Analysis of the preferred conformations and determination of the concentrations of ephedrine and pseudoephedrine based on hollow fiber liquid-phase microextraction

CHEN Xuan, BAI Xiaohong*, WANG Xiaofeng, WANG Jing, BU Wei

Department of Pharmacy, Shanxi Medical University, Taiyuan 030001, China

Abstract: The preferred conformations of the ephedrine and pseudoephedrine in Ephedra sinaica Stapf and rat urine were analyzed by the hollow fiber liquid-phase microextraction (HF-LPME) and their extraction mechanisms were illuminated. The method of the separation of the ephedrine and pseudoephedrine and the determination of their concentrations with high performance liquid chromatography (HPLC) were established. The optimal experimental conditions were as follows: the organic phase carrier was the hollow fiber of polyvinylidene fluoride (PVDF). The MOF-503 organic solvent was n-hexanol; the extraction time was 35 min; the stirring rate was 1200 r/min; the sample phase was the NaOH solution; 5 mol/L of the analyte; the acceptor was 0.01 mol/L H₂SO₄ solution. The extracts were analyzed by HPLC. Under the optimal conditions, the method is convenient and highly sensitive. In Ephedra sinaica Stapf, the linear ranges of ephedrine and pseudoephedrine were 5 – 100 μg/L, and the detection limits were 1.9 μg/L and 1.2 μg/L, respectively. In rat urine, the linear ranges were 100 – 5 × 10⁴ μg/L, and the detection limits were 30 μg/L and 42 μg/L, respectively. The obtained results indicated that the method can be successfully applied for the extraction and determination of the ephedrine and pseudoephedrine in Ephedra sinaica Stapf and rat urine.

* Corresponding author. E-mail: bzh246@163.com

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Key words: hollow fiber liquid-phase microextraction, preferred conformation extraction mechanism, ephedrine, pseudoephedrine, *Ephedra sinica* Stafî, rat urine

1  
1.1  
1.1.1  

Agilent 1200, G1314B  

MOF 503: 0.8 mm, 0.2 μm  

MIF 503: 0.8 mm, 0.1 μm  

UEIS 503: 0.8 mm, 0.2 μm  

UPIS 503: 0.8 mm, 0.2 μm  

171237-200505, 171241-200506  

1.2  
1.2.1  

2 mg [C] 100 mL  

1.2.2  

0.4 g [C] 40 mL 1 mol/L [C] -20% [C] 1:10 [C] v/v 12 h 100  

W 40 kHz [C] h 100 mL 1 mol/L [C] -20% [C] 1:10 [C] v/v 10 mL 1 mol/L [C] -20% [C] 1:10 [C] v/v 10 [C] 10  

1.2.3  

Wistar 200 ± 5.5 g [C]  

12 h [C] 1 mg [C] 24 h [C] -20 ℃ [C]  

1.2.4  

2.5 mL [C] 5 mol/L [C] 1.5 mL [C] 10 cm [C] 30 s [C] 0.01 mol/L [C] 50 μL [C] 1 200 r/min [C] 35 min [C]  

1.2.5  

HPLC  

Agilent Eclipse XDB-C18 150 mm × 4.6 mm [C] 5 μm [C] 3.97 [C] v/v 1.0 mL/min [C] 210 nm [C] 20 μL  

2  

2.1  
2.1.1  

MOF 503 > MIF 503 > UP-IS 503 > UEIS 910 > MOF 503  

20 mg/L
Organic solvent selection

Extraction efficiency was studied for water and urine samples using various organic solvents such as alcohols, hydrocarbons, and aromatic hydrocarbons. As shown in Fig. 1, aliphatic alcohols showed the highest extraction efficiency, with shorter chain alcohols having lower efficiency than longer chain alcohols. The highest efficiency was observed with n-hexanol. The extraction efficiency gradually decreased with increasing carbon atom number. Therefore, n-hexanol was selected as the solvent.

Sample phase NaOH concentration selection

Extraction efficiency was also evaluated with different NaOH concentrations in the sample phase. As illustrated in Fig. 2, the efficiency rose significantly with an increase in NaOH concentration until reaching 0.01 mol/L, beyond which it remained constant. Therefore, a NaOH concentration of 0.01 mol/L was selected.

接收相的选择

母液相中氢氧化钠浓度的选择

考察了样品相中氢氧化钠浓度对水和尿液中两分析物萃取效率的影响,结果见图。当氢氧化钠浓度小于 0.01 mol/L 时,萃取效率随着浓度的增大显著提高,当氢氧化钠浓度大于 0.01 mol/L 时,萃取效率不再增加。故选择样品相中的氢氧化钠浓度为 0.01 mol/L。

接收相的选择

考察了接收相分别为盐酸、醋酸、硫酸、磷酸溶液时对分析物萃取效率的影响。结果表明,硫酸溶液明显优于其他溶液,且浓度为 0.01 mol/L (27 µg/L) 时萃取效率最高。故选择 0.01 mol/L 硫酸溶液为接收相。

萃取时间的选择

考察了萃取时间对两分析物萃取效率的影响。结果表明,分析物在萃取到平衡后,之后随着萃取时间的延长萃取效率明显下降。因此选择萃取时间为 35 min。

搅拌速度的选择

考察了搅拌速度为 300 ~ 1800 r/min 时对萃取效率的影响。结果表明,随着搅拌速度的提高,萃取效率提高。因此选择搅拌速度为 35 min。

2.1.3

研究了中空纤维类型对萃取效率的影响。中空纤维类型包括不同孔径的纤维,孔径越小,萃取效率越高。故选择 5 mol/L 的中空纤维为萃取纤维。

2.1.4

研究了 0.001 ~ 1.0 mol/L pH 3 的萃取效率。 pH 3 对实验结果影响较小,故选择 0.01 mol/L pH 2。
2.2.1

The average recovery rate of pseudoephedrine is 98.4% ± 1.2%, and the average recovery rate of ephedrine is 96.8% ± 2.4%. The RSD of the recovery rate is 6.0% ± 6.6%.


table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Analyte</th>
<th>Regression equation</th>
<th>r</th>
<th>Linear range[μg/L]</th>
<th>Detection limit[μg/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>ephedrine</td>
<td>$y = 246532x + 7158.1$</td>
<td>0.9953</td>
<td>5 – 100</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>pseudoephedrine</td>
<td>$y = 302807x + 5667.3$</td>
<td>0.9958</td>
<td>5 – 100</td>
<td>1.2</td>
</tr>
<tr>
<td>Urine</td>
<td>ephedrine</td>
<td>$y = 426.12x - 25.413$</td>
<td>0.9929</td>
<td>100 – 50 × 10⁴</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>pseudoephedrine</td>
<td>$y = 396.92x - 14.769$</td>
<td>0.9985</td>
<td>100 – 50 × 10⁴</td>
<td>42</td>
</tr>
</tbody>
</table>

y [peak area, x [mass concentration] μg/L.

2.2.2

HPLC analysis was performed on urine samples. The average recovery rate of pseudoephedrine is 95.2% ± 2.4%, and the average recovery rate of ephedrine is 93.4% ± 2.6%. The RSD of the recovery rate is 6.0% ± 6.6%.

Fig. 4 HPLC chromatograms of a) Ephedra sinica Stapf and b) urine sample

2.4 3p-HF-LPME

2.5 3p-HF-LPME

Fig. 5 Supermolecule system in microextraction process

Fig. 6 Preferred conformations of ephedrine and pseudoephedrine
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第

陈

璇,等:基于中空纤维液相微萃取的麻黄碱和伪麻黄碱优势构象的确定及含量测定

结果比较,证实了生物大分子对构象有一定的影响,二者可能发生转换。这一研究结果还有待于用光谱法进一步证实。

样品基质对麻黄碱和伪麻黄碱的萃取和测定的影响

由图可知:麻黄碱和伪麻黄碱在尿液中经萃取和测定所产生的峰高信号比在水中低。其主要原因是:(1) 尿液中大量含氮生物大分子可与麻黄碱和伪麻黄碱通过氢键形成含药物大分子而被中空纤维阻挡,不能进入接收相中,使测定的灵敏度降低,二者在尿液中的萃取效率和浓缩倍数明显低于水中;(2) 尿液中大量内源性化合物和极性小分子使的基线噪声增大,信噪比减小,使二者在尿液中的检出限比在水中高倍。因此即使麻黄碱和伪麻黄碱的测定浓度相同,在水和尿液中所形成的峰高信号也会有明显的区别。

结论

本文在探讨麻黄碱和伪麻黄碱萃取机理的同时,分析和阐述了二者在不同样品基质中可能的优势构象及构象变化,并结合实现了二者的定量分析。研究表明:通过萃取过程中电荷转移超分子的形成,能够使麻黄碱和伪麻黄碱获得较高的萃取效率和富集倍数。此外,在麻黄碱和伪麻黄碱的条件下优化过程中和方法学考察时必须考虑样品基质的重要影响。