Determination of perfluorinated compounds in human urine by ultra high performance liquid chromatography-tandem mass spectrometry

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Abstract A method for the analysis of 12 perfluorinated compounds (PFCs) in human urine by ultra high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was developed and validated. One mL 2% formic acid in methanol was added into the urine. After ultrasonication and centrifugation, the samples were purified by a solid phase extraction column and examined by UPLC-MS/MS. The target compounds were quantified by stable isotope dilution technique. The linear range was 0.05 – 50 μg/L for the 12 PFCs and the correlation coefficient ≥ 0.992. The detection level of 12 PFCs were in the range of 0.44 – 3.47 ng/L. The matrix recoveries of the method for the 12 PFCs in three spiked levels 20, 100, 500 ng/L ranged from 80.3% to 116.2%. The relative standard deviations (RSDs) n = 5 were between 5.5% and 13.8%. The sensitive and accurate method was successfully applied to the analysis of PFCs in human urine.

Key words ultra high performance liquid chromatography-tandem mass spectrometry, UPLC-MS/MS, perfluorinated compounds, urine
并参考人体和环境基质的分析方法，本研究采用固相萃取法（PSA）和固相微萃取（SPME）技术来提取和净化尿液中的目标化合物。根据尿液基质特点，优化提取条件，建立了人尿液中 spiked 溶液为全氟己酸的超高效液相色谱-串联质谱（UPLC-MS/MS）测定方法。根据尿液基质特点，优化提取条件，建立了人尿液中 spiked 溶液为全氟己酸的超高效液相色谱-串联质谱（UPLC-MS/MS）测定方法。

### 1.3 实验条件

<table>
<thead>
<tr>
<th>化合物</th>
<th>质谱模式</th>
<th>离子源</th>
<th>质荷比范围</th>
<th>电喷雾电压</th>
<th>干扰离子</th>
<th>杂质离子</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFHxS</td>
<td>产品离子</td>
<td>ESI-</td>
<td>M+</td>
<td>-90 V</td>
<td>60 80</td>
<td>70 80</td>
</tr>
<tr>
<td>PFOS</td>
<td>产品离子</td>
<td>ESI-</td>
<td>M+</td>
<td>-90 V</td>
<td>90 100</td>
<td>100 110</td>
</tr>
<tr>
<td>PFOA</td>
<td>产品离子</td>
<td>ESI-</td>
<td>M+</td>
<td>-90 V</td>
<td>90 100</td>
<td>100 110</td>
</tr>
<tr>
<td>PFDS</td>
<td>产品离子</td>
<td>ESI-</td>
<td>M+</td>
<td>-90 V</td>
<td>90 100</td>
<td>100 110</td>
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</tbody>
</table>

### Table 1 MS parameters of target compounds and internal standards

<table>
<thead>
<tr>
<th>化合物</th>
<th>质量转移比</th>
<th>锥电压</th>
<th>冲击能量</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFHxS</td>
<td>399–80</td>
<td>50</td>
<td>35</td>
</tr>
<tr>
<td>PFOS</td>
<td>499–80</td>
<td>50</td>
<td>35</td>
</tr>
<tr>
<td>PFOA</td>
<td>499–99</td>
<td>50</td>
<td>35</td>
</tr>
<tr>
<td>PFDS</td>
<td>599–80</td>
<td>50</td>
<td>48</td>
</tr>
<tr>
<td>PFHxA</td>
<td>503–80</td>
<td>50</td>
<td>48</td>
</tr>
<tr>
<td>PFHxS®</td>
<td>315–270</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>PFODa</td>
<td>463–419</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>PFNA</td>
<td>468–423</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>PFDA</td>
<td>515–470</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>PFUda</td>
<td>563–519</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>MPFDoa</td>
<td>613–569</td>
<td>15</td>
<td>13</td>
</tr>
</tbody>
</table>

### 1.4 样品制备

样品收集包括血液、血清、母乳和其他生物材料。血液样本来自非职业暴露人群。尿液样本购自浙江湖州英美生物科技有限公司。纯度高于 80% 的超纯水用于制备 spiked 溶液。人工合成尿液购自浙江湖州英美生物科技有限公司。样品处理在冷冻高离心机中进行。样品以 10% 甲酸水溶液、人工合成尿液和甲酸水溶液为流动相，以乙酸为脱溶剂，流速为 1.0 mL/min，柱温设定为 50 °C。检测模式：多反应监测；离子源：电喷雾离子源（ESI-）；质谱参数见表 1。
色谱第卷

## 样品前处理

取尿液样品于聚丙烯离心管中，加入定量内标溶液，甲酸甲醇溶液，超声后在下以离心，收集的上清液用固相萃取柱净化。先用氨水甲醇、甲醇、水活化柱，上样后分别用甲酸水溶液和甲酸水溶液甲醇，淋洗，真空抽干柱后再用甲醇进行第二次淋洗，最后用氨水甲醇洗脱目标化合物，洗脱液经水浴氮气吹干后用甲醇水溶液定容为，用尼龙过滤膜除去杂质后进行分析。

### 结果与讨论

#### 空白基质的选择

采用小鼠尿液进行实验，发现其存在较高的本底，因此不能用作本实验的空白基质。人工合成尿液中不存在本底污染，同时不含蛋白质，而由于可与蛋白质结合，使用不含蛋白质的人工尿液无法准确评价方法的提取净化能力。因此本实验通过对不同人的尿液筛选，在样本中筛选出无本底的人体尿液作为本实验的空白基质。

#### 前处理条件的选择

目前血液样品常用的前处理方法是离子对萃取法，而本实验中采用离子对萃取法处理尿液时，因尿液的取样量大，浓缩过程繁琐耗时，因此参考文献对母乳和水样的处理方法，采用作为尿液的提取与富集方法。

#### 提取液优化

分别用甲酸水、纯乙腈、甲酸乙腈、纯甲醇、甲酸甲醇、%019/38甲醇溶液作为尿液样品的提取液，比较不同提取溶液对目标物的提取效率。在本实验室以前建立的母乳提取方法中使用得到了很好的提取效果。但同样以甲酸水作为提取液处理尿液时，目标物的保留时间会发生明显的变化，并且内标的回收率较低，用其提取的尿液基质中05;的响应明显低于标准溶液且05;的保留时间缩短了约%019/38。因此甲酸水不适于提取尿液中的0536。其余种提取液的基质加标（加标质量浓度为%019/38）回收试验结果见图，从中可看出甲酸甲醇溶液提取效率较好，目标物的回收率的范围为%019/38-%019/38，而且不同目标物之间无明显差异。
2.3

6 2 μg/L
50% 200 μL 100 μg/L > 1
< 1

2 μg/L

2.4

4 Y X

0.05 ~ 50 μg/L

0.992

RSD n = 6
5.5% ~ 13.8%

2.5

LOD 5 LOQ

3

10

LOD 12 LOD

LOD 2

2.6

80%

20 100 500 ng/L

1.4 7.1

9 PFCAs 3 PFSAs

RSD 3

2.7

PFCs

n = 5

Table 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>Background ng/L</th>
<th>20 ng/L</th>
<th>100 ng/L</th>
<th>500 ng/L</th>
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<tbody>
<tr>
<td></td>
<td>Recovery/%</td>
<td>RSD/%</td>
<td>Recovery/%</td>
<td>RSD/%</td>
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<tr>
<td>PFHxA</td>
<td>&lt; LOD</td>
<td>93.6</td>
<td>114.5</td>
<td>9.4</td>
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<tr>
<td>PFHpA</td>
<td>&lt; LOD</td>
<td>116.2</td>
<td>111.5</td>
<td>9.3</td>
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<tr>
<td>PFOA</td>
<td>3.82</td>
<td>89.7</td>
<td>97.3</td>
<td>7.1</td>
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<tr>
<td>PFNA</td>
<td>&lt; LOD</td>
<td>95.9</td>
<td>91.4</td>
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<td>PFDA</td>
<td>1.28</td>
<td>94.6</td>
<td>89.0</td>
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<td>91.3</td>
<td>5.5</td>
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<td>92.7</td>
<td>88.6</td>
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<td>PPTeD</td>
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<td>88.9</td>
<td>84.2</td>
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<tr>
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<td>112.8</td>
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<td>PFDS</td>
<td>&lt; LOD</td>
<td>97.0</td>
<td>106.3</td>
<td>10.2</td>
</tr>
</tbody>
</table>

2.4

Y X

4 6

100% 3

7.4 ~ 47.3 ng/L

PFOA PFNA

PFDA

PFOA PFOS

PFCs

3

4

Table 4

<table>
<thead>
<tr>
<th>Sample</th>
<th>PFOA</th>
<th>PFNA</th>
<th>PFDA</th>
<th>PFOS</th>
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<tbody>
<tr>
<td>S1</td>
<td>4.4</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
<td>7.4</td>
</tr>
<tr>
<td>S2</td>
<td>14.7</td>
<td>1.9</td>
<td>1.73</td>
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<tr>
<td>S3</td>
<td>7.9</td>
<td>1.6</td>
<td>&lt; LOD</td>
<td>14.6</td>
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<td>S4</td>
<td>17.0</td>
<td>2.8</td>
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<tr>
<td>S5</td>
<td>6.9</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
<td>8.5</td>
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<tr>
<td>S6</td>
<td>15</td>
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<td>3.1</td>
<td>47.3</td>
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</tbody>
</table>
图3 MRM色谱图，显示尿液中检测到的PFCs

引文：