Determination of hydrazine in smokeless tobacco products by GC-MS

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Overview
Determination of hydrazine in smokeless tobacco products

- Why quantify hydrazine in tobacco products?
- Method summary
- Linearity
- Recovery
- LOQ and LOD
Why?

Determination of hydrazine in smokeless tobacco products

- First identified in tobacco and cigarette smoke by Hoffmann et al\(^1\)

- Identified in Smoking and Tobacco Control Monograph 2 as a carcinogenic agent in smokeless tobacco products\(^2\)

- IARC Monograph 89
  - Concluded that smokeless tobacco is a Group 1 (known human) carcinogen

- IARC Monograph 71 categorised hydrazine as a Group 2B possible human carcinogen

- No published data on hydrazine in contemporary smokeless tobacco products

- The development of a method for hydrazine analysis in smokeless tobacco products was conducted as part of a project characterising levels of toxicants in contemporary smokeless tobacco products

\(^1\) Liu, Schmeltz & Hoffmann, Anal. Chem., 1974, 46 (7), pp 885-889

\(^2\) Brunneman and Hoffmann, Smoking and Tobacco Control Monograph No.2 Chapter 3, pg 96
External References

Determination of hydrazine in smokeless tobacco products

- Validation of an existing method for contemporary smokeless tobacco matrices in partnership with GC Labs UK
- SANCO/3030/99 rev.4 ‘Guidance for generating and reporting methods of analysis…’ (GLP approach) selected by GC Labs

- Specificity
  - Impurities should contribute < 3% to total target peak

- Linearity
  - Extend over nominal range (±20%) in normal matrices

- Accuracy
  - Can use assessment of interference and precision

- Precision
  - Must report mean, %RSD and number of determinations
The stages of validation
Determination of hydrazine in smokeless tobacco products

- Establish the linear dynamic range of response
- Determine efficiency of derivatisation
- Analysis of ‘control’ tobacco products
- Assess accuracy, precision and LOQ
- Demonstrate confirmation of chemical identity
Method Overview

Determination of hydrazine in smokeless tobacco products

- Tobacco extracted using 80/20 MeOH/0.1N HCl
  - 2g in 50mL

- Extract reacted with pentafluorobenzaldehyde
  - Hydrazine reacts to form pentafluorobenzaldehyde azine

- Partitioned into hexane

- Analysed by GC-MS
  - External standard method
Equipment

Determination of hydrazine in smokeless tobacco products

- Varian 3800/Saturn 4D GC/MS
- Ion trap detector
- **Settings**
  - 30m x 0.25mm x 0.25µm Zebron ZB-5 column
  - 2µL splitless injection at 200°C
  - Oven Ramp: 70°C to 250°C at 15°C/min and hold for 3 min
  - He flow rate of 1.0mL/min
  - Acquisition Range of 40 – 550m/z
  - Quantitative ions 369m/z and 388m/z
The stages of validation
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Linearity: Standard Calibration Curve in Blank solvent

Determination of hydrazine in smokeless tobacco products

\[ y = 1028045.6374x - 1886.8800 \]
\[ R^2 = 0.9996 \]
Linearity: Standard chromatogram
Determination of hydrazine in smokeless tobacco products
The stages of validation

Determination of hydrazine in smokeless tobacco products

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Hydrazine is derivatised with pentafluorobenzaldehyde to form the azine of pentafluorobenzaldehyde.

- Hydrazine derivatised exactly according to the method
  - In blank solvent

- 104% recovery
  - Mean of 2 experiments

- Data confirm efficiency of partition into hexane
The stages of validation
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Accuracy and Precision
Determination of hydrazine in smokeless tobacco products

- Analysis of five ‘control’ tobacco products
  - Dry snuff, loose snus, plug, chewing tobacco and tablet

- Tobacco samples spiked with hydrazine at three different levels
  - Approximately 0.5µg/g, 0.05µg/g and 0.025µg/g

- Results of recovery experiments define accuracy and precision
- Lowest acceptable recovery defines LOQ
## Accuracy and Precision; Recovery Data

**Determination of hydrazine in smokeless tobacco products**

<table>
<thead>
<tr>
<th>Tobacco Product</th>
<th>Spike Level (µg/g hydrazine)</th>
<th>Mean Recovery (%)</th>
<th>RSD (%)</th>
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<tbody>
<tr>
<td><strong>Dry Snuff</strong></td>
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<tr>
<td>0.5300</td>
<td>87.5</td>
<td>5.12</td>
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<td>0.0265</td>
<td>96.7</td>
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<td><strong>Loose Snus</strong></td>
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</table>
Accuracy and Precision
Determination of hydrazine in smokeless tobacco products

- **Acceptance criteria:**
  - Accuracy: recovery data between 70% and 110%
  - Precision: RSD < 20%

- **Accuracy:**
  - All tobacco types pass criteria at 0.025μg/g with the exception of tablet tobacco
  - All tobacco types pass criteria at 0.05μg/g

- **Precision:**
  - All tobacco types display good precision at all spiked levels

- **LOQ:**
  - 0.05μg/g for tablet tobacco products
  - 0.025μg/g for dry snuff, loose snus, plug and chewing tobacco
The stages of validation

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Identification

Determination of hydrazine in smokeless tobacco products

Mass Spec of Standard Solution

Mass Spec of Recovery Solution
Summary of method and performance

Determination of hydrazine in smokeless tobacco products

- The validated method is suitable for the determination of hydrazine in 5 different contemporary smokeless tobacco product types

- The data demonstrate fulfilment of SANCO/3030/99 rev. 4

- Linear range of 0.8ng/mL – 170ng/mL hydrazine
  - Equivalent to 1.6ng/g – 340ng/g

- Recovery experiments show accuracy and precision fall within defined acceptance criteria

- LOQ at 25ng/g except for tablet products at 50ng/g
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