DETERMINATION OF THEOBROMINE IN PROCESSED TOBACCO PRODUCTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Introduction

Theobromine is the primary methylxanthine found in cocoa, a commonly used additive to tobacco products. Methods most commonly used for the determination of theobromine in tobacco products involve the extraction of a sample with solvents such as water, mixture of KOH-methanol-water, aqueous solutions of ammonium hydroxide and Na3PO4. The aqueous extracts are usually analyzed by high performance liquid chromatography (HPLC) using C18 reversed phase column for theobromine separation and UV detector for quantification. Although these methods are applicable on most tobacco samples with good results, it has been our experience that in certain cases theobromine peak cannot be effectively separated from tobacco matrix by C18 reversed phase column. In this study, an analytical method was developed and validated for the determination of theobromine in different types of tobacco products, based on extraction with water by sonication, followed by HPLC analysis with a propyl-linked pentafluorophenyl (PFP) column, which allows for better separation of tobacco matrix interference from the theobromine peak.

Luna PFP Column (150 x 4.6mm, 3µm) vs Lichrosphere RP C18 Column (250 x 4mm, 5µm)

Sample Preparation and HPLC-UV Operating Conditions

Sample Preparation

Mix 1g tobacco sample with 20mL water

Varian HPLC System Parameters

Injection volume: 20µL
UV detector wavelength: 280nm
Luna PFP column: 4.6 x 150mm, 3µm
Column temperature: 30°C
Column flow rate: 0.8mL/min

Mobile phase isocratic-gradient:

- 20.0: 100% A, 0% B
- 19.0: 50% A, 50% B
- 18.0: 10% A, 90% B
- 0.0: 1% Acetic acid in water

HPLC-UV analysis

Mobile phase A - Methanol
Mobile phase B - 1% Acetic acid in water

Calibration Curve and Method Detection Limits

Limit of Detection (LOD) 0.377µg/g
Limit of Quantification (LOQ) 1.26µg/g

Method Recovery from Fortified Tobacco Samples

<table>
<thead>
<tr>
<th>Spiked amount</th>
<th>3R4F Mean</th>
<th>SD (%)</th>
<th>CRP-3 Mean</th>
<th>SD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25.4µg/g</td>
<td>94.8</td>
<td>8.3</td>
<td>96.7</td>
<td>6.6</td>
</tr>
<tr>
<td>108µg/g</td>
<td>97.0</td>
<td>5.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Method Precision (Sample: Marlboro Light KS)

<table>
<thead>
<tr>
<th>Mean (µg/g)</th>
<th>RSD (%)</th>
<th>Mean (µg/g)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.8</td>
<td>8.2</td>
<td>12.6</td>
<td>10.5</td>
</tr>
</tbody>
</table>

Theobromine Levels (µg/g as is) in Cigarette and Smokeless Tobacco

Sample name
Zhong Nanhai light KS 86.5 ± 1.8
Marlboro Flavor Plus 13.9 ± 1.7
Marlboro Light KS 11.8 ± 1.0
Camel KS 78.8 ± 2.3
Marlboro Gold Original 16.7 ± 2.6
Merit Enriched Flavor KS 26.7 ± 3.1
Natural American Spirit < 1.26
3R4F < 1.26
CRP-1 (Snus pouch) < 1.26
CRP-2 (Moist snuff) 4.33 ± 0.32
CRP-3 (Dry snuff) 8.49 ± 0.48
CRP-4 (Loose leaf) < 1.26

Conclusions

- A simple and reproducible method has been developed and validated for the analysis of theobromine in processed tobacco products.
- Water with sonication provides effective extraction efficiency for theobromine from tobacco products: recoveries from spiked tobacco sample range from 94.8 to 97.0% with RSD under 8.5%.
- The utilization of a PFP column allows for better separation of tobacco matrix interference from the theobromine peak, which ensure more accurate and robust method.
- The method is applicable to the analysis of theobromine in all tobacco products.