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

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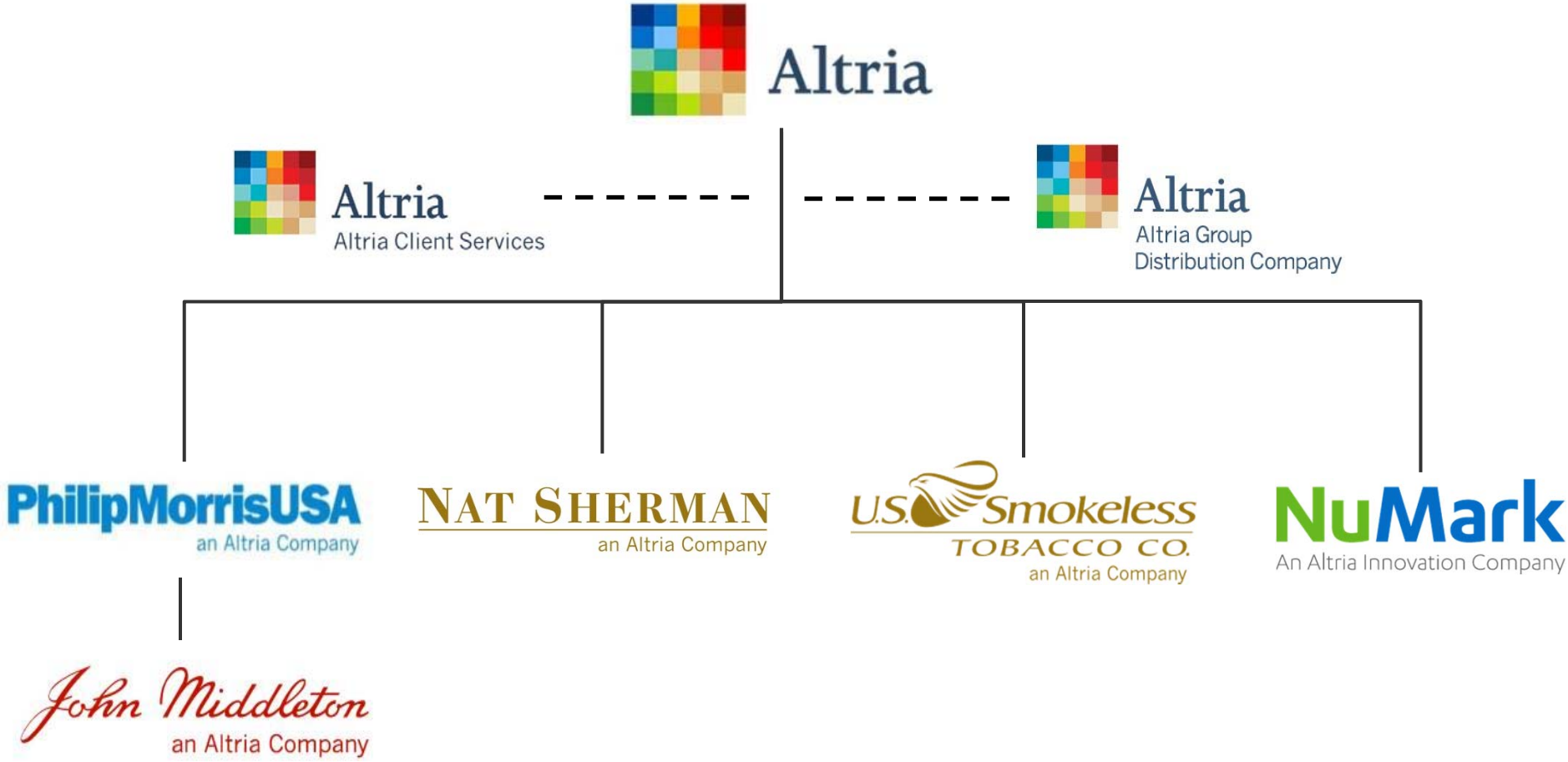
# Altria Operating Structure

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* For illustrative purposes only. Not actual corporate structure; does not reflect all Altria subsidiaries

# Altria Center for Research & Technology (CRT)


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# CRT

- **Purpose**:  Promote collaboration and creativity to develop technologies that improve Altria's companies' current product portfolio and lead to innovative new products.
- **Facility**: 450,000 square-foot facility in the Virginia Biotechnology Research Park opened in 2007
  - Office and meeting space
  - Consumer Opinion Center
  - ~13,006 square meters of laboratory space

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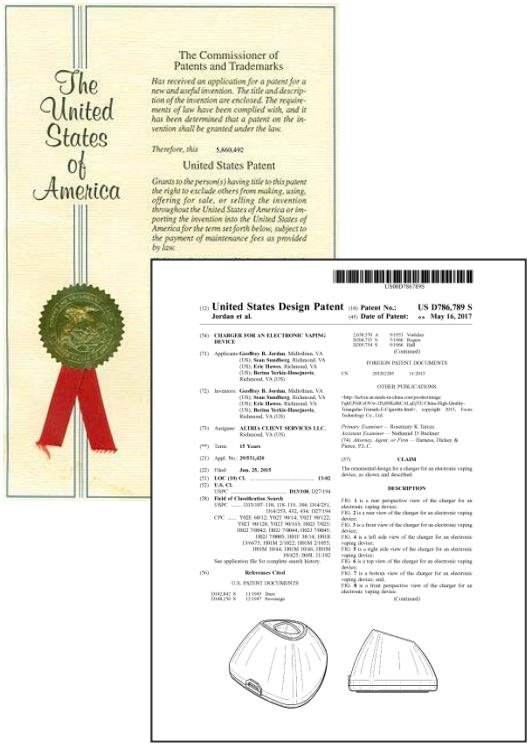
Talent recruited from academia, regulatory agencies, or other private sector firms
Citizens from 16 different countries
400+ employees, including scientists and engineers
110 employees have PhDs
32 different languages spoken

# CRT Research

## Since opening…

### 600+ Patents




### 200+ Publications


### 200+ Presentations

Global Forum on Nicotine

SOT Society of Toxicology

ENDS ELECTRONIC NICOTINE DELIVERY SYSTEMS

GTNF 2017 Global Tobacco & Nicotine Forum Dynamic dialogue, expanding perspectives.

CORESTA

SOCIETY FOR RESEARCH ON NICOTINE & TOBACCO

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## Our Aspiration Statement

Altria aspires to be the U.S. leader in authorized, non-combustible, reduced-risk products.

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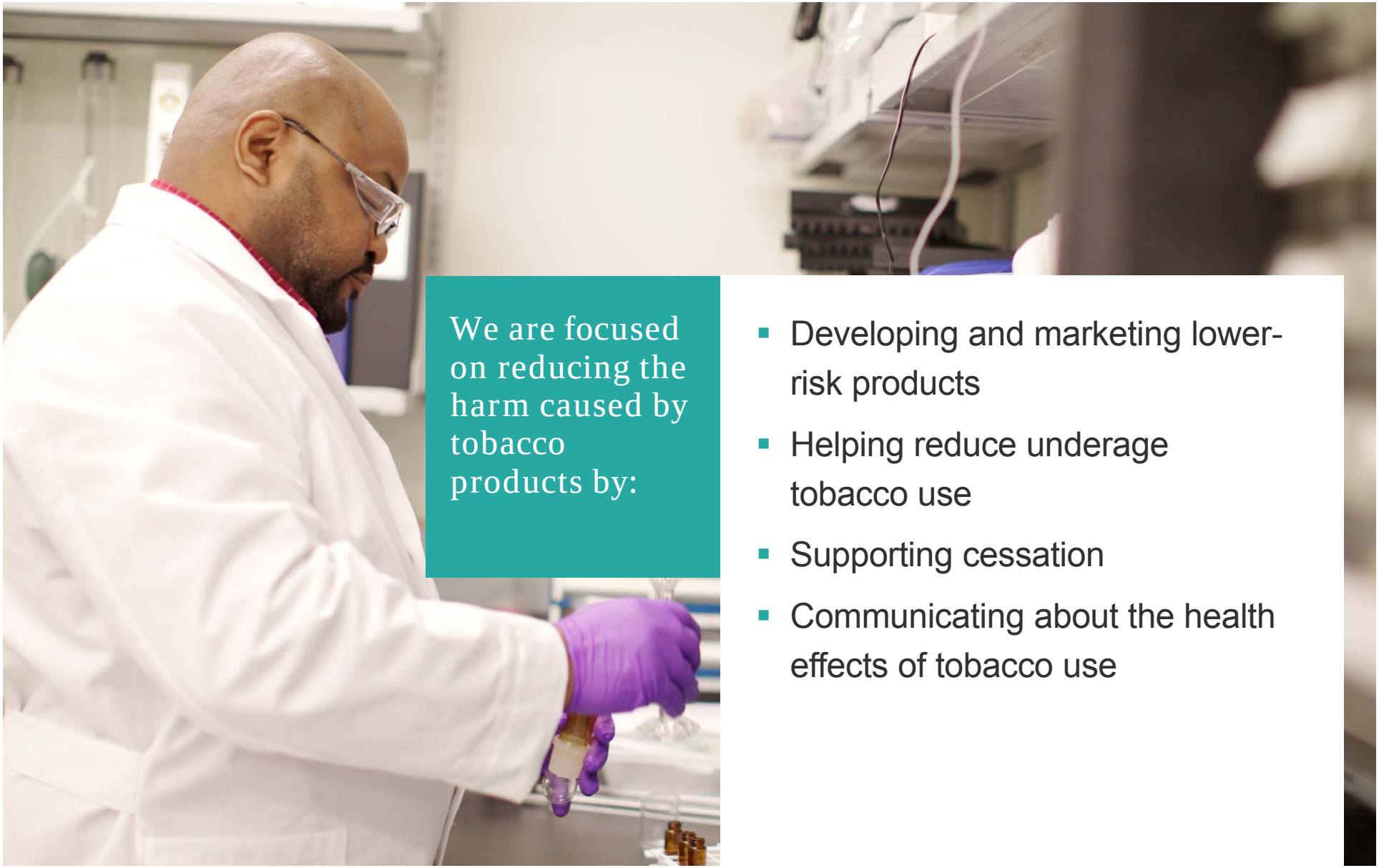

We are focused on reducing the harm caused by tobacco products by:

- Developing and marketing lower-risk products
- Helping reduce underage tobacco use
- Supporting cessation
- Communicating about the health effects of tobacco use

http://www.altria.com/Responsibility/Tobacco-Harm-Reduction/Pages/default.aspx?src=topnav 3/22/16

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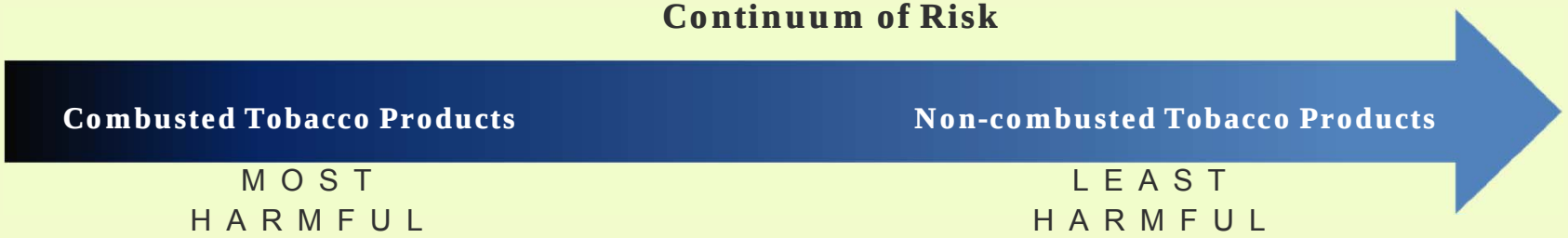
# FDA Recognizes Continuum of Risk

*"We must recognize the potential for innovation to lead to less harmful products, which, under FDA's oversight, could be part of a solution. While there's still much research to be done on these products and the risks that they may pose, they may also present benefits that we must consider."*

Dr. Scott Gottlieb
FDA Commissioner

**Continuum of Risk**




July 28, 2017: Protecting American Families: Comprehensive Approach to Nicotine and Tobacco
https://www.fda.gov/NewsEvents/Speeches/ucm569024.htm

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# Product Landscape






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

Altria Client Services | Helen Miller| Scientist I | Sotax Seminar 2018

# Introduction

**Smokeless Tobacco Analysis:**

- Typical analysis of smokeless tobacco products is based on forced extraction of constituents from tobacco but this does not provide information for constituent release over time.

- There are currently no standardized methods to make comparisons of constituent release for smokeless tobacco products.

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# 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**

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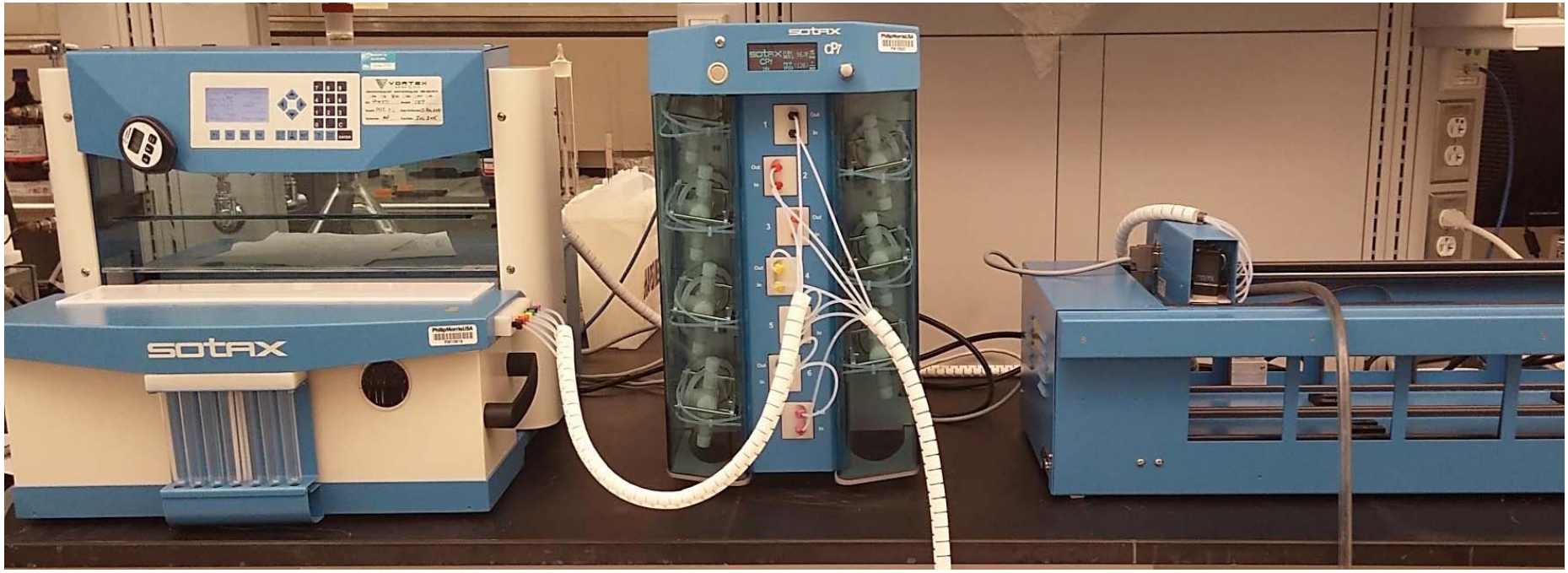
# Method

## USP4 Dissolution Apparatus - SOTAX CE7 Smart USP4

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

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# Method

- USP4 Dissolution Cell Setup

1 mm beads on bottom
3 mm beads on top

Direction of Flow

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# 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 37°C
  - 1 mm glass beads on bottom
  - 3 mm glass beads on top
  - 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 |

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# 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 |

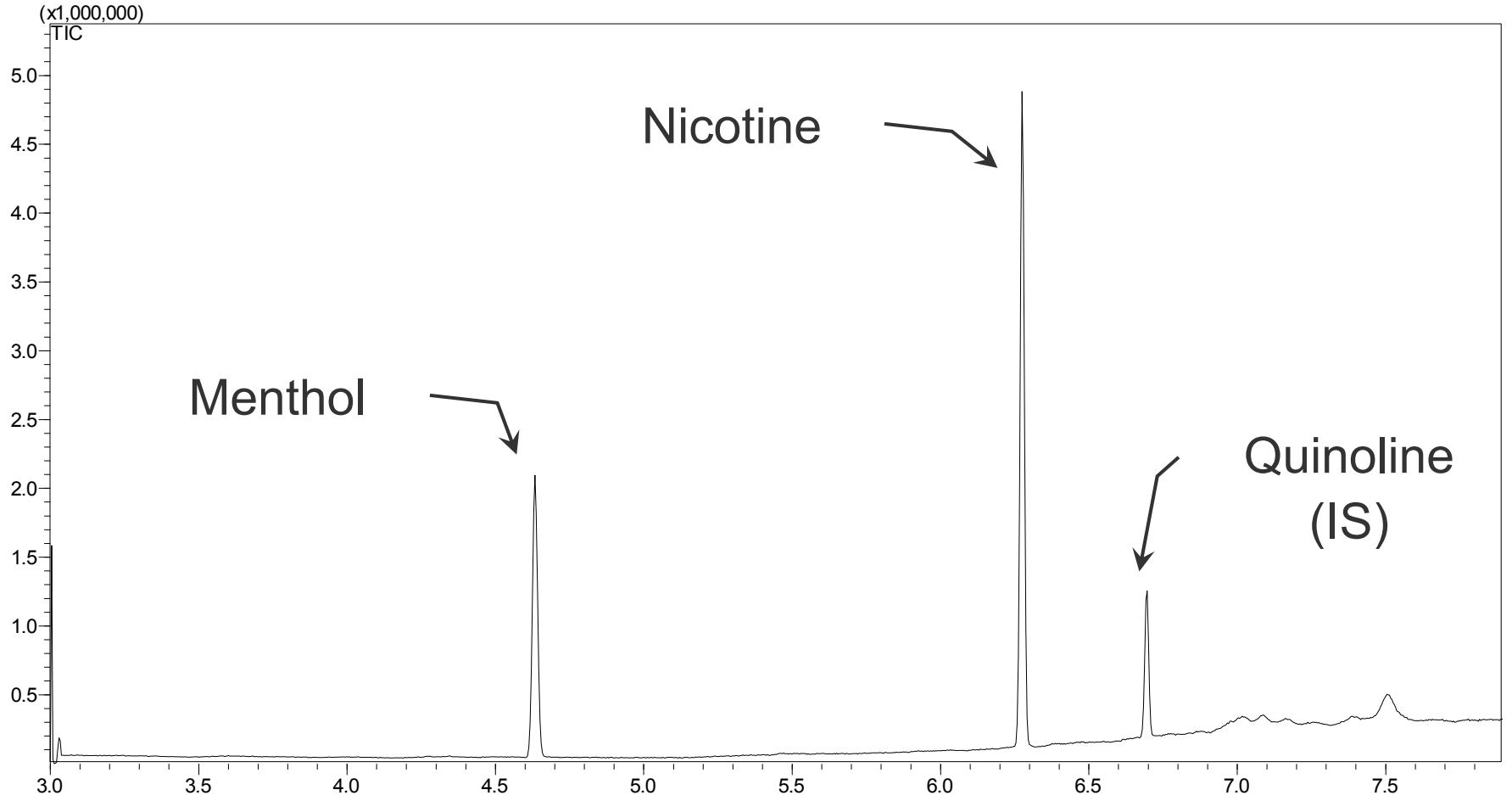
## Mass Spectrometer – Selected Ion Monitoring

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

## Calibration

| | | |
|---|---|---|
| Menthol | 2.50 – 75.0 µg/mL | Linear no Weighting |
| Nicotine | 5.00 – 250 µg/mL | Linear no Weighting |

# GC/MS Chromatogram (TIC) - Example

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# 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)

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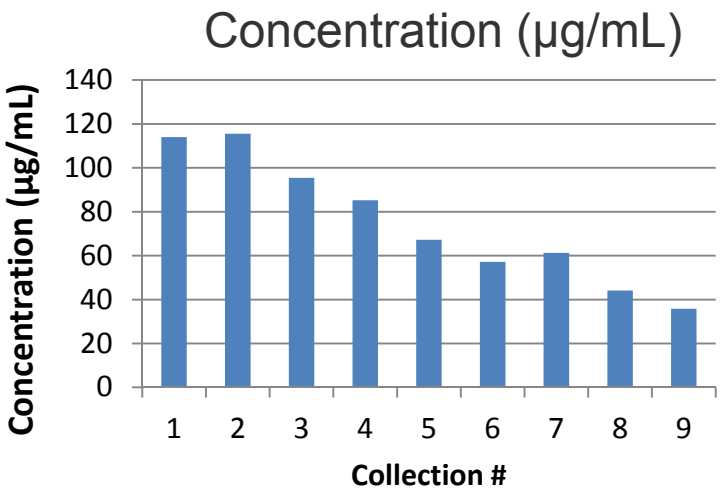
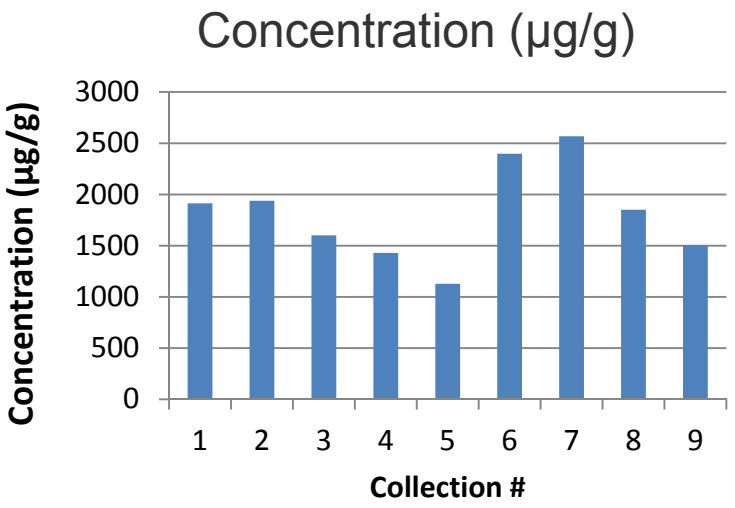
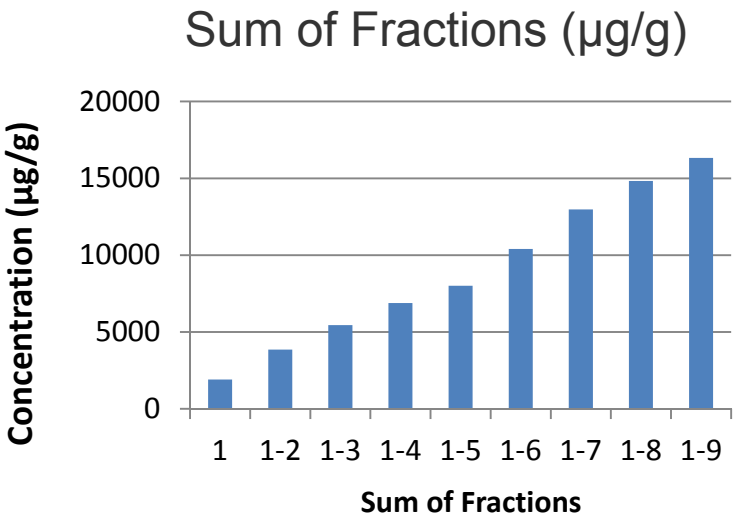
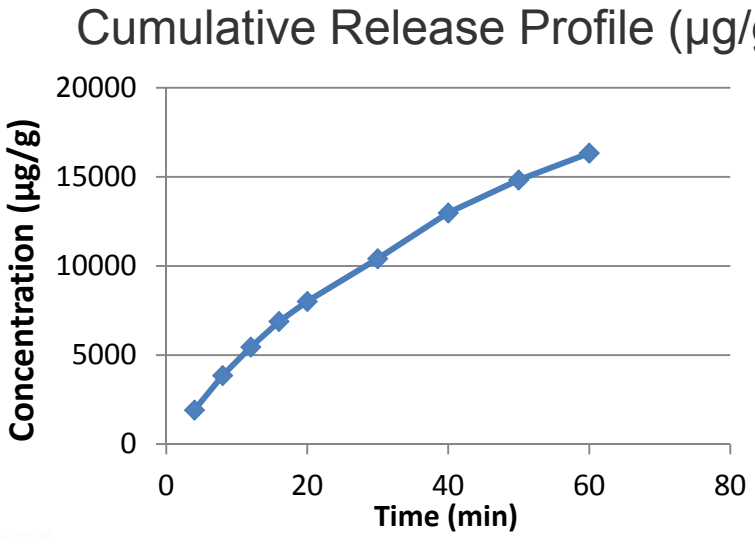
# Results – Calculations for Cumulative Release Profile

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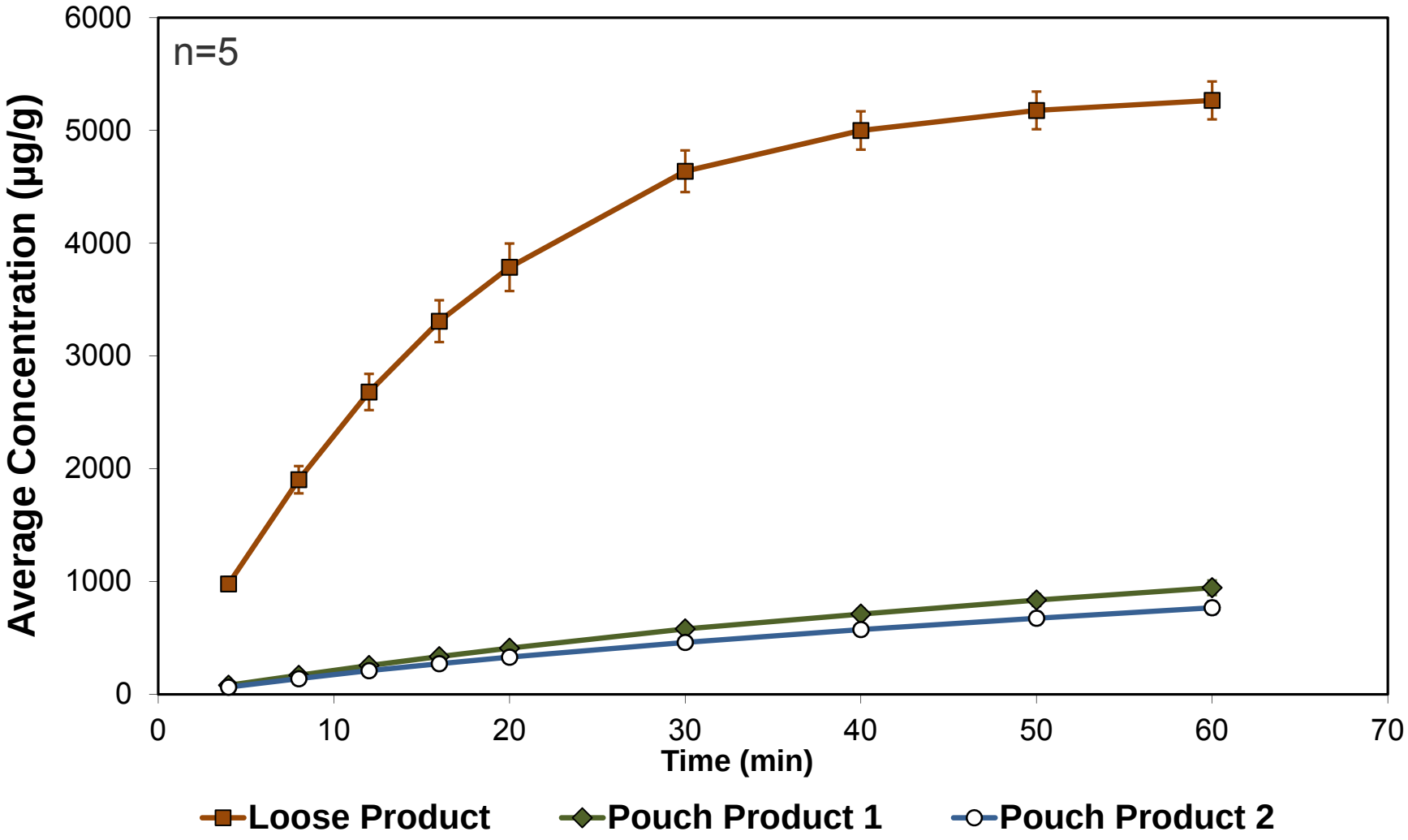
# Results –Flavor Release (Menthol in Mint Products)

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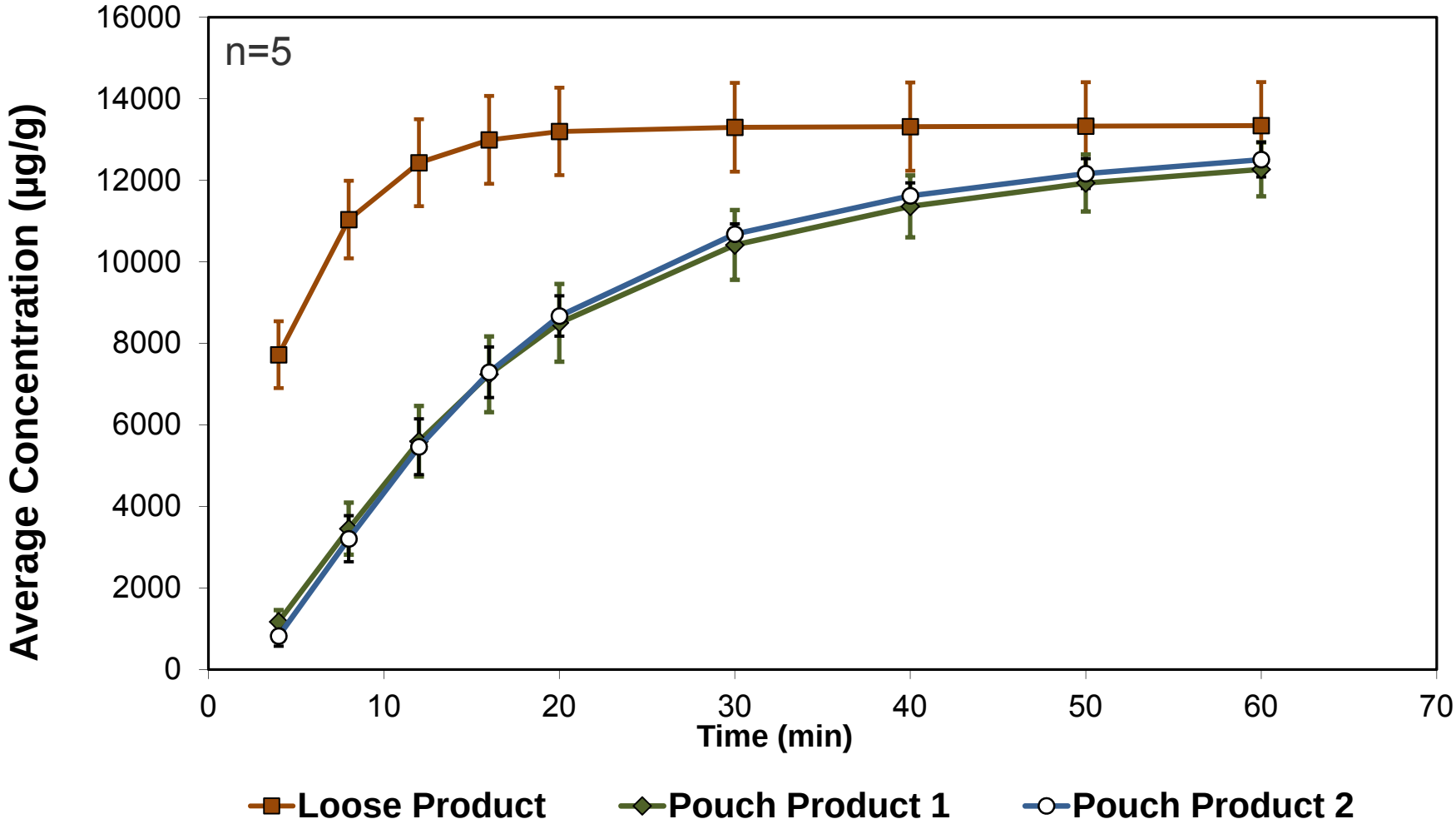
# Results – Nicotine Release (µg/g)

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# 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

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