U.S. Pharmacopeia Dissolution Technique for the Determination of Nicotine and Flavor Release from Smokeless Tobacco Products

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Introduction

Smokeless Tobacco Analysis:

- Analysis of smokeless tobacco products is based on forced extraction of constituents from tobacco
- This does not provide information for constituent release under consistent conditions
- There are no standardized methods to make comparisons of constituent release for smokeless tobacco products

Loose

Pouch

Snus

Objective

- Evaluate USP4* dissolution apparatus as a potential technique to evaluate nicotine and flavor release from moist smokeless tobacco (MST) products (loose and pouch) and snus products under consistent conditions
  - Allows for a way to compare multiple products
  - Not meant to replicate human exposure

Loose

Pouch

Snus

*United States Pharmacopeia

Method

USP4 Dissolution Apparatus - SOTAX CE7 Smart USP4

Cell Holder **Pump** **Fraction Collector**

Method

USP4 Dissolution Apparatus - SOTAX CE7 Smart USP4

Image used: http://www.sotax.com/products/dissolution-testing/usp-4/dissolution/off-line-open/?no_cache=1

Method

- USP4 Dissolution Cell Setup

Figure 2a: Loose Product

1 mm beads on bottom
3 mm beads on top

Direction of Flow

Figure 2b: Snus

1 mm beads on bottom
Pouch held with clip

Method

Artificial Saliva (pH 6.8)

Ingredient	Per 1000 mL
Magnesium Chloride Hexahydrate ($MgCl \cdot 6H_2O$)	0.17 g
Potassium Hydrogen Phosphate anhydrous ($K_2HPO_4 \cdot H_2O$)	0.68 g
Sodium Chloride (NaCl)	0.33 g
Potassium Chloride (KCl)	0.75 g
Calcium chloride dihydrate ($CaCl \cdot 2H_2O$)	0.15 g
Potassium Carbonate (K_2CO_3)	0.53 g
Type I Water (De-ionized)	1000 mL
Hydrochloric acid	To pH 6.8 ± 0.1

German Institute for Standardization (DIN) Recipe is based upon German standard DIN v53160-1, "Determination of the Colour Release of Articles of Daily Use, Part1: Resistance to Artificial Saliva", section 4.2, October 2002.

Method - SOTAX CE7 Smart USP4

- USP4 parameters
  - Flow rate 4.0 mL/min
  - Temperature 37ºC
  - 1 mm glass beads on bottom
  - 3 mm glass beads on top or clip
  - 1.0 gram of tobacco or 1 pouch

Table 1: Sotax Collection

Fraction Number	Fraction Collection Time (min)	Fraction Collection Duration (min)	Volume Collected (mL)
1	4	4	16
2	8	4	16
3	12	4	16
4	16	4	16
5	20	4	16
6	30	10	40
7	40	10	40
8	50	10	40
9	60	10	40

Method and GC/MS parameters

Sample Preparation

1. Transfer 1 mL of each fraction into an extraction vial and add 250 µL of 2N NaOH.
2. Add 1 mL of methylene chloride extraction solution containing Internal Std.
3. Vortex for 45 minutes
4. Transfer methylene chloride to an autosampler vial for analysis by GC/MS.

Gas Chromatograph

Column	DBWax-ETR, 30m x 0.25mm x 0.25µm d_f
Injection Volume	1 µL
Inlet Temp	250°C
Inlet Mode	Split (5:1)
Analysis Time	8.0 min

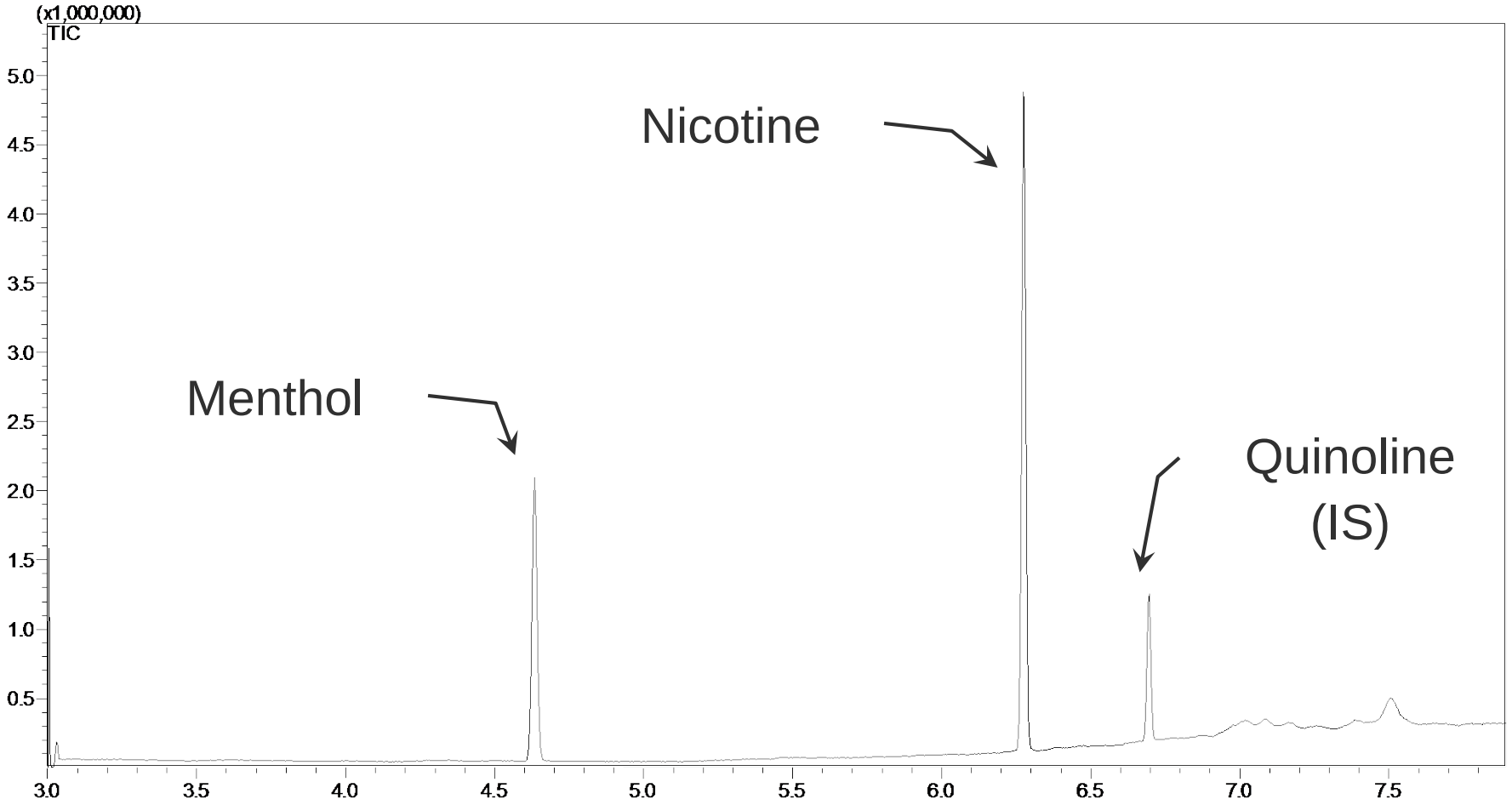
Mass Spectrometer – Selected Ion Monitoring

Menthol (m/z)	71 (quant), 81, 95
Quinoline - ISTD (m/z)	129 (quant), 102
Nicotine (m/z)	84 (quant), 133

Calibration

Menthol	0.50 – 10.0 µg/mL	Linear no Weighting
Nicotine	1.00 – 200 µg/mL	Linear no Weighting

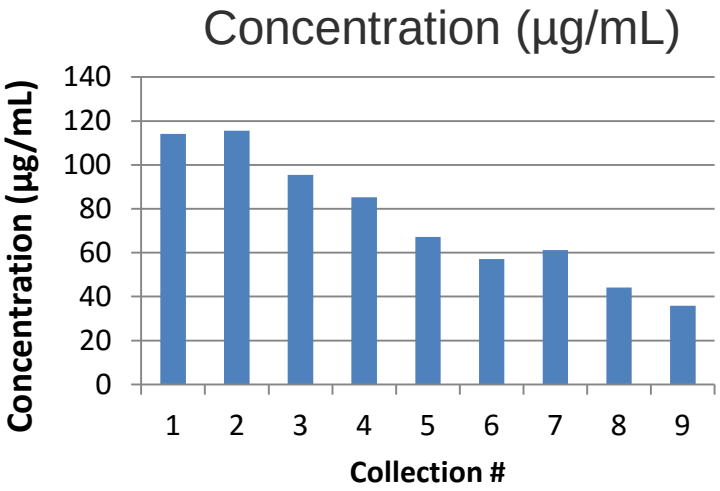
GC/MS Chromatogram (TIC) - Example

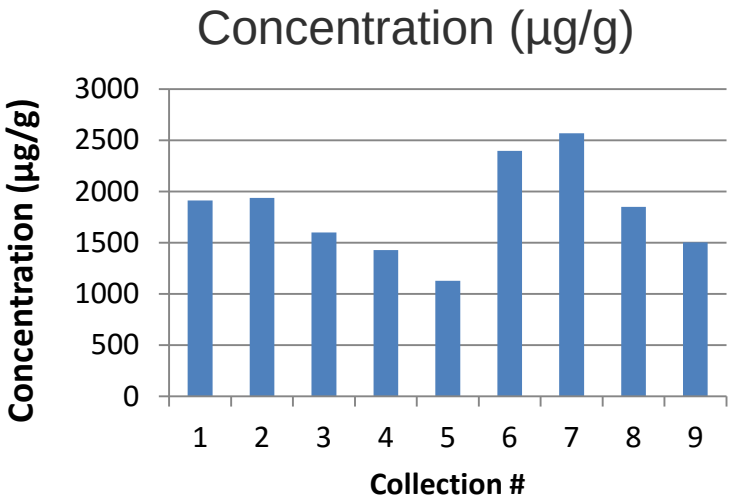


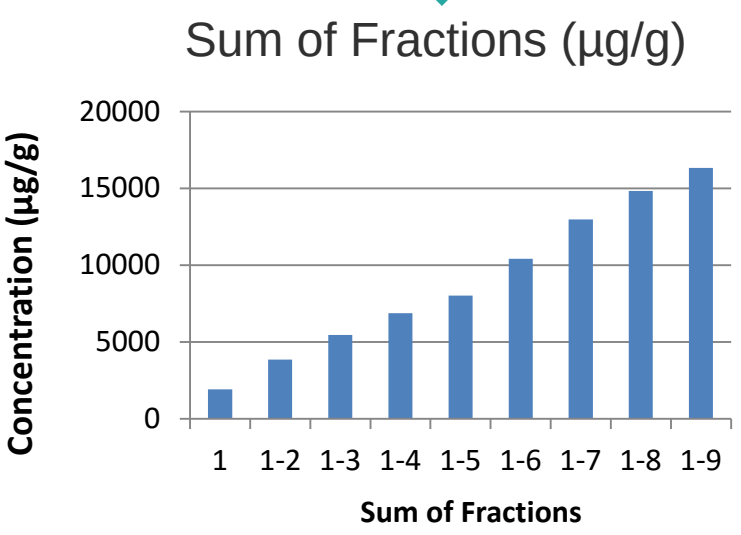
Method Validation

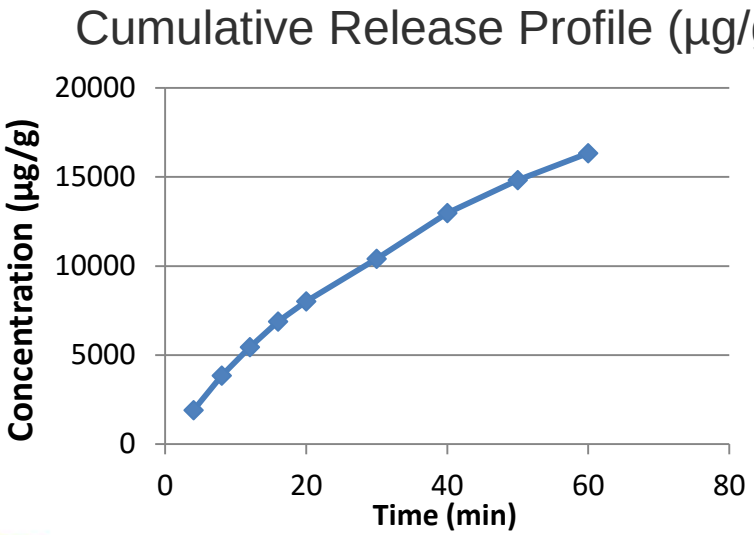
- Calibration
  - R^2 > 0.997 on all days
  - % RCR < 9.2%
- Accuracy - evaluated at 3 levels for each product type
  - Menthol: between 97.0% and 113%
  - Nicotine: between 85.2% and 112%
- Precision – < 3.0%
- Specificity – No interferences were observed at the retention time or m/z of any of the analytes
- LOQ – Menthol 0.176 µg/mL, Nicotine 0.442 µg/mL
- LOD – Menthol 0.053 µg/mL, Nicotine 0.133 µg/mL
- Stability – Dissolution samples and final extracts were stable for up to 4 days at 0-4 ^{o}C (refrigerated)

Results – Calculations for Cumulative Release Profile









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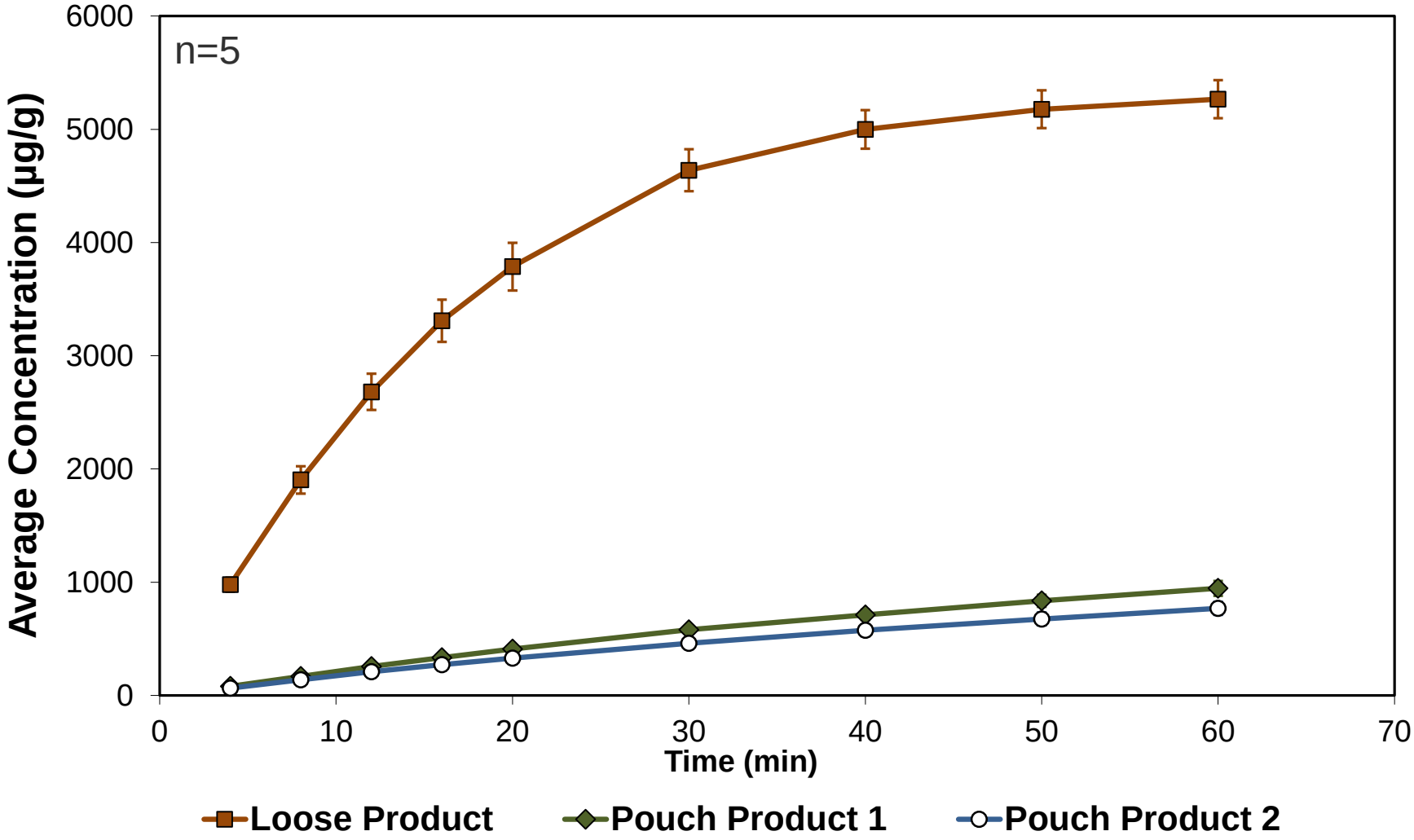
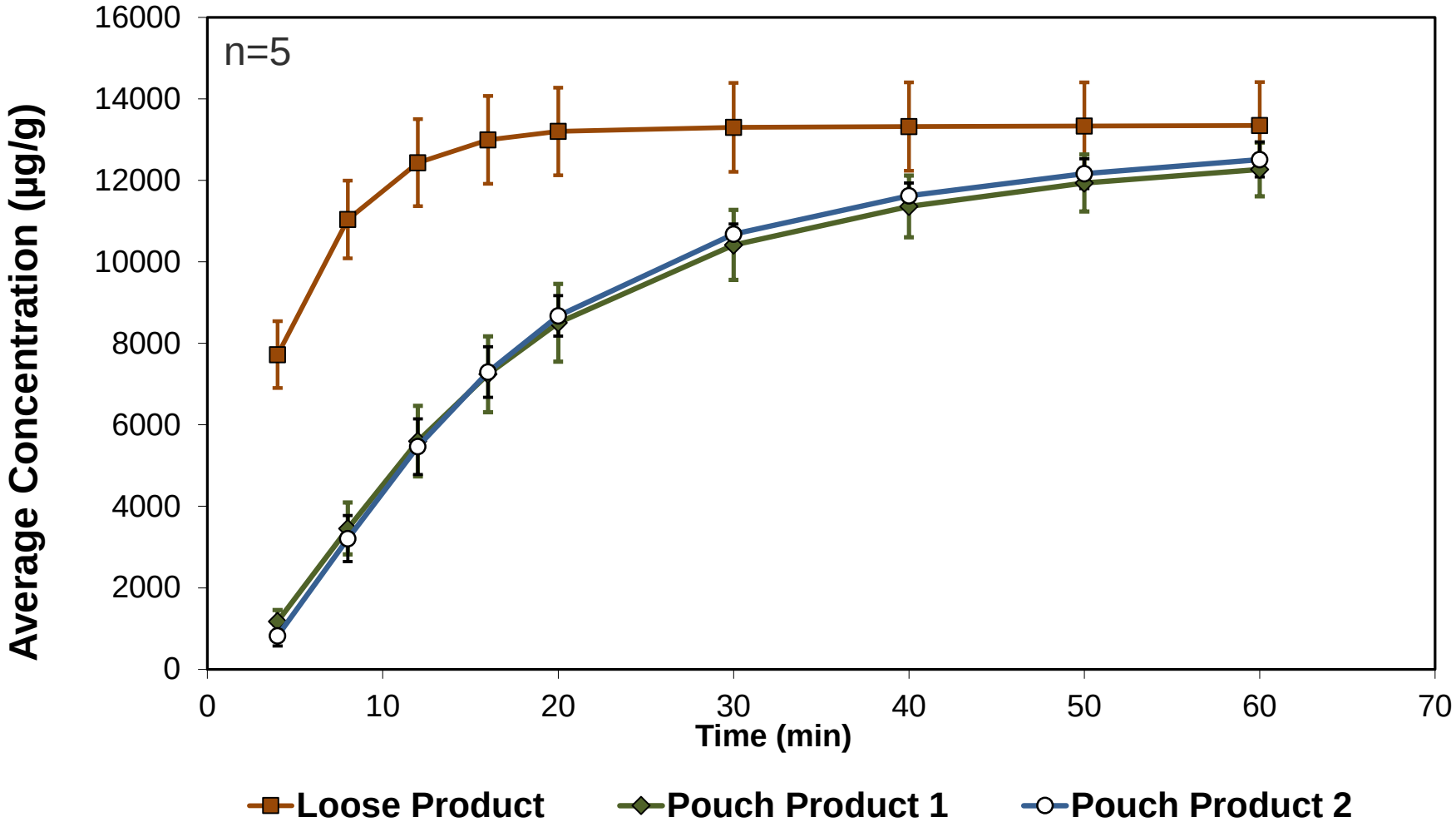
Results –Flavor Release (Menthol in Mint Products)



Results – Nicotine Release (µg/g)



Summary

- USP4 – Allows for cumulative release profiles to be generated for both loose and pouch smokeless tobacco products under consistent conditions
- Analysis of fractions was performed by GC/MS for nicotine and menthol
- Analytical method was fully validated for analysis of nicotine and menthol in artificial saliva
- This technique demonstrates excellent reproducibility and can be applied to measure a variety of constituents that are released from smokeless tobacco for comparative and regulatory reporting purposes

This presentation may be accessed @
www.altria.com/ALCS-Science