Determination of four carcinogens in mainstream cigarette smoke by liquid chromatography-tandem mass spectrometry

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Abstract N-nitrosornocotinie NNN N64 methylnitrosamino[]-I 3-pyrildyl[]-1-butanoine
NNK N-nitrosatabine NAT and N-nitrosobasine NAB are the most abundant carcinogens identified in tobacco and tobacco smoke. The accurate quantifications of NNN NNK NAT and NAB are necessary to evaluate its impact on the public health. A liquid chromatography-electrospray tandem mass spectrometry LC-ESI MS/MS method was developed to simultaneously determine NNN NNK NAT and NAB in mainstream cigarette smoke. Mainstream smoke was collected in a Cambridge filter pad and then was extracted by 10 mL 100 mmol/L ammonium acetate after 100 μL of mixed deuterated internal standards was added. Then the extract was detected by using positive electrospray ionization on a tandem mass spectrometer in multiple reaction monitoring MRM mode. NNN NNK NAT and NAB were separated on a Zorbax Eclipse XDB-C18 column with the gradient elution using mobile phase A 0.1% acetic acid in water and mobile phase B 0.1% acetic acid in methanol. The detection limits for NNN NNK NAT and NAB were 0.019 0.002 0.008 and 0.007 μg/L respectively. The recoveries were varied from 84.9% to 104.5% for Chinese Virginia cigarettes and the relative standard deviations n = 8 ranged from 2.96% to 6.65%. This proposed approach provides a higher sensitivity and specificity is suitable for the determination of NNN NNK NAT and NAB in mainstream cigarette smoke.

Key words liquid chromatography-electrospray tandem mass spectrometry LC-ESI MS/MS N-nitrosornocotinie N-nitrosatabine mainsteam cigarette smoke Chinese Virginia cigarettes blended cigarettes

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液相色谱-质谱联用（LC-MS/MS）技术用于检测烤烟主流烟气中上述有害物质的含量，具有快速、选择性强、灵敏度高、重现性好的优点，既能够对烟气中上述有害物质的释放量进行准确的定量，又可以满足解决的主要问题之一。以前多采用液液萃取法和固相萃取法来净化样品，尽管这些方法能有效地纯化样品，但样品前处理过程复杂，选择性较差，因此限制了它的进一步应用。特别是固相萃取方法样品前处理过程复杂，选择性较差，因此限制了它的进一步应用。目前关于热能分析法（TEDIA）和Thermo Fisher公司生产的气相色谱-质谱联用（GC-MS）、液相色谱-质谱联用（LC-MS/MS）等检测方法被广泛用于卷烟烟气中的有害物质的检测，但是没有较为合适的方法来对其进行准确的定量。

### 实验部分

**储备液及工作液：** 分别用甲醇准确配制10 μg/ml的单标准储备液及工作液：分别用甲醇准确配制1 μg/ml的单内标储备液，储存于棕色玻璃瓶中，于-20℃下保存。取一定量的各单标准储备液进行混合，作为混合标准溶液（亚硝基甲氨基）、亚硝基降烟碱（亚硝基新烟草碱、亚硝基假木贼碱）和混合型卷烟的检测方法，应用最为广泛，但没有较为合适的方法来对其进行准确的定量。对于基质复杂的烟气样品，如何降低杂质干扰和基质效应是固相萃取方法的专一性和灵敏度均高，但样品前处理过程复杂，选择性较差，因此限制了它的进一步应用。特别是固相萃取方法样品前处理过程复杂，选择性较差，因此限制了它的进一步应用。目前关于热能分析法（TEDIA）和Thermo Fisher公司生产的气相色谱-质谱联用（GC-MS）、液相色谱-质谱联用（LC-MS/MS）等检测方法被广泛用于卷烟烟气中的有害物质的检测，但是没有较为合适的方法来对其进行准确的定量。

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Table 1  Some parameters of mass spectrometry for NNN|NNK|NAT | NAB and the internal standards in multiple reaction monitoring mode

<table>
<thead>
<tr>
<th>Compound</th>
<th>Parent ion/product ion</th>
<th>Collision energy/ eV</th>
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</thead>
<tbody>
<tr>
<td>NNN</td>
<td>178.1/148.2</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>178.1/120.1</td>
<td>15</td>
</tr>
<tr>
<td>NNN-d4</td>
<td>182.2/152.1</td>
<td>15</td>
</tr>
<tr>
<td>NNK</td>
<td>208.1/122.1</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>208.1/106.1</td>
<td>16</td>
</tr>
<tr>
<td>NNK-d4</td>
<td>212.1/126.1</td>
<td>16</td>
</tr>
<tr>
<td>NAT</td>
<td>190.1/160.1</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>190.1/106.1</td>
<td>15</td>
</tr>
<tr>
<td>NAT-d4</td>
<td>194.1/164.1</td>
<td>15</td>
</tr>
<tr>
<td>NAB</td>
<td>192.1/162.2</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>192.1/133.1</td>
<td>17</td>
</tr>
<tr>
<td>NAB-d4</td>
<td>196.1/166.1</td>
<td>17</td>
</tr>
</tbody>
</table>

* quantiative ion pair.

1.4

ISO 8243[2006]†

ISO 8042[1999]†

ISO 3308[2000]†

NNN|NNK|NAT | NAB†

5 mL 100 μL 10 mL 50 mL

100 mmol/L 30 min 0.45 μm

2.1 LC-MS/MS

NNN|NNK|NAT | NAB

NNN|NNK|NAT | NAB

10 mmol/L 0.1% A 0.1% B

NNN|NNK|NAT | NAB

NNN|NNK|NAT | NAB

40°C

NNN|NNK|NAT | NAB

NNN|NNK|NAT | NAB

Fig. 1  MRM chromatograms of a mixture of NNN|NNK|NAT | NAB standards and their corresponding internal standards[2] 20 μg/L†
2.2 算,得到相应的线性回归方程。

2.3 要存在于卷烟主流烟气的粒相物中,因此选择采用乙酸铵水溶液萃取。

乙酸铵水溶液萃取

表

2.4 烟气的滤片上分别按照低、中、高配比较高,而晾晒烟烟叶中

烟气中

15

90% ~ 105%

2.5

15

10

5

1

10

1

1

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<table>
<thead>
<tr>
<th>Sample</th>
<th>Cigarette type</th>
<th>NNN</th>
<th>NNK</th>
<th>NAT</th>
<th>NAB</th>
<th>Total yield</th>
<th>Tar yield/mg</th>
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</thead>
<tbody>
<tr>
<td>8</td>
<td>Chinese Virginia</td>
<td>10.50</td>
<td>12.62</td>
<td>15.18</td>
<td>9.80</td>
<td>48.10</td>
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<tr>
<td>9</td>
<td>Chinese Virginia</td>
<td>2.13</td>
<td>5.48</td>
<td>5.08</td>
<td>4.56</td>
<td>17.25</td>
<td>4.83</td>
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<tr>
<td>10</td>
<td>Chinese Virginia</td>
<td>4.78</td>
<td>8.86</td>
<td>10.12</td>
<td>4.09</td>
<td>27.85</td>
<td>8.39</td>
</tr>
<tr>
<td>11</td>
<td>Chinese Virginia</td>
<td>7.24</td>
<td>11.62</td>
<td>13.54</td>
<td>6.04</td>
<td>38.44</td>
<td>7.91</td>
</tr>
<tr>
<td>13</td>
<td>Chinese Virginia</td>
<td>4.64</td>
<td>10.00</td>
<td>9.48</td>
<td>3.66</td>
<td>27.78</td>
<td>8.60</td>
</tr>
<tr>
<td>15</td>
<td>Chinese Virginia</td>
<td>8.58</td>
<td>12.02</td>
<td>13.18</td>
<td>10.28</td>
<td>44.06</td>
<td>5.93</td>
</tr>
</tbody>
</table>

N5 NNN N9 NNK N5 NAT N5 NAB

20%~10%~18%~10%~18%

<table>
<thead>
<tr>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC-MS/MS</td>
<td>NNN NNK NAT NAB</td>
</tr>
</tbody>
</table>

3.3


Moldoveanu S C, Borgerding M. Beitr Tabakforsch Int 2008:22:1319


