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 Na_3PO_4. The aqueous extracts are usually analyzed by high performance liquid chromatography (HPLC) using C_{18} reversed phase column for theobromine separation and UV detector for quantification. Although those methods are applicable on most tobacco samples with good results, it has been our experience that in certain cases theobromine peak can not be effectively separated from tobacco matrix by C_{18} 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.

Sample Preparation and HPLC-UV Operating Conditions

Sample Preparation

Mix 1g tobacco sample with 20mL water

Sonicate at 60ºC for 60min

Wrist shake for 30min

Filter through a syringe filter

HPLC-UV analysis

Varian HPLC-UV System Parameters

Injection volume: 20µL
UV detector wavelength: 280nm
Luna PFP column : 4.6 x150mm, 3µm
Column temperature: 30ºC
Column flow rate: 0.8mL/min
Mobile phase isocratic-gradient:

Time (minutes)	Composition	
0.0	10% A	90% B
18.0	10% A	90% B
19.0	50% A	50% B
20.0	100% A	0% B
23.0	100% A	0% B
24.0	10% A	90% B

Equilibrate 6 minutes at end

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

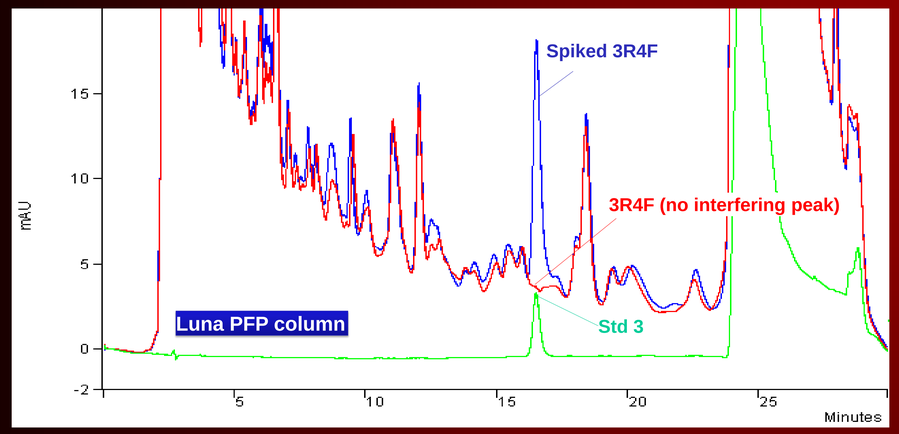
Method Recovery from Fortified Tobacco Samples

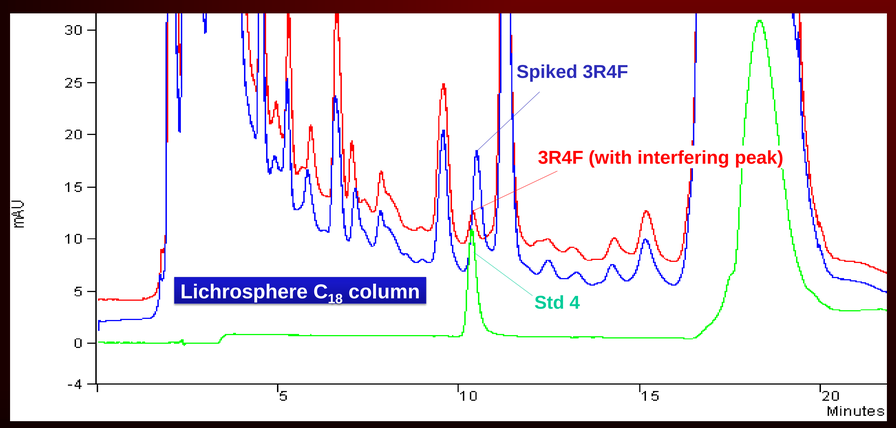
Spiked amount	3R4F (n=6) Mean	3R4F (n=6) SD (%)	CRP-3 (n=3) Mean	CRP-3 (n=3) SD (%)
25.4µg/g	94.8	8.3	96.7	6.6
108µg/g	97.0	5.5		

Method Precision (Sample: Marlboro Light KS)

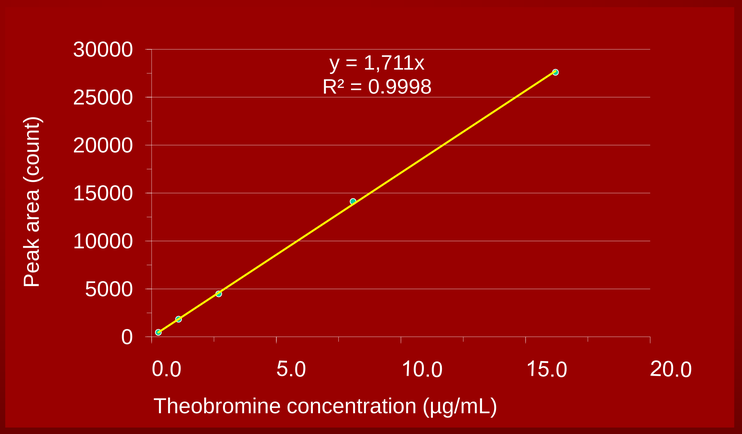
Intra-day (n=3)		Inter-day (n=6)	
Mean (µg/g)	RSD (%)	Mean (µg/g)	RSD (%)
11.8	8.2	12.6	10.5

Luna PFP Column (150 x 4.6mm, 3µm) vs Lichrosphere RP C_{18} Column (250 x 4mm, 5µm)





Calibration Curve and Method Detection Limits



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

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

Sample name	Mean ± SD (n = 3)
Zhong Nanhai light KS	86.5 ± 1.8
Marlboro Flavor Plus	13.9 ± 1.7
Marlboro Light KS	11.8 ± 1.0
Camel KS	78.6 ± 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 utliization of a PFP column allows for better separation of tobacco matrix interference from theobromine peak, which ensure more accurate and robust method.
- The method is applicable to the analysis of theobromine in all tobacco products.