA + TMCS

EtG [ ]

5 µL [ ]

2.2.1 BSTFA + TMCS 50 µL [ ]

50 µL [ ]

50 60 70 80 90 [ ]

70 [ ]

EtG [ ]
2.2.2

- 100 μL BSTFA + TMCS
- 70 °C, 1 h
- 20°C, 30 min, 50 μL
- 40 min, 20°C, 30 min

2.3 EtG

2.4

- EtG 0.4 mg/L
- 18.73 min
- 2, 3

2.5

- EtG
- D₃-EtG
- 0.5 1.0 1.5 mg/L
- 5
- RSD
- 1

Table 1

<table>
<thead>
<tr>
<th>Spiked [mg/L]</th>
<th>Recovery (%)</th>
<th>RSD/ %</th>
</tr>
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<tbody>
<tr>
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<td>Intra-day</td>
<td>Inter-day</td>
</tr>
<tr>
<td>0.5</td>
<td>108.7</td>
<td>3.4</td>
</tr>
<tr>
<td>1.0</td>
<td>95.9</td>
<td>3.2</td>
</tr>
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<td>1.5</td>
<td>92.5</td>
<td>2.8</td>
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</tbody>
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Fig. 1 Chromatograms of a urine sample spiked with EtG 200 μg/L [a] extracted by direct method and [b] SPE method.

Fig. 2 Chromatograms of [a] a blank urine and [b] a urine sample spiked with EtG 0.4 mg/L.

Fig. 3 Mass spectrum of a urine sample spiked with EtG after derivatization.

Table 1 Spiked recoveries and precisions of EtG in urine n = 5.

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第6期 于天晓,等:固相萃取气相色谱质谱法检测尿液中乙基葡萄糖醛酸苷

实际样本测定
男性受试者(30岁,65 kg)饮酒后收集尿液样本,分别检测尿液中乙醇含量和乙醛含量。经检测发现实际尿液样本中乙醇的质量浓度为440 mg/L,乙醛的质量浓度为116 μg/L。具体结果见图2。

稳定性实验
在实际应用中,由于某些原因采集到的样本不能被及时检测,因此实验考察了保留时间对乙醛体在尿液中含量的影响。实验选择乙醛阳性样本和添加乙醛(0.5 mg/L)的空白尿液样本各1例,分装后分别置于室温和冰箱保存,每隔1 d检测一次,连续检测5次,观察两个样本在相同时间内不同温度条件下响应值的变化情况(如图3)。

图2 实际尿液样本的色谱图
图3 保留时间对尿液中乙醛浓度的影响

3 结语
本实验建立了运用SPE-GC-MS检测尿液中乙醛的方法。本方法样本用量少且便于采集,预处理简单,有较好的灵敏度和线性关系,回收率高,时间短,对法医鉴定和临床检验中乙醛的定性定量分析有重要意义。

参考文献: