In Vitro Dissolution Testing of Nicotine Release from Smokeless Tobacco Products

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Substantial Equivalence (SE) Pathway "905"

Compare Predicate vs. New Product

Demonstrates if the new product has:

- Same characteristics as the predicate
- Product does not raise different questions of public health

US FDA: Abbreviated HPHC List*

Cigarette Smoke	Cigarette Filler
NNK	NNK
NNN	NNN
Nicotine	Nicotine
Acetaldehyde	Arsenic
Crotonaldehyde	Cadmium
Formaldehyde	Ammonia
Acrolein	
Acrylonitrile	
Benzene	**Smokeless Tobacco**
1,3-Butadiene	NNK
Isoprene	NNN
Toluene	Nicotine (total and free)
4-Aminobiphenyl	Acetaldehyde
1-Aminonaphthalene	Crotonaldehyde
2-Aminonaphthalene	Formaldehyde
Ammonia	Arsenic
Benzo[a]pyrene	Cadmium
Carbon monoxide	Benzo[a]pyrene

"Reporting Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke Under Section 904(a)(3) of the Federal Food, Drug, and Cosmetic Act" (Guidance for the Industry, March 2012).

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Substantial Equivalence (SE) for Smokable Tobacco Products

Predicate vs. New Product

- Comparison of HPHCs in cigarette filler and in smoke (route of exposure)
- Smoking machines allow for a comparison of multiple products
- Standardized smoking protocols: ISO, CORESTA & HC
- Not meant to replicate human exposure

Cigarette **Smoking Machine**

ISO: International Organization for Standardization **CORESTA:** Cooperation Center for Scientific Research Relative to Tobacco **HC:** Health Canada.

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Substantial Equivalence (SE) for Smokeless Tobacco Products

Cigarette

Smoking Machine

Loose

Snus

Pouch

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Dissolution Testing

In vitro laboratory test method designed to demonstrate how efficiently an active ingredient is extracted out of a solid oral dosage into solution

Applications in Pharmaceutical Industry

- Guide product design
- Quality control testing
- Product to product performance comparison
- Develop *in-vivo*/*in-vitro* correlation (IVIVC)

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Dissolution Methodology Considerations

- Apparatus
- Dissolution Media
- Analytical Method Development & Validation

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Dissolution Apparatus

Multiple commercial & non-commercial options available for measuring release of constituents from oral dosage forms

Example: U.S. Pharmacopeia Apparatus

Name	Apparatus Type
USP-1	Basket
USP-2	Paddle
USP-3	Reciprocating cylinder
USP-4	Flow-through cell
USP-5	Paddle over disk
USP-6	Cylinder
USP-7	Reciprocating holder

ALCS Dissolution Method

SOTAX CE7 Smart Flow-Through Cell Apparatus (USP-4)

- Flow rate 4.0 mL/min
- Temperature 37°C

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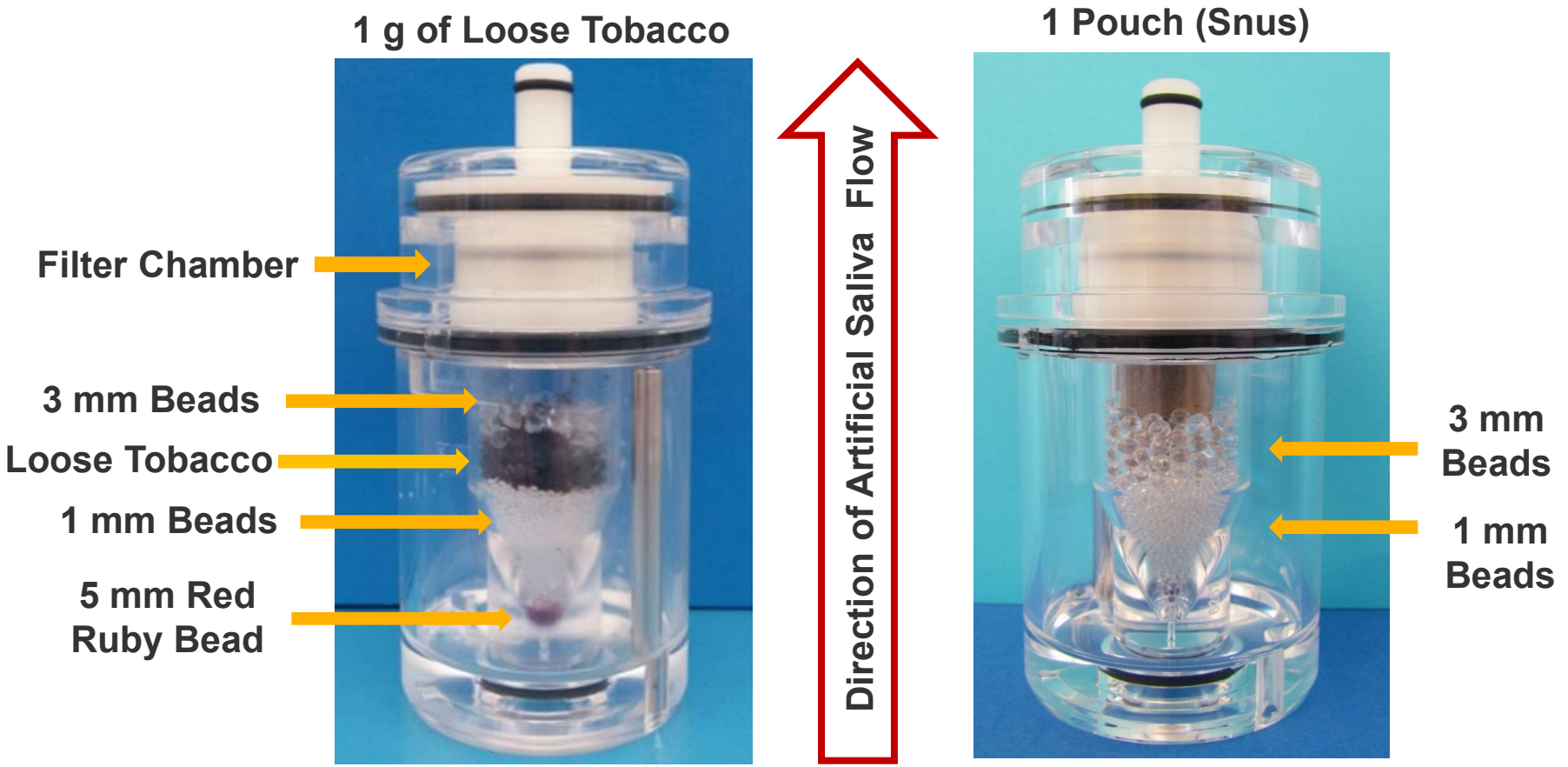
ALCS Dissolution Method

Flow-Through Cell in USP-4 Apparatus



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ALCS Dissolution Method

Artificial Saliva Composition*

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.

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ALCS Dissolution Method

Fraction Collection Protocol

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

- Flow rate 4.0 mL/min
- Temperature 37°C

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Analytical Method: Determination of Nicotine in Dissolution Fractions

Sample Preparation in Autosampler Vials

1. 0.1 mL of the dissolution fraction
2. 0.8 mL of artificial saliva
3. 0.1 mL of ISTD (Ethyl Benzoate, 1 mg/mL)
4. Cap, vortex and analyze by UPLC-PDA*

ISTD: Internal Standard
UPLC-PDA: Ultra-high Performance Liquid Chromatography Photodiode Array

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Analytical Method: Determination of Nicotine in Dissolution Fractions

Instrument: Waters Acquity I-Class UPLC system coupled to Photodiode Array Detector
Column: BEH C18, 2.1 x 100 mm, 1.7 µm, Waters Corporation
Guard column: BEH C18 VanGuard, 2.1 x 5 mm, 1.7 µm, Waters Corporation

UPLC Parameter	Setting
Run Time	12 min
Injection Volume	10 µL
Autosampler Temperature	5 °C
Column Temperature	45 °C
Mobile Phase A	10 mM Ammonium Acetate pH 10
Mobile Phase B	Acetonitrile
Pump Program	Gradient Elution
Flow Rate	0.5 mL /min

Compound	PDA Setting
Nicotine	260 nm
Ethyl benzoate	260 nm

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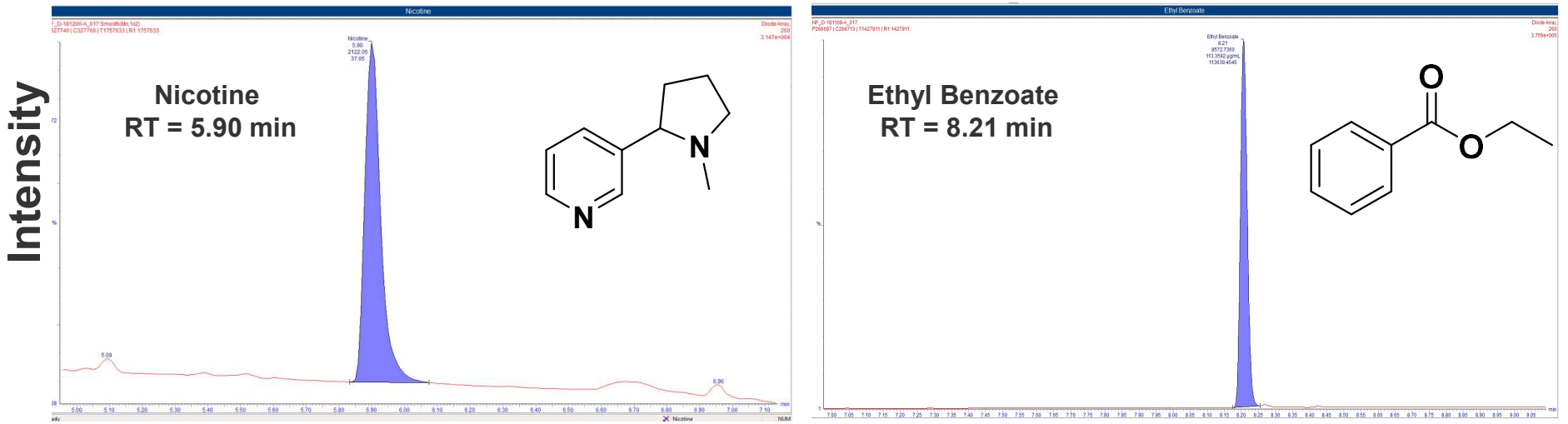
Analytical Method: Determination of Nicotine in Dissolution Fractions

UPLC-PDA Chromatograms of Nicotine and Ethyl Benzoate (ISTD)



ISTD: Internal Standard
UPLC-PDA: Ultra-high Performance Liquide Chromatography Photodiode Array

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Analytical Method: Determination of Nicotine in Dissolution Fractions

Method Validation

Parameter	Outcome
Calibration (0.5-100 µg/mL)	▪ R^2 > 0.998 on all days ▪ %RCR < 10%
Accuracy - 3 fortification levels in triplicate for each product type	96.2% - 102%
Repeatability (Intra-day precision, n = 6)	< 2.0%
Intermediate Precision (Inter-days precision, n = 18)	< 6.0%
Specificity	No interferences observed at the retention time of nicotine or IS
LOQ	0.5 µg/mL
Stability - Dissolution samples and final extracts	Stable for up to 15 days when stored in amber glass vials at 0-4°C (refrigerated)

Analysis of Products for Regulatory Submissions

Predicate vs. New Product

12 replicates per product*

- Requires two runs for each product (7 cell holders per unit)
  1. 6 Reps + 1 IQCS (CRP 2.1, American style loose moist snuff)
  2. 6 Reps + 1 reagent blank
- 9 fractions collected for each sample replicate

**Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Dosage Forms, FDA Center for Drug Evaluation and Research (CDER),1997.*

- **IQCS:** Internal Quality Control Sample

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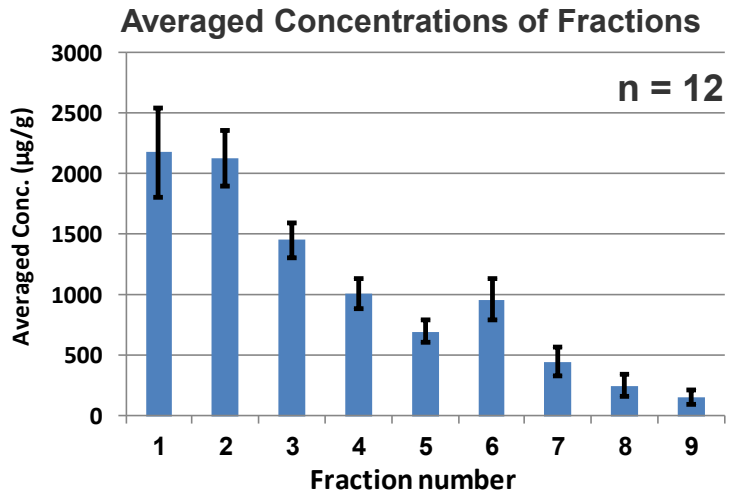
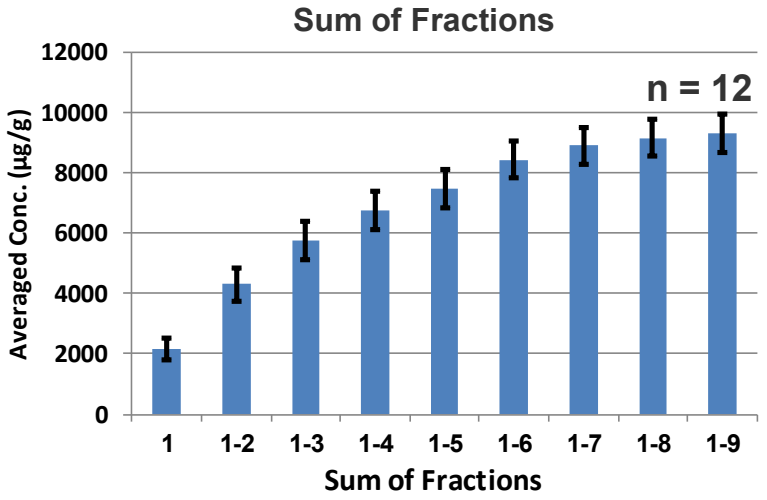
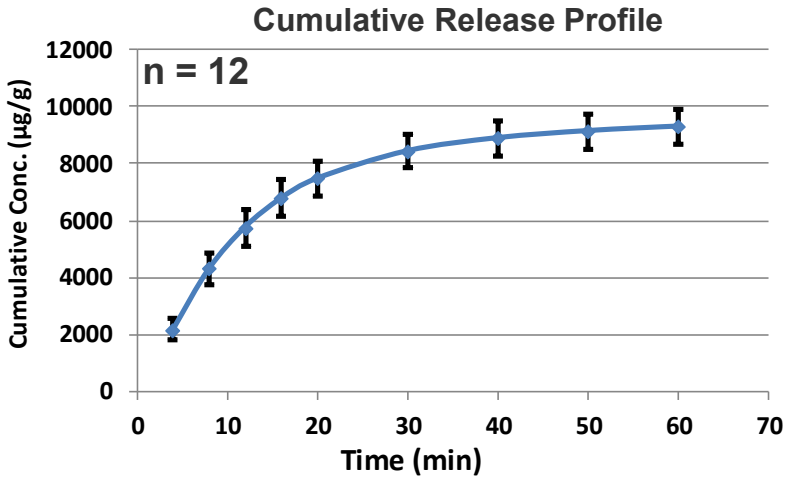
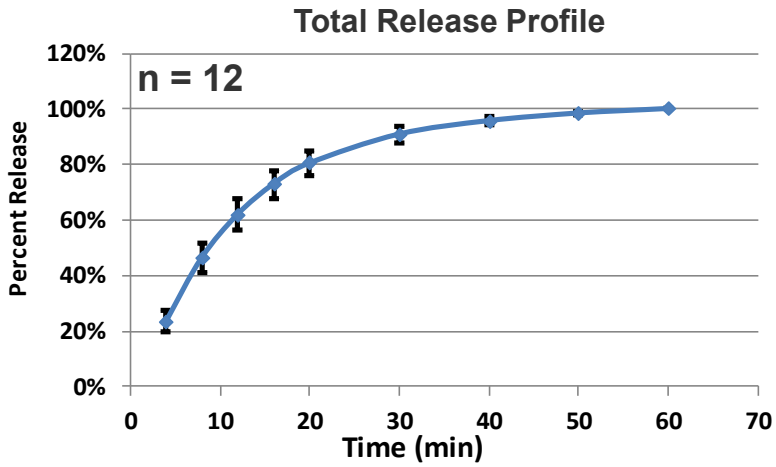
Calculations for Cumulative Release Profile









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CORESTA Smokeless Tobacco Reference Products (CRPs)

CRP 1.1 (Swedish style snus pouch)

CRP 2.1 (American style loose moist snuff)

CRP 4 (loose-leaf chewing tobacco)

CRP 4.1 (chopped loose-leaf chewing tobacco)

https://www.coresta.org/coresta-smokeless-tobacco-reference-products

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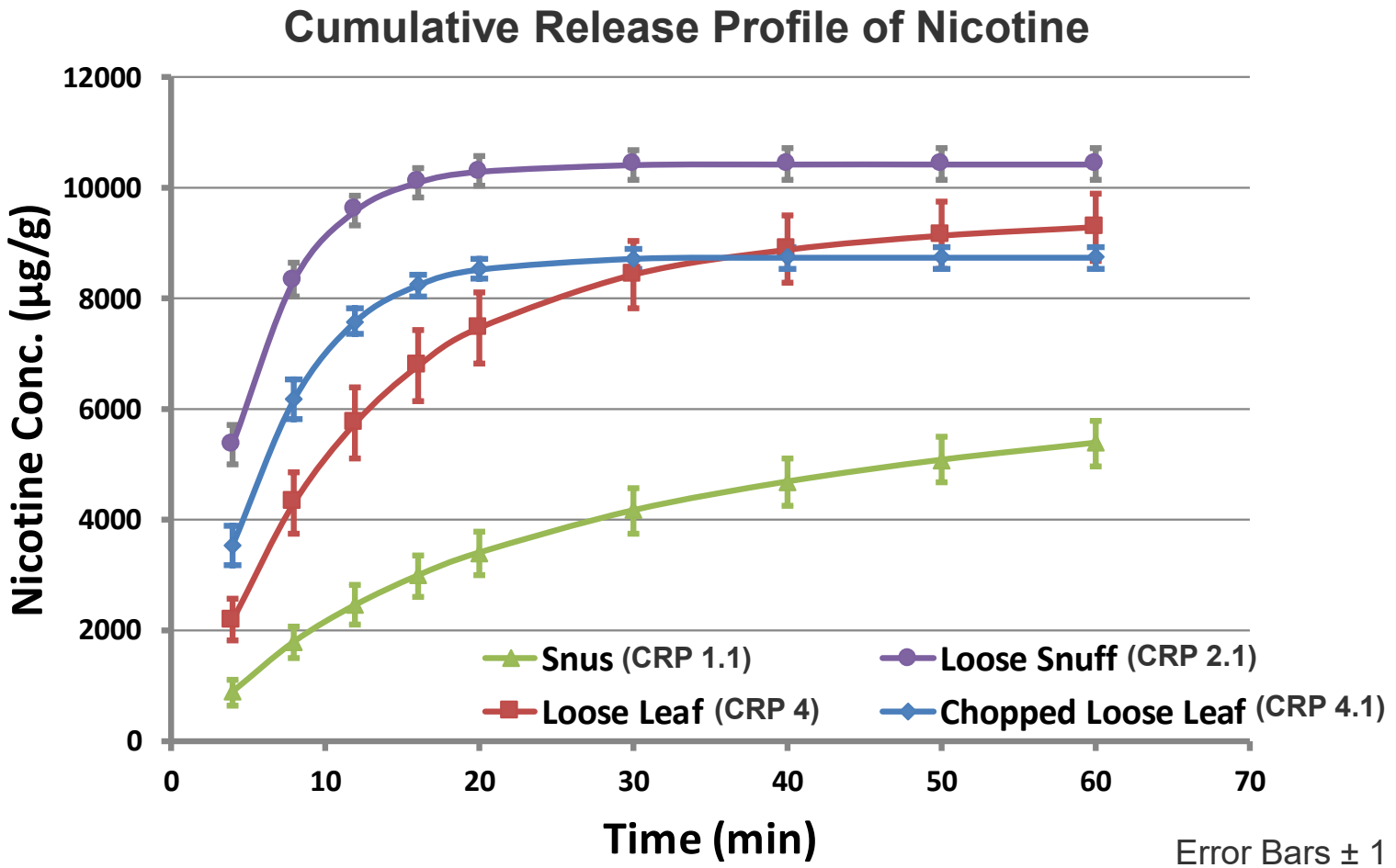
Results

CORESTA Smokeless Tobacco Reference Products (CRPs)



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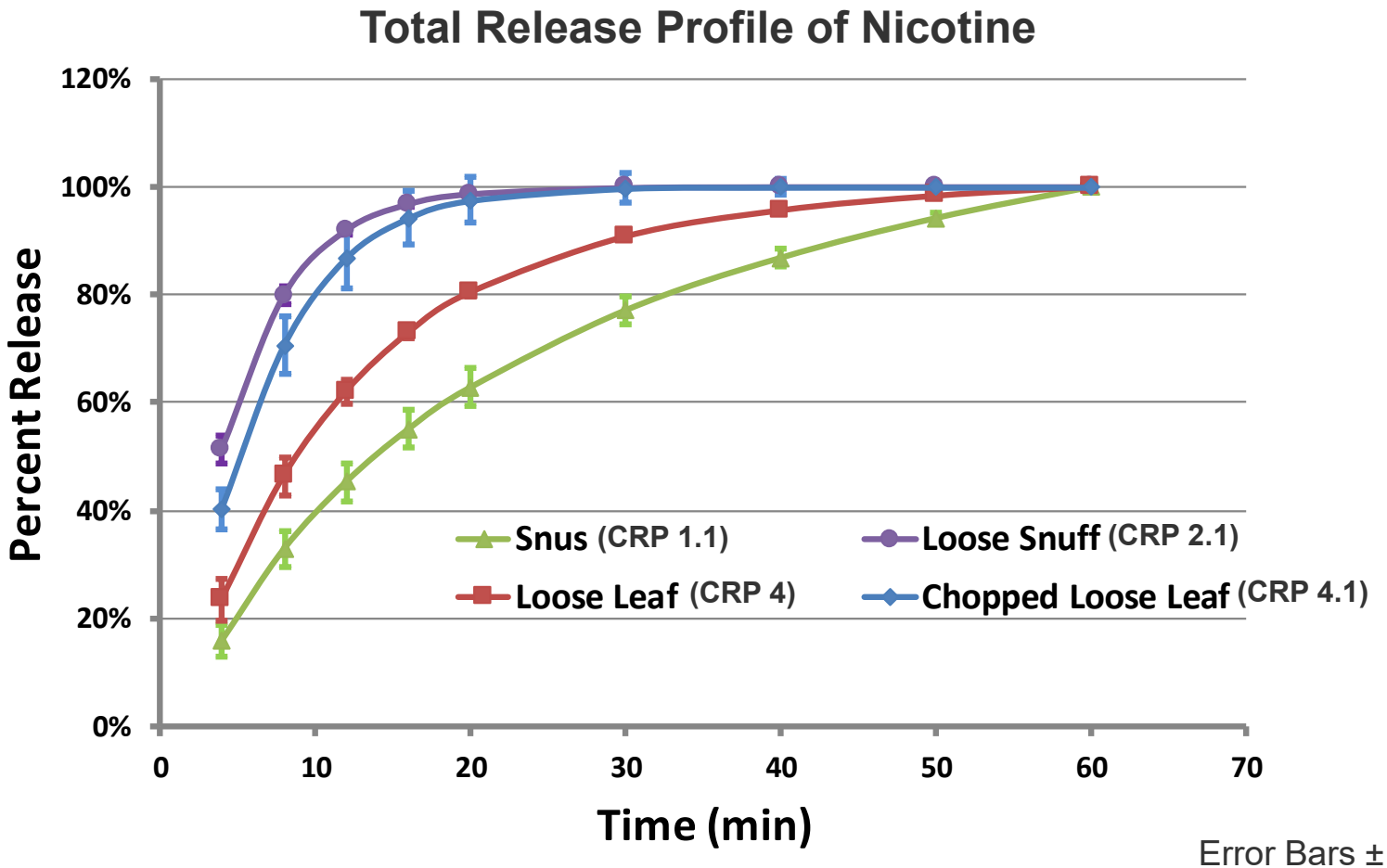
Results

CORESTA Smokeless Tobacco Reference Products (CRPs)



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Dissolution Profiles Comparison

Approach based on FDA guidance from CDER*

- Determine nicotine release profiles for predicate and new product (12 replicates per product)
- Use mean dissolution values from both curves at each time point to calculate difference factor (f_1) and similarity factor (f_2)

f_1 values up to 15 (0-15) and f_2 values of 50 or greater (50-100) ensure similarity or equivalence between two products

**Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Dosage Forms, FDA Center for Drug Evaluation and Research (CDER),1997.*

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Product to Product Comparison using f_1 and f_2

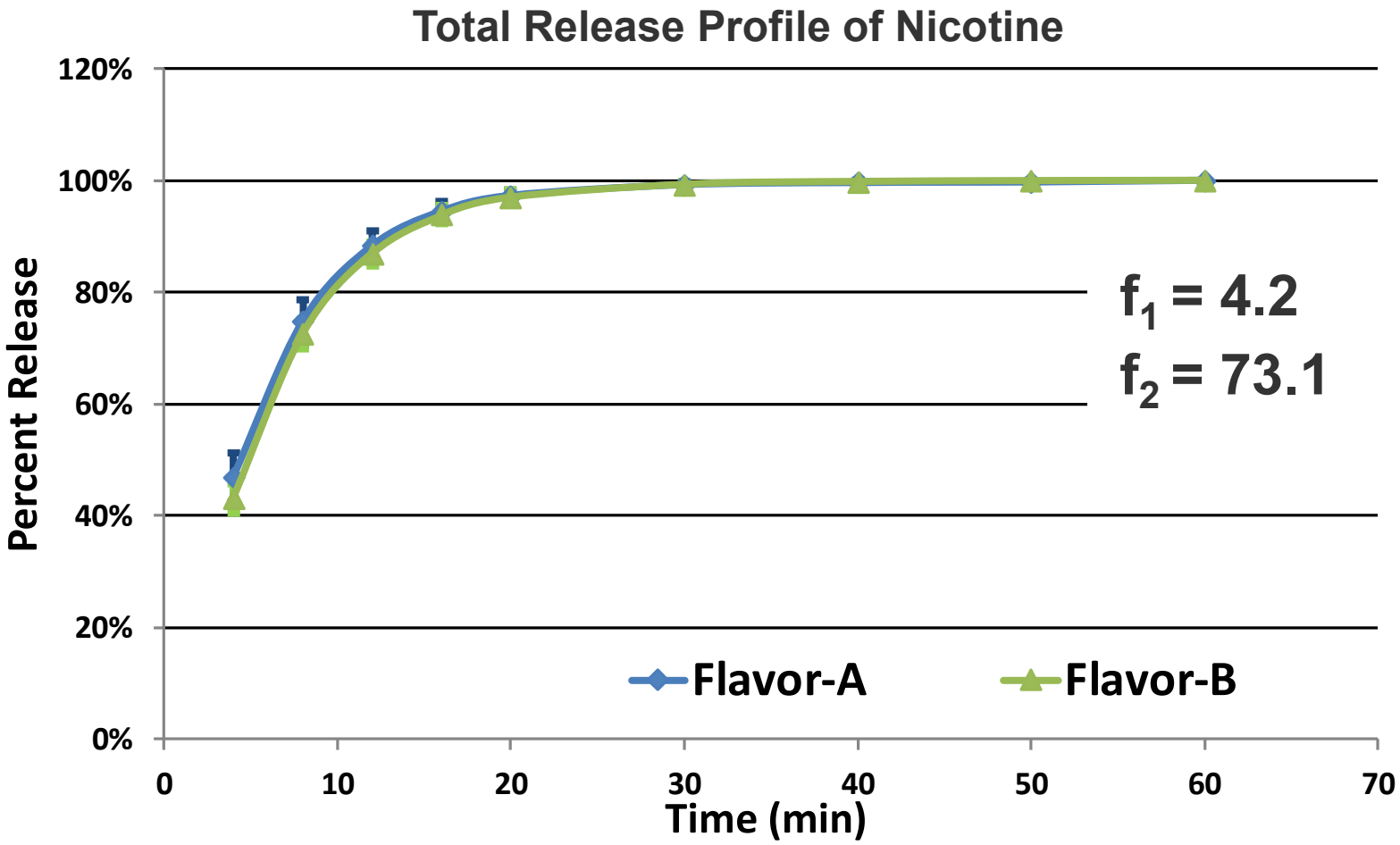
Compared Products	f_1	f_2	Equivalency
CRP 2.1 vs CRP 4.1	11.3	52.7	Yes
CRP 2.1 vs CRP 4	27.5	30.0	No
CRP 2.1 vs CRP 1.1	39.2	21.9	No
CRP 4.1 vs CRP 4	23.1	35.3	No
CRP 4.1 vs CRP 1.1	36.1	24.8	No
CRP 4 vs CRP 1.1	20.2	42.2	No

CRP 1.1 (Swedish style snus pouch)
CRP 2.1 (American style loose moist snuff)
CRP 4 (loose-leaf chewing tobacco)
CRP 4.1 (chopped loose-leaf chewing tobacco).

Equivalent:
f1 values up to 15 (0-15)
f2 values of 50 or greater (50-100)

Results

Commercial Products with Different Flavors



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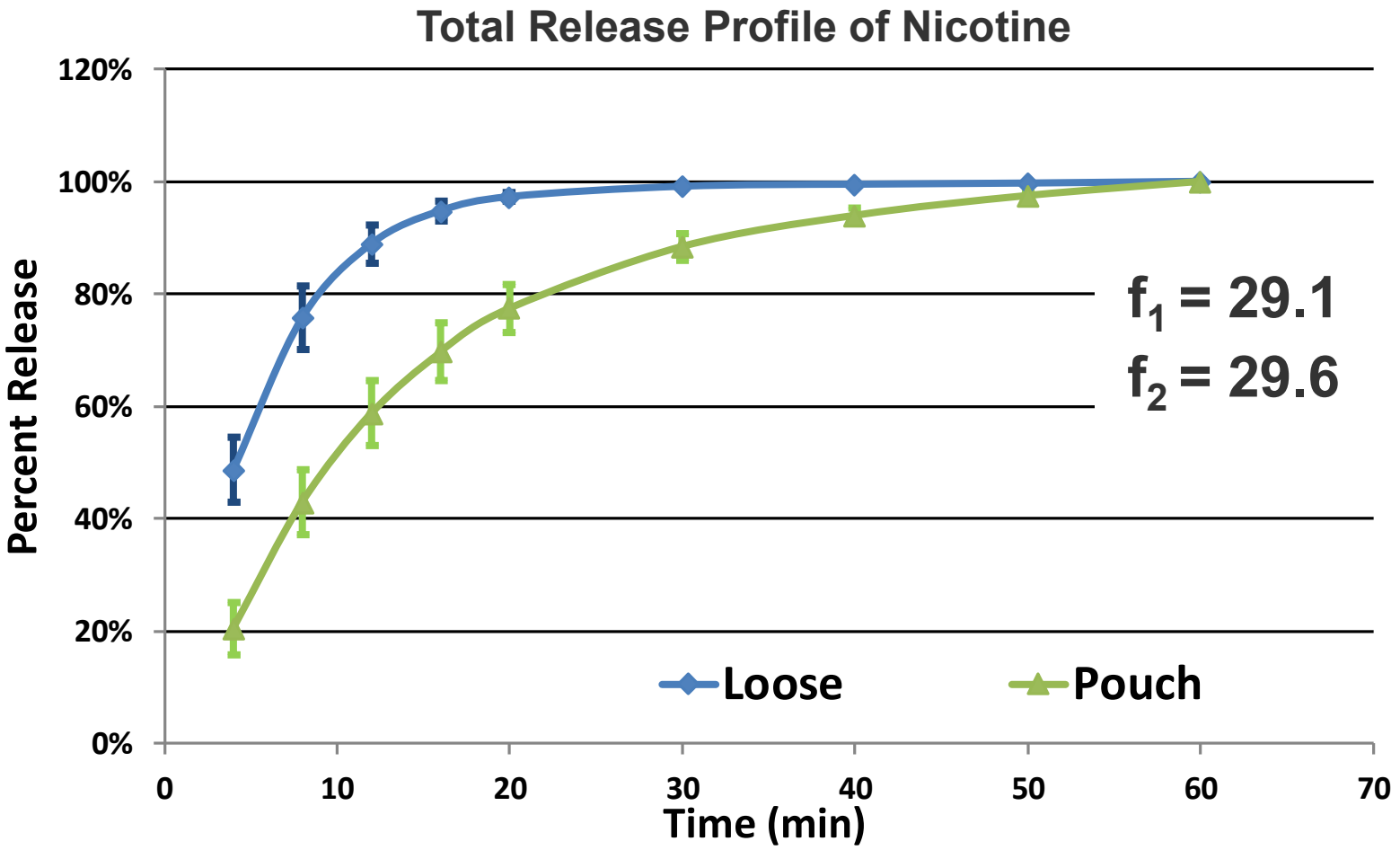
Results

Loose vs Pouched Commercial Products



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Summary

- An efficient method for the dissolution testing and analytical quantitation of nicotine was validated for a variety of smokeless tobacco products using a USP-4 flow-through cell apparatus and UPLC-PDA
- Percent of total release profiles, and f_1 and f_2 calculations were used to evaluate the similarity and differences between the smokeless tobacco products
- The nicotine release profiles are dependent on the form and cut of the smokeless tobacco products
- Validated dissolution methodologies can be an important tool for smokeless tobacco product assessments and product-to-product comparisons

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

Results

f_1 and f_2 Calculations

$$f_1 = \left\{\left[\sum_{t=1}^{n} |R - T|\right] / \left[\sum_{t=1}^{n} R\right]\right\} \text{ X } 100$$

$$f_2 = 50 \cdot \log\left[\frac{100}{\sqrt{1 + \frac{\sum_{t=1}^{t=n}[R_t - T_t]^2}{n}}}\right]$$

R_t and T_t are the cumulative percentage dissolved at each of the selected n time points of the two products.

f_1 values up to 15 (0-15) and f_2 values of 50 or greater (50-100) ensure similarity or equivalence between two products.

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