Non-targeted Analysis of Selected Hoffmann Toxicants in Smoke Condensate by Cryoprobe 1H NMR

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67th Tobacco Science Research Conference, Williamsburg, VA, 15-18 September 2013
Presentation No. 53
Outline

- Introduction
- Screening analytical techniques
- Nuclear Magnetic Resonance
  - Advantages and limitations
  - Whole tobacco smoke screening
- Results
- Next steps
Introduction

- Tobacco smoke – extremely complex matrix containing 6000 + substances with largely different physico-chemical properties
  
  challenging task for determination of constituents

- Analytical strategies for measurement of constituents
  
  • Targeted analysis (established methods)
  
  • Exploratory analysis (screening techniques)
The Lists

- Acetaldehyde
- Acrolein
- Formaldehyde
- Benzo[a]pyrene
- Carbon monoxide (CO)
- 1,3-Butadiene
- Benzene
- FCTC
- NNK
- NNN
- Nicotine
- Acrylamide
- o-Anisidine
- Benzo[b]fluoranthene
- A-α-C (2-amino-9H-fluoreno[2,3-b]indole)
- Benzo[a]anthracene
- Benz[b]kfluoranthene
- Benzo[a]pyrene
- (3-Aminobiphenyl)
- 1-Aminonaphthalene
- Acetone
- Beryllium
- Caffeic acid
- Hydrazine
- Indeno[1,2,3-cd]pyrene
- Methyl ethyl ketone
- MeA-a-C (2-Amino-3-methyl)-9H-pyrido[2,3-b]indole
- 5-Methylchrysene
- Naphthalene
- Nitrobenzene
- Benz[a]anthracene
- Chlorinated dioxins/furans
- Cobalt
- Coumarin
- Phenol
- (Hydroquinone)
- Cyclopenta[c,d]pyrene
- N-Nitrosodiethylamine
- N-Nitrosomorpholine (NMOR)
- Dibenz[a,h]anthracene
- Dibenz[a,e]pyrene
- Dibenz[a,h]pyrene
- Dibenzo[a,e]pyrene
- Dibenzo[a,h]pyrene
- 2,6-Dimethylaniline
- Ethyl carbamate (urethane)
- Ethylene oxide
- Furan
- N-Nitrososarcosine (NSAR)
- Glu-P-1 (2-Amino-6-methylidipyridino[1,2-a:3',2'-d]imidazole)
- Glu-P-2 (2-Aminodipyridino[1,2-a:3',2'-d]imidazole)
- Trp-P-1 (3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole)
- Trp-P-2 (1-Methyl-3-amino-5H-pyrido[4,3-b]indole)
- PhiP (2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine)
- Vinyl acetate
- Propylene oxide
- iQ (2-Amino-3-methylimidazo[4,5-f]quinoline)
- Polonium-210
- Nickel
- Selenium
- Uranium-235
- N-Nitrosodiethanolamine (NDELA)
- Benz[a]pyrene
- N-Nitrososomethylmethyamine
- N-Nitrosoamine (NPI)
- N-Nitrososopiperidine (NPS)

Numbers:
- 10
- 9
- 21
- 63

Agencies:
- HC
- FDA
- N-Nitrosodiethanolamine (NDELA)
Analytical approaches

**Established Methods**
- Targeted analysis of a single compound / suites of substances of similar physico-chemical properties
- Multi step analytical procedure
- Usually fully validated
- High confidence in the results
- Often time consuming
- Labour intensive
- Limited flexibility

**Screening Techniques**
- Identification and semi-quantification of extended suites of target analytes
- Fingerprinting and comparison at a greater level of detail
- Only “partial” validation
- Non-targeted screening of multiple analyte groups in complex matrices
- Method development tool for confirmatory methods

**Limited scope**

**Broad scope**
Chromatographic screening techniques

- GC×GC-TOFMS
- GC-HRTOFMS
- LC-HRTOFMS
Nuclear Magnetic Spectroscopy

- Detailed information about molecules and their environment

**Principle**
Radiofrequency waves are used to excite nuclei in a magnetic field at their resonance frequency (RF)

- Four sequential steps:
  - The net alignment of the magnetic nuclear spins with an applied constant magnetic field
  - The perturbation of this alignment by employing radio frequency (RF) pulses
  - Measuring the current induced as the nuclei relax to realign with the magnetic field (Free induction decay)
  - Fourier transform of the time domain data to generate the frequency domain NMR

Benefits of NMR

- Targeted and non-targeted analysis
- Robust (minimal matrix effects)
- Non destructive
- Fast answers and high confidence in chemical identity
- Structural information
- NMR library
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Fast screening approach for identification and semi-quantification of tobacco smoke constituents

Characterisation of tobacco smoke condensate

Confirmatory technique and guided method development

Compatible with industry standards sample preparation strategies
Development of NMR capability: BAT and FERA collaboration

- Food and Environment Research Agency (FERA)

Cryoprobe $^1$H NMR
Sample preparation workflow

Whole smoke

= Particulate phase

CFP trapped particulate

Solvent Extracted

Gas phase

Volatile bubbled through impinger

Combined extract

Data interpretation

1H NMR
Results

Feasibility study
- 33 Hoffmann analytes representing different chemical classes excluding inorganics, metals and NFDPM
  - 20 analytes readily detected in tobacco smoke condensate
  - 13 analytes not detected $\Rightarrow$ 12 analytes could be detected at higher concentration levels by fortifying experiments (butyraldehyde - no resolvable peaks)
  - NMR database

Validation
- Validation of the methodology - overspiking experiments
- Verification of the robustness methodology - “blind” test samples
## Detectability of the analytes in 3R4F smoke condensate

<table>
<thead>
<tr>
<th>Compound in 3R4F whole smoke condensate</th>
<th>Present &gt; LOQ. Detectable by NMR.</th>
<th>Detectable by NMR at higher concentration levels.</th>
<th>Not detectable by NMR.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaldehyde</td>
<td></td>
<td>1,3-butadiene</td>
<td>Butyraldehyde</td>
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<tr>
<td>Isoprene</td>
<td></td>
<td>N-nitrosoanatabine</td>
<td></td>
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<tr>
<td>Nicotine</td>
<td></td>
<td>N-nitrosoanabasine</td>
<td></td>
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<tr>
<td>Acetone</td>
<td></td>
<td>N-nitrosonornicotine ketone</td>
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<tr>
<td>Acrolein</td>
<td></td>
<td>Benzo(a)pyrene</td>
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<tr>
<td>Toluene</td>
<td></td>
<td>4-aminobiphenyl</td>
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<tr>
<td>Catechol</td>
<td></td>
<td>Resorcinol</td>
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<tr>
<td>Hydroquinone</td>
<td></td>
<td>Quinoline</td>
<td></td>
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<tr>
<td>Formaldehyde</td>
<td></td>
<td>N-nitrosonornicotine</td>
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<tr>
<td>Acrylonitrile</td>
<td></td>
<td>1-aminonaphthalene</td>
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<tr>
<td>Propionaldehyde</td>
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<td>2-aminonaphthalene</td>
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<tr>
<td>Crotonaldehyde</td>
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<td>3-aminobiphenyl</td>
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<tr>
<td>Butanone</td>
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<td>Benzene</td>
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<tr>
<td>Pyridine</td>
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<tr>
<td>Phenol</td>
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<tr>
<td>Styrene</td>
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<tr>
<td>o-Cresol (2-methylphenol)</td>
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<td></td>
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</tr>
<tr>
<td>m-Cresol (3-methylphenol)</td>
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<tr>
<td>p-Cresol (4-methylphenol)</td>
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</tr>
</tbody>
</table>
NMR profile of 3R4F (5 cigarettes smoked, ISO)
NMR profile of 3R4F (5 cigarettes smoked, ISO)

**Advantages**
- All information in one output
- Profile sample specific
- Repeatable
- Robust

**Limitations**
- Sensitivity (currently 3-10µg/cig)
- Not for very volatile species
- Not for metals
Differentiation of matrices

Sample 1: NFPDM ~ 9 mg/cig

Sample 2: NFPDM ~ 4 mg/cig

Graph showing differentiation of matrices with peaks for Acrolein, Propionaldehyde, Acetaldehyde, Methanol, Acetone, Butanone, and other compounds in the 0-5 ppm and 5-10 ppm ranges.
Repeatability

Acrolein
RSD 7%

Crotonaldehyde
RSD 9%

Isoprene
RSD 6-8%

Hydroquinone
RSD 8%

Catechol
RSD 11%

Acrylonitrile
RSD 6-11%

1,3 Butadiene
RSD 5-8%

Chemical shift (ppm)
Summary

- 33 Hoffmann analytes tested
  - 20 readily detected
  - 12 analytes detectable at higher concentration
  - Butyraldehyde - no resolvable peaks

- Robust and repeatable methodology
- Sample (matrix) specific
- Quantifiable
Next steps

- Increase sensitivity
- Expanding NMR database (spectral libraries for more substances)
- Further characterisation of the tobacco smoke condensate (data mining)
- Use NMR as complimentary technique to MS
- Implementation of NMR guided approach for other techniques and method development
Acknowledgements

Giampaolo Venditti, James Donarski
Thank you for your attention... Questions?