Non-targeted analysis of tobacco smoke constituents by TOF-MS

Justin Frosina, Jasper van Heemst

Presented by Christopher Proctor

GR&D, British American Tobacco, Southampton, UK

67th Tobacco Science Research Conference, Williamsburg, VA, 15-18 September 2013
Presentation 71
Overview

- **Introduction**
  - Why non-targeted analysis

- **GC-HRTOFMS**
  - Improving our existing methods

- **GC×GC-TOFMS**
  - Improving separation and identification

- **LC-HRTOFMS**
  - New capability

- **Summary**
Overview

• **Introduction**
  • Why non-targeted analysis
  • GC-HRTOFMS
    • Improving our existing methods
  • GC×GC-TOFMS
    • Improving separation and identification
  • LC-HRTOFMS
    • New capability
  • Summary
Introduction

Why non-targeted analysis?

- Rodgman and Perfetti 2013 – 9000+ compounds identified in tobacco and tobacco smoke
  - 6000+ in smoke alone
- FDA HPHC list – 93+ compounds
Introduction

Non-targeted analysis role in the screening process

Non-targeted

Targeted

Control Samples

Test Samples

Volatiles

Semi Volatiles

Non Volatiles

Volatiles

Semi Volatiles

Non Volatiles

Target List

Process Finished

Targeted Method for quantification

Library Search

Library Hit

No Library Hit

Decision on way to proceed (Unidentified spectra/RT saved)

Process finished

Significant driver of change

Not a significant driver of change

Concentration increased

Concentration unchanged

Compounds of interest

Compound not of interest
Introduction

Quadrupole mass spectrometers

Scanning detector – limited sensitivity
Poor mass discrimination
Speed constrains data quality

Positive

Negative

Images from Crawford Scientific
Introduction

Time of Flight mass spectrometers

Array detector – improved sensitivity
Greater mass selectivity
Much faster, more complete data
Overview

• Introduction
  • Why non-targeted analysis

• **GC-HRTOFMS**
  • Improving our existing methods

• **GC×GC-TOFMS**
  • Improving separation and identification

• **LC-HRTOFMS**
  • New capability

• Summary
High Resolution MS

- The higher the resolution, the better the mass discrimination
- Improve TOF resolution by increasing flight path length

High Resolution MS

LECO Pegasus HRT

- GC coupled to a multi-reflectron Time-of-Flight mass analyser
- Up to 50,000 mass resolution (e.g. can separate 50.000 and 50.001 m/z)
- < 1 ppm mass accuracy – calculation of empirical formula
- Up to 200Hz scanning rate – aids peak deconvolution
GC-MS (Quadrupole)

Improving our existing methods

• Data acquisition
GC-HRTOFMS

Improving our existing methods

• Data acquisition
## GC-MS (Quadrupole)

### Improving our existing methods

- Reporting the comparison of two samples

<table>
<thead>
<tr>
<th>Peak</th>
<th>RT (min)</th>
<th>Compound</th>
<th>Area</th>
<th>% of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.50</td>
<td>Vinyl pyridine + Benzaldehyde + Methylfurfural</td>
<td>41909236</td>
<td>3.5</td>
</tr>
<tr>
<td>2</td>
<td>13.26</td>
<td>Phenol + Benzonitrile + Ethylmethylpyrazine</td>
<td>18796725</td>
<td>1.6</td>
</tr>
<tr>
<td>3</td>
<td>14.03</td>
<td>Limonene + Formylpyrrole</td>
<td>17539057</td>
<td>1.5</td>
</tr>
<tr>
<td>4</td>
<td>14.64</td>
<td>Corylon</td>
<td>19605840</td>
<td>1.6</td>
</tr>
<tr>
<td>5</td>
<td>16.30</td>
<td>Cresol + Guaiacol</td>
<td>46161890</td>
<td>3.8</td>
</tr>
<tr>
<td>6</td>
<td>16.39</td>
<td>Cresol + ?</td>
<td>24768196</td>
<td>2.1</td>
</tr>
<tr>
<td>7</td>
<td>18.61</td>
<td>?</td>
<td>37985543</td>
<td>3.2</td>
</tr>
<tr>
<td>8</td>
<td>19.54</td>
<td>Naphthalene</td>
<td>22186922</td>
<td>1.8</td>
</tr>
<tr>
<td>9</td>
<td>21.12</td>
<td>Methylindole + Dihydrobenzofuran?</td>
<td>32029748</td>
<td>2.7</td>
</tr>
<tr>
<td>10</td>
<td>22.84</td>
<td>Indanone +?</td>
<td>18798473</td>
<td>1.6</td>
</tr>
<tr>
<td>11</td>
<td>22.99</td>
<td>Methyleneapthalene</td>
<td>19972645</td>
<td>1.7</td>
</tr>
<tr>
<td>12</td>
<td>23.42</td>
<td>Methyleneapthalene</td>
<td>17911289</td>
<td>1.5</td>
</tr>
<tr>
<td>13</td>
<td>23.57</td>
<td>Methoxyvinylphenol + Quinoline</td>
<td>28976030</td>
<td>2.4</td>
</tr>
<tr>
<td>14</td>
<td>24.55</td>
<td>Tracetin + Nicotine (s)</td>
<td>11134488</td>
<td>9.3</td>
</tr>
<tr>
<td>15</td>
<td>24.75</td>
<td>Butylbutyrate +?</td>
<td>29244414</td>
<td>2.2</td>
</tr>
<tr>
<td>16</td>
<td>25.59</td>
<td>Methyl indole</td>
<td>18798473</td>
<td>1.6</td>
</tr>
<tr>
<td>17</td>
<td>26.21</td>
<td>Dimethylnaphthalene</td>
<td>19972645</td>
<td>1.7</td>
</tr>
<tr>
<td>18</td>
<td>26.31</td>
<td>Trimethyldodecatriene</td>
<td>17911289</td>
<td>1.5</td>
</tr>
<tr>
<td>19</td>
<td>26.95</td>
<td>Myosmine + Biphenyl</td>
<td>34629794</td>
<td>2.9</td>
</tr>
<tr>
<td>20</td>
<td>27.07</td>
<td>Eugenol + Dimethylnaphthalene + ?</td>
<td>31323535</td>
<td>2.6</td>
</tr>
<tr>
<td>21</td>
<td>28.18</td>
<td>Nicotyrine</td>
<td>68537216</td>
<td>5.7</td>
</tr>
<tr>
<td>22</td>
<td>29.73</td>
<td>Dipyridyl</td>
<td>32453037</td>
<td>2.7</td>
</tr>
<tr>
<td>23</td>
<td>30.39</td>
<td>Megastigmatrienone + Trimethylnaphthalene + Fluorene +?</td>
<td>21598169</td>
<td>1.8</td>
</tr>
<tr>
<td>24</td>
<td>31.62</td>
<td>Dimethylbiphenyl</td>
<td>30103623</td>
<td>2.5</td>
</tr>
<tr>
<td>25</td>
<td>32.62</td>
<td>Long chain hydrocarbon + Trimethylpyridopyrimidinone</td>
<td>48955489</td>
<td>4.1</td>
</tr>
<tr>
<td>26</td>
<td>35.24</td>
<td>Neophytadiene</td>
<td>34651370</td>
<td>28.8</td>
</tr>
<tr>
<td>27</td>
<td>36.83</td>
<td>Long chain hydrocarbon</td>
<td>32085569</td>
<td>2.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Peak</th>
<th>RT (min)</th>
<th>Compound</th>
<th>Area</th>
<th>% of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.75</td>
<td>Ethanol</td>
<td>508699174</td>
<td>22.2</td>
</tr>
<tr>
<td>2</td>
<td>5.80</td>
<td>Propylene Glycol</td>
<td>18711925</td>
<td>0.8</td>
</tr>
<tr>
<td>3</td>
<td>12.50</td>
<td>Cyclobutapyridine + Methylfurfural + Benzaldehyde</td>
<td>51508406</td>
<td>2.2</td>
</tr>
<tr>
<td>4</td>
<td>13.09</td>
<td>Phenol + Trimethylbenzene</td>
<td>19433998</td>
<td>0.8</td>
</tr>
<tr>
<td>5</td>
<td>16.15</td>
<td>Cresol + Pyridinecarbonitrile</td>
<td>25356164</td>
<td>1.1</td>
</tr>
<tr>
<td>6</td>
<td>16.28</td>
<td>Cresol + Guaiacol</td>
<td>27491725</td>
<td>1.2</td>
</tr>
<tr>
<td>7</td>
<td>19.10</td>
<td>Ethylphenol</td>
<td>20403247</td>
<td>0.9</td>
</tr>
<tr>
<td>8</td>
<td>20.96</td>
<td>Dihydrobenzofuran + Trimethylphenol</td>
<td>37250283</td>
<td>1.6</td>
</tr>
<tr>
<td>9</td>
<td>21.12</td>
<td>Dihydrobenzofuran + Methylindole</td>
<td>20692552</td>
<td>0.9</td>
</tr>
<tr>
<td>10</td>
<td>22.99</td>
<td>Methylnaphthalene + Indole</td>
<td>28554814</td>
<td>1.2</td>
</tr>
<tr>
<td>11</td>
<td>24.18</td>
<td>Triacetin (s)</td>
<td>27787736</td>
<td>12.1</td>
</tr>
<tr>
<td>12</td>
<td>24.50</td>
<td>Nicotine (s)</td>
<td>48545834</td>
<td>21.2</td>
</tr>
</tbody>
</table>

## Ambiguous compound identity

- Unknown variability of response
Improving our existing methods

- Reporting the comparison of two samples

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Name</th>
<th>Type</th>
<th>Match</th>
<th>Concentration</th>
<th>R.T. (s)</th>
<th>Expected R.T. (s)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>Pyrazine, methyl-</td>
<td>Match</td>
<td>818.2</td>
<td>98.35</td>
<td>278.997</td>
<td>278.05</td>
<td>169421</td>
</tr>
<tr>
<td>33</td>
<td>Phenol</td>
<td>Match</td>
<td>913.6</td>
<td>108.28</td>
<td>417.392</td>
<td>417.057</td>
<td>2902762</td>
</tr>
<tr>
<td>75</td>
<td>Phenol, 2-methyl-</td>
<td>Match</td>
<td>851.6</td>
<td>85.61</td>
<td>102.294</td>
<td>102.267</td>
<td>504347</td>
</tr>
<tr>
<td>149</td>
<td>1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-</td>
<td>Match</td>
<td>812.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>165</td>
<td>Hydroquinone</td>
<td>Match</td>
<td>863.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>194</td>
<td>Triacetin</td>
<td>Match</td>
<td>953</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>201</td>
<td>Phenol, 2,6-dimethoxy-</td>
<td>Match</td>
<td>901.3</td>
<td>102.88</td>
<td>750.125</td>
<td>750.204</td>
<td>577524</td>
</tr>
<tr>
<td>204</td>
<td>Nicotine</td>
<td>Match</td>
<td>872.6</td>
<td>113.62</td>
<td>754.369</td>
<td>754.551</td>
<td>99841246</td>
</tr>
<tr>
<td>241</td>
<td>Naphthalene, 1,7-dimethyl-</td>
<td>Match</td>
<td>647.8</td>
<td>102.27</td>
<td>813.809</td>
<td>813.951</td>
<td>144073</td>
</tr>
<tr>
<td>242</td>
<td>Pyridine, 3-(3,4-dihydro-2H-pyrrol-5-yl)</td>
<td>Match</td>
<td>805.2</td>
<td>104.66</td>
<td>815.181</td>
<td>815.415</td>
<td>1631988</td>
</tr>
<tr>
<td>390</td>
<td>Scopoletin</td>
<td>Match</td>
<td>814.6</td>
<td>107.12</td>
<td>1173.549</td>
<td>1173.636</td>
<td>934853</td>
</tr>
<tr>
<td>Peak 52/0.667ug/ml spiked 3R4F v13</td>
<td>Not Found</td>
<td>Not Found</td>
<td>0</td>
<td>0</td>
<td>455.763</td>
<td>455.763</td>
<td>0</td>
</tr>
<tr>
<td>Peak 391/0.667ug/ml spiked 3R4F v13</td>
<td>Not Found</td>
<td>Not Found</td>
<td>0</td>
<td>0</td>
<td>1249.739</td>
<td>1249.739</td>
<td>0</td>
</tr>
<tr>
<td>105</td>
<td>Isophorone</td>
<td>Out of Tolerance</td>
<td>838.4</td>
<td>65.96</td>
<td>556.654</td>
<td>556.313</td>
<td>457065</td>
</tr>
<tr>
<td>133</td>
<td>Naphthalene</td>
<td>Out of Tolerance</td>
<td>689</td>
<td>32.63</td>
<td>618.141</td>
<td>618.522</td>
<td>1508168</td>
</tr>
<tr>
<td>142</td>
<td>Hexachlorobutadiene</td>
<td>Out of Tolerance</td>
<td>918.8</td>
<td>55.64</td>
<td>639.188</td>
<td>639.963</td>
<td>428163</td>
</tr>
<tr>
<td>180</td>
<td>Naphthalene, 2-methyl-</td>
<td>Out of Tolerance</td>
<td>908.8</td>
<td>57.55</td>
<td>714.235</td>
<td>714.458</td>
<td>790815</td>
</tr>
<tr>
<td>230</td>
<td>2-Nitroaniline</td>
<td>Out of Tolerance</td>
<td>726.3</td>
<td>52.54</td>
<td>798.924</td>
<td>799.117</td>
<td>152523</td>
</tr>
<tr>
<td>257</td>
<td>Acenaphthylene</td>
<td>Out of Tolerance</td>
<td>857.6</td>
<td>53.89</td>
<td>839.552</td>
<td>839.716</td>
<td>943698</td>
</tr>
<tr>
<td>287</td>
<td>Dibenzo(furand)</td>
<td>Out of Tolerance</td>
<td>849.4</td>
<td>54.76</td>
<td>889.963</td>
<td>890.209</td>
<td>1252960</td>
</tr>
<tr>
<td>398</td>
<td>Fluoranthene</td>
<td>Out of Tolerance</td>
<td>790.4</td>
<td>63.93</td>
<td>1244.88</td>
<td>1245.108</td>
<td>940401</td>
</tr>
<tr>
<td>405</td>
<td>Pyrene</td>
<td>Out of Tolerance</td>
<td>690.2</td>
<td>61.11</td>
<td>1275.951</td>
<td>1276.221</td>
<td>970739</td>
</tr>
<tr>
<td>414</td>
<td>Benz[a]anthracene</td>
<td>Out of Tolerance</td>
<td>807.4</td>
<td>54.19</td>
<td>1453.683</td>
<td>1453.758</td>
<td>252816</td>
</tr>
<tr>
<td>428</td>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>Out of Tolerance</td>
<td>701</td>
<td>51</td>
<td>1784.889</td>
<td>1784.565</td>
<td>104564</td>
</tr>
<tr>
<td>70</td>
<td>Peak 70</td>
<td>Peak</td>
<td>0</td>
<td>0</td>
<td>480.3</td>
<td>0</td>
<td>161051</td>
</tr>
<tr>
<td>209</td>
<td>Peak 209</td>
<td>Peak</td>
<td>0</td>
<td>0</td>
<td>759.03</td>
<td>0</td>
<td>81354</td>
</tr>
<tr>
<td>278</td>
<td>Peak 278</td>
<td>Peak</td>
<td>0</td>
<td>0</td>
<td>871.084</td>
<td>0</td>
<td>17774</td>
</tr>
</tbody>
</table>
Improving Existing Methods

Quad-MS

~ 40 compounds detected and tentatively identified, 1 day processing time

HR-TOFMS

~ 1000 compounds detected, ~500 tentatively identified, 15 min processing time
Overview

• Introduction
  • Why non-targeted analysis

• GC-HRTOFMS
  • Improving our existing methods

• GC\times GC-TOFMS
  • Improving separation and identification

• LC-HRTOFMS
  • New capability

• Summary
GC×GC-TOFMS

Improving separation and identification

- **Collaboration with University of Liege**
  - Characterisation of tobacco smoke
Improving separation and identification

GC×GC-TOFMS

Alkanes
n-Alkyl acids
n-Alkyl acid methyl esters
-Alkyl acid amides
Terpenes and Steroids
Phenols
Aldehydes
PAHs
Naphthalenes
Alkyl substituted naphthalenes
GC×GC-TOFMS
Calculating statistical differences

multivariate statistics (e.g. PCA)

Fisher Ratio
Overview

- Introduction
  - Why non-targeted analysis
- GC-HRTOFMS
  - Improving our existing methods
- GC×GC-TOFMS
  - Improving separation and identification
- LC-HRTOFMS
  - New capability
- Summary
LC-HRTOFMS

New Capability

- LC coupled to quadrupole/Time-of-Flight mass analyser (Q-TOF)
- Resolution of 40 000
- < 1 ppm mass accuracy
- Capable of analysing a range of up to 10,000 m/z
LC-HRTOFMS

Improved Scan Sensitivity

Standard Peak Finding  3R4F ACN Extract

Peak Deconvolution
Summary

- Non-targeted analysis reduces discrimination in data collection
- The combination of GC and LC-HRTOFMS with deconvolution allows comprehensive smoke ‘fingerprinting’
- GC×GC-TOFMS provides additional identification and classification information
- Information rich data from a number of instrument platforms – common data analysis platform desirable
Acknowledgements

• **University of Liege**
  • Dr. Michal Brokl
  • Dr. Jef Focant

• **BAT**
  • Louise Bishop
  • Maria Carradus
Thank you for your attention... Questions?