



**BRITISH AMERICAN
TOBACCO**

Determination of hydrazine in smokeless tobacco products by GC-MS

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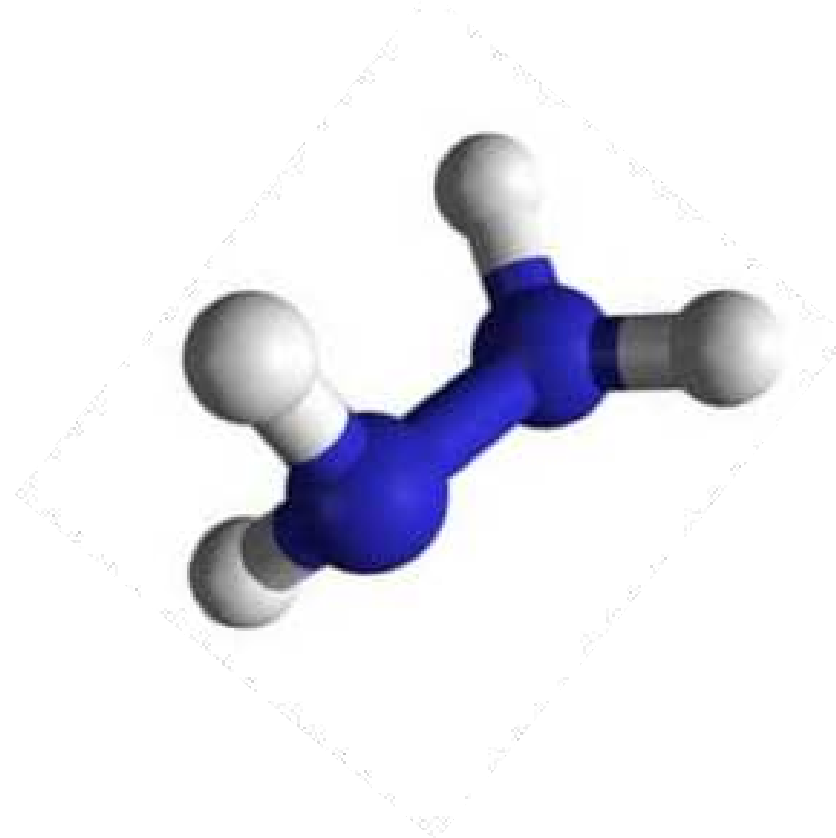
BAT Group Research and Development with G C Laboratories UK

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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 products²
- 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

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

²Brunneman 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 ($\pm 20\%$) 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 0.25 μ m Zebron ZB-5 column

- 2 μ L splitless injection at 200 $^{\circ}$ C

- Oven Ramp: 70 $^{\circ}$ C to 250 $^{\circ}$ C at 15 $^{\circ}$ 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





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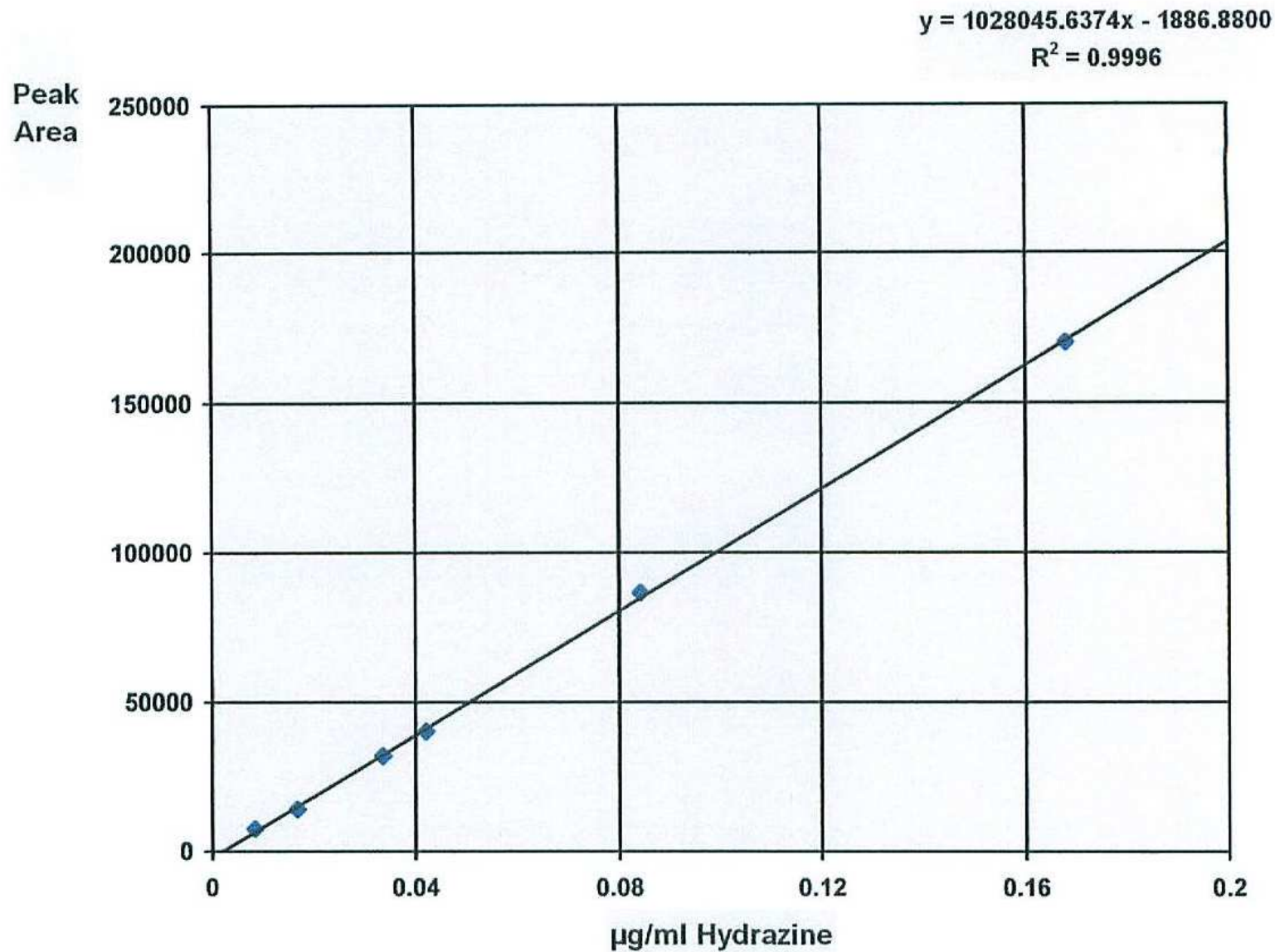
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Linearity: Standard Calibration Curve in Blank solvent

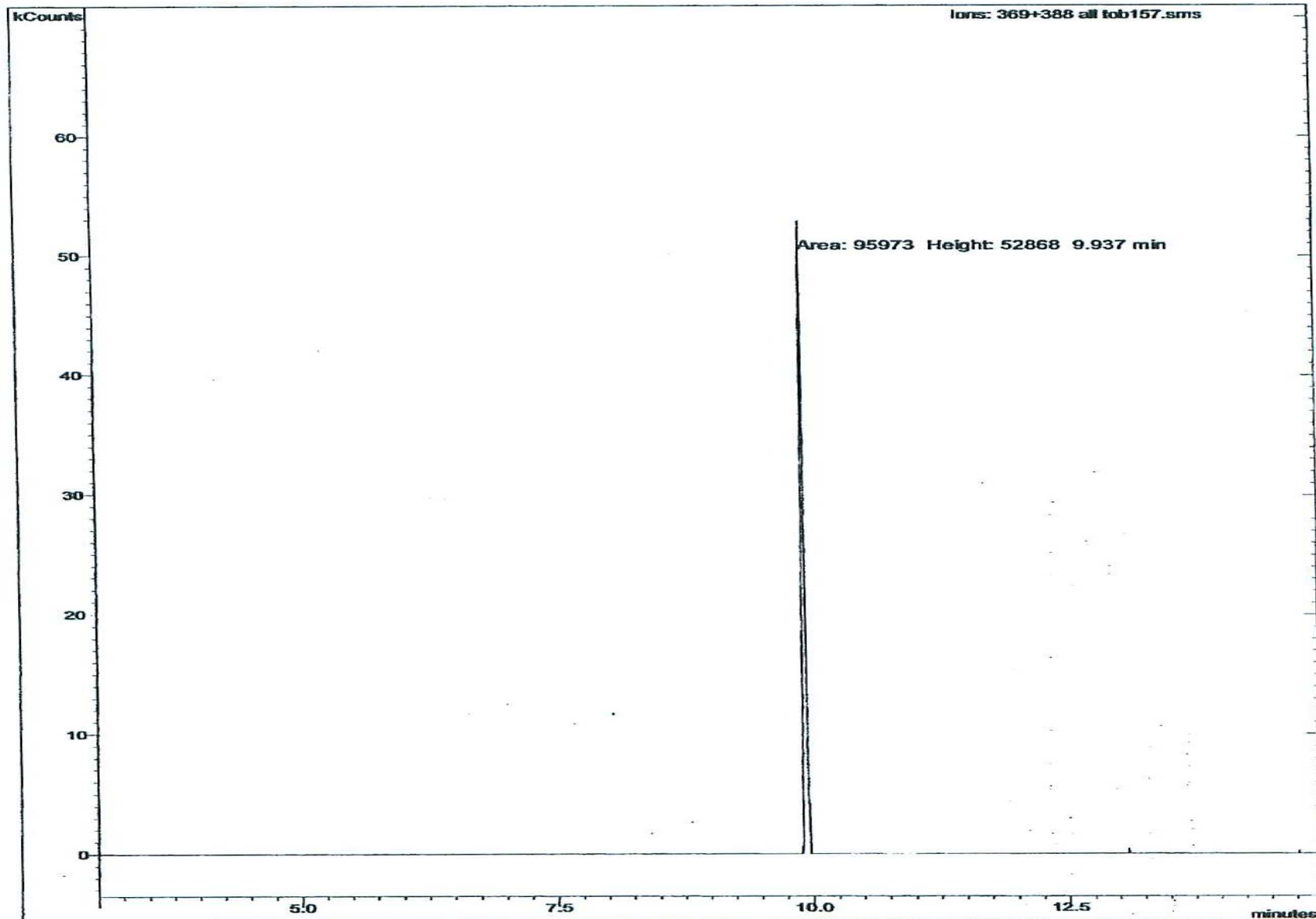
Determination of hydrazine in smokeless tobacco products





Linearity: Standard chromatogram

Determination of hydrazine in smokeless tobacco products





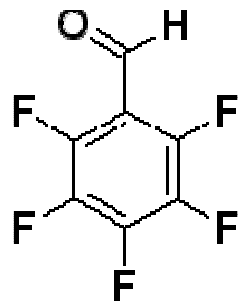
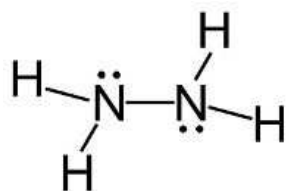
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Derivatisation Efficiency

Determination of hydrazine in smokeless tobacco products



- 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



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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 $0.5\mu\text{g/g}$, $0.05\mu\text{g/g}$ and $0.025\mu\text{g/g}$
- 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

Tobacco Product	Spike Level ($\mu\text{g/g}$ hydrazine)	Mean Recovery (%)	RSD (%)
Dry Snuff	0.5300	87.5	5.12
	0.0530	105.2	4.66
	0.0265	96.7	11.9
Loose Snus	0.5300	75.7	8.34
	0.0530	96.1	6.87
	0.0265	83.1	3.33
Plug	0.5300	85.2	4.07
	0.0530	95.3	8.47
	0.0265	92.2	6.20
Chewing Tobacco	0.5300	90.5	12.0
	0.0530	98.3	3.22
	0.0265	99.7	9.11
Tablet	0.5300	77.0	5.00
	0.0530	74.1	6.86
	0.0265	64.0	11.3



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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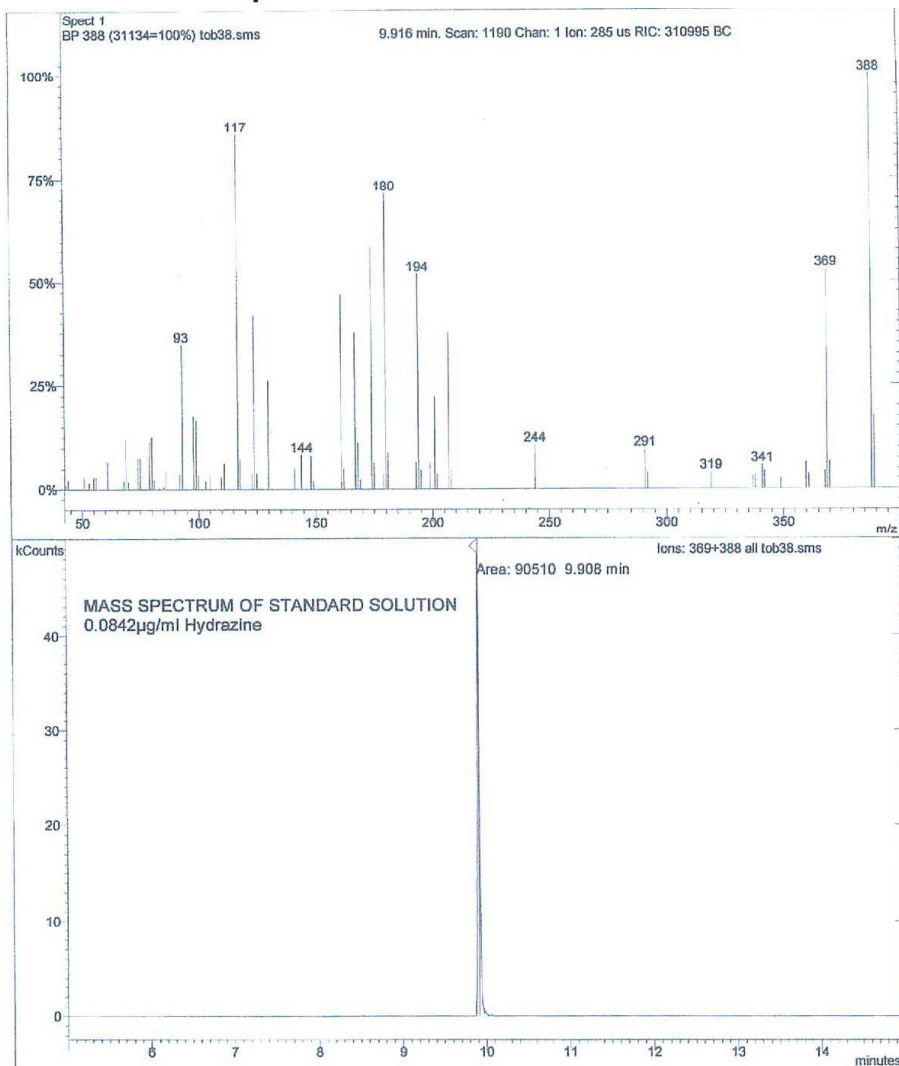
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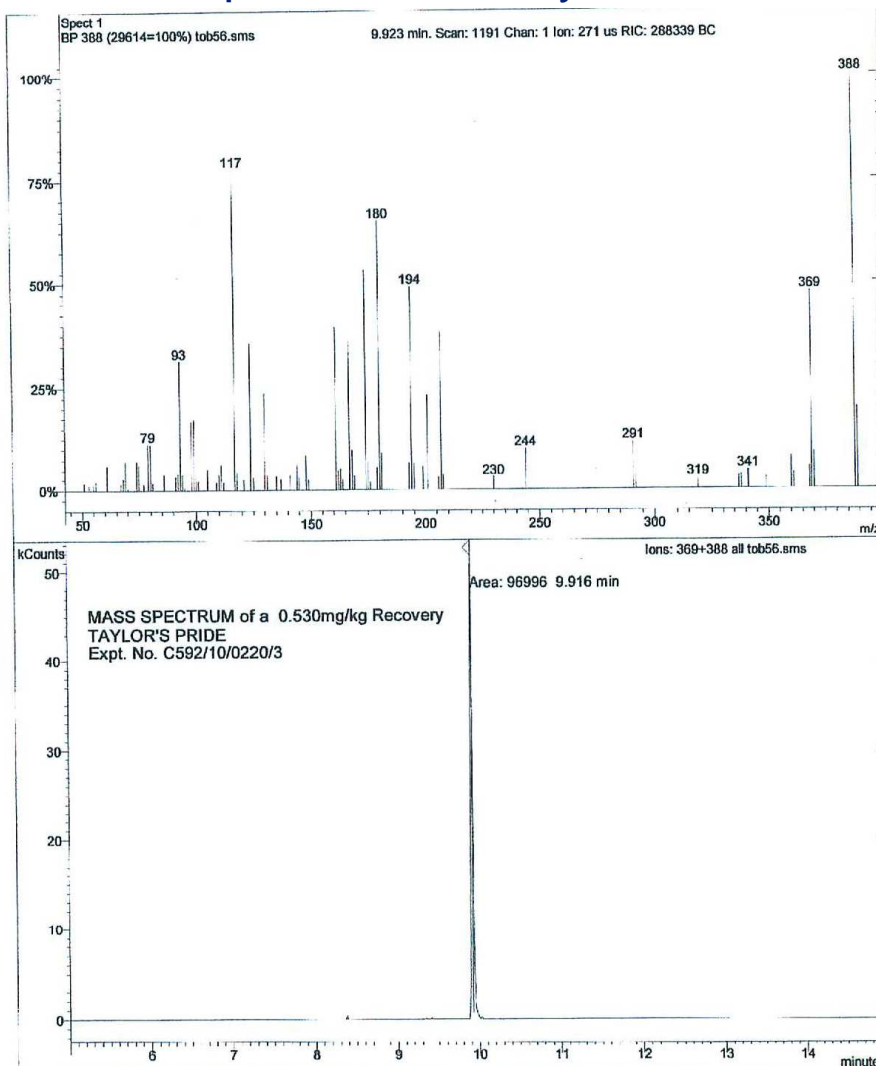
Identification

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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