Determination of 17 Free Amino Acids in Tobacco Products using UPLC with UV Detection

Cathy X. Jin, Celeste T. Wilkinson, John Miller, Jason W. Flora, and Naren Meruva
Altria Client Services, 601 East Jackson Street, Richmond, VA 23219

INTRODUCTION

- Free amino acids play an important role in smokeless tobacco taste and cigarette smoke flavor.
- Amino acid levels change during tobacco aging, curing, and manufacturing.

OBJECTIVE

- To develop and validate a rapid and robust method for the analysis of free amino acids in tobacco products using UPLC with UV detection

Serine (Ser)	Alanine (Ala)	Valine (Val)
Arginine (Arg)	Proline (Pro)	Isoleucine (Ile)
Glycine (Gly)	Cysteine (Cys)	Leucine (Leu)
Aspartic acid (Asp)	Lysine (Lys)	Phenylalanine (Phe)
Glutamic acid (Glu)	Tyrosine (Tyr)	Tryptophan (Trp)
Threonine (Thr)	Methionine (Met)	

METHODS

Sample Preparation

- Weigh 1.5 g tobacco sample
- Add 50 mL Milli-Q water; shake 30 min at 250 rpm
- Pipette 700 µL extract into 300 µL 0.1 N HCl; mix well
- Derivatize acidified extract with Waters AccQ-Tag™ derivatization kit, which has 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC)
  - Transfer 70 µL borate buffer to sample vial
  - Add 10 µL 2 mM norvaline in 0.1 N HCl (ISTD) and 10 µL standard or sample; vortex 10 sec
  - Add 20 µL of reconstituted derivatization reagent
  - Cap vial; vortex 10 sec
  - Let vial stand 1 min at room temperature
  - Heat vial in heating block 10 min at 55°C

Waters ACQUITY® UPLC

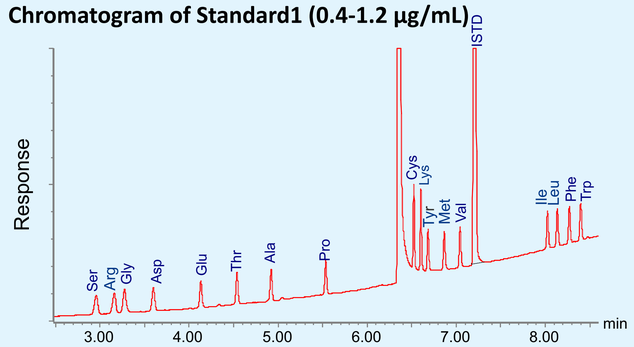
- Analytical column AccQ-Tag™ ULTRA C18, 1.7 µm, 2.1 x 100 mm column
- UV detection, 260 nm
- Mobile Phase AccQ-Tag™ A & B, gradient elution
- Sample temperature: 10°C
- Column temperature: 55°C
- Injection volume: 1 µL
- Flow rate: 0.7 mL/min
- Run time: 12 min

Time, min.	%A	%B
Initial	99.9	0.1
0.54	99.9	0.1
5.74	90.9	9.1
8.74	78.8	21.2
9.04	40.4	59.6
9.14	10.0	90.0
10.14	10.0	90.0
10.24	99.9	0.1
12.00	99.9	0.1

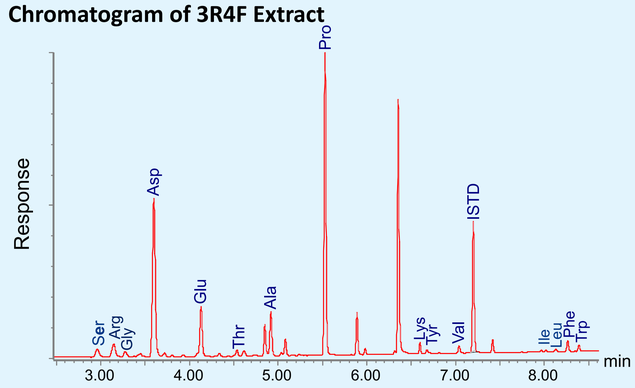
ABSTRACT

Free amino acids play an important role in smokeless tobacco taste and cigarette smoke flavor. Amino acid levels change during tobacco aging, curing and tobacco manufacturing process. The objective of this research was to develop and validate a rapid and robust method for the analysis of 17 free amino acids in tobacco products using ultra performance liquid chromatography (UPLC) with ultraviolet (UV) detection. Tobacco samples were extracted with MilliQ water and an aliquot of extract was acidified with 0.1N HCl. The acidified extract was derivatized using Waters AccQ·Fluor Reagent (6-aminoquinoyl-N-hydroxysuccinimidyl carbamate (AQC) and the derivatives were analyzed using UPLC with UV detection. An Acquity UPLC BEH C18 column (1.7 µm particle size) was used and the mobile phases were Waters AccQ·Tag Ultra Eluent A and Eluent B. All requirements for method validation were met including linearity, accuracy, precision, limits of detection (LOD), limits of quantitation (LOQ), method robustness, and standard and sample extract stability. For example, the linearity was demonstrated with a calibration range of 5 to 900 pmol/µL (0.3 to 180 µg/mL) with a coefficient of determination of R2>0.995 for each amino acid. The recoveries for 17 amino acids in tobacco ranged between 90%-112%. The limit of quantitation (LOQ) ranged from 3.0 to 13 µg/g in tobacco for the various amino acids. Once validated, this method was used to evaluate CORESTA reference tobacco products CRP1, CRP2, CRP3, CRP4 and 3R4F tobacco filler where amino acid levels were detected from below LOQ to 6000 µg/g.

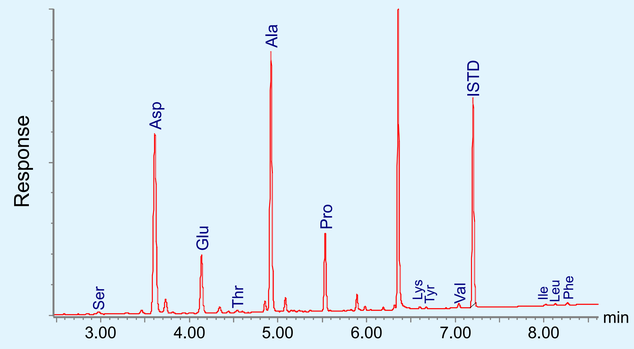
Chromatogram of Standard1 (0.4-1.2 µg/mL)



Chromatogram of 3R4F Extract



Chromatogram of CRP2 Extract

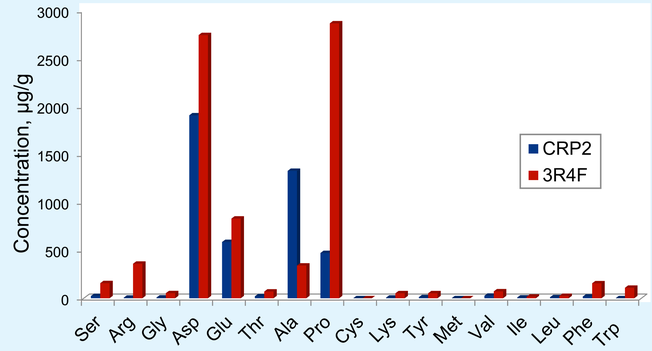


RESULTS

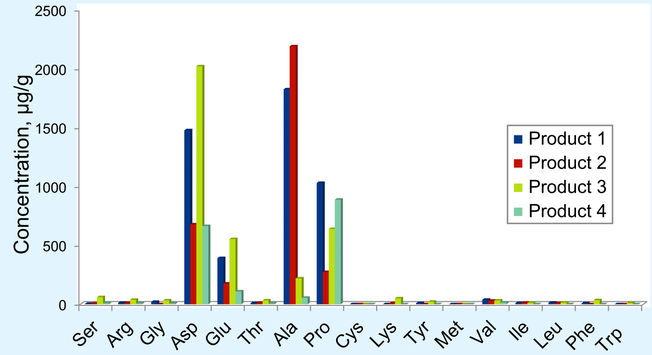
Validation Summary

Parameter	Result
Calibration	Standard range from 0.3 µg/mL to 180 µg/mL R^2>0.995, n=3 days
Accuracy	Average recoveries for all analytes ranged from 90% to 112%
Precision	Instrument precision %RSD: 0.3-3% Intraday %RSD: 3R4F 2-7%, CRP2 3-9% Interday %RSD: 3R4F 3-11%, CRP2 4-10%
Limit of quantitation	3-13 µg/g in tobacco
Sample stability	Samples were stable for more than 3 days (% difference from day 0 <15%)

Amino Acids in References CRP2 and 3R4F



Amino Acids in 4 Commercial Products



SUMMARY

- A rapid and robust method for the analysis of amino acids in tobacco products has been developed and validated.
- This method can be used to better understand the role of free amino acids in smokeless tobacco and cigarette smoke flavor.
- This method can also be used to better understand how amino acid levels change during tobacco aging, curing and manufacturing.

Corresponding author for copy of poster: Narendra.K.Meruva@Altria.com