

Comparison of Two Apparatuses for Nicotine Dissolution Testing in Smokeless Tobacco and Tobacco-Free Nicotine Containing Portioned Products

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Apparatus 1 (USP1)

- Apparatus 1 Basket (USP1) Sotax AT Xtend

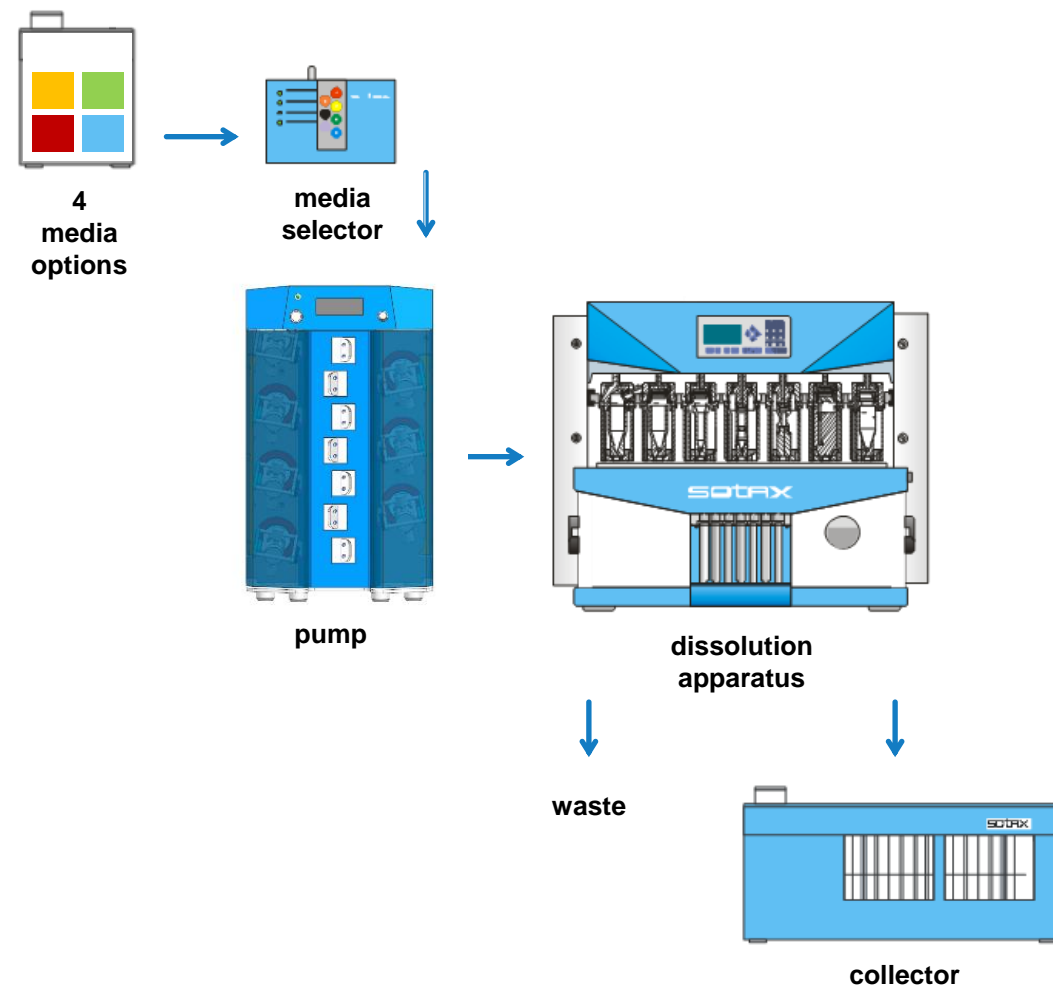
Parameters	Description
Apparatus	USP 1 (40 Mesh Basket)
Medium	12mM NH ₄ H ₂ PO ₄ , pH 7.4
Volume	500mL
Speed	50 RPM (0 to 60 min), 250 RPM (60 to 90 min)
Temperature	37°C ± 0.5°C
Sample	n = 5 and 1 Control in vessel 6
Filtration	0.45um nylon filter
Sampling profile (min)	1, 3.5, 6, 8.5, 11, 15, 20, 30, 60, 90



Apparatus 4 (USP4)

- Apparatus 4 flow-through cell (USP4) Sotax CE7 Smart
- Open loop system with offline HPLC measurement using parameters described in the following paper: John H. Miller, et al.; *Method development and validation of dissolution testing for nicotine release from smokeless tobacco products using flow-through cell apparatus and UPLC-PDA. (2020)*

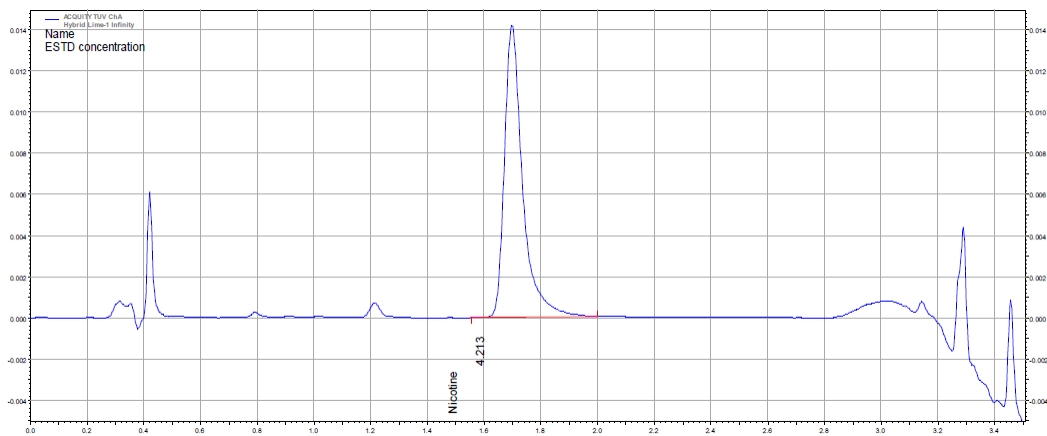
Parameters	Description
Apparatus	USP 4 (flow-through cell) setup as open system
Cell type	22.6mm diameter
Cell setup	Laminar flow with 1 spoon of glass beads, product loaded, and then 1 spoon of glass beads on top of product
Medium	12mM NH ₄ H ₂ PO ₄ , pH 7.4
Volume	Infinite sink continuous flow
Speed rate	4 mL/min
Temperature	37°C ± 0.5°C
Sample	n = 6 and 1 Control in Cell 7
Filtration	2.7µm filter
Sampling profile (min)	4, 8, 12, 16, 20, 30, 40, 50, 60
Fraction Collection	100% throughout the run



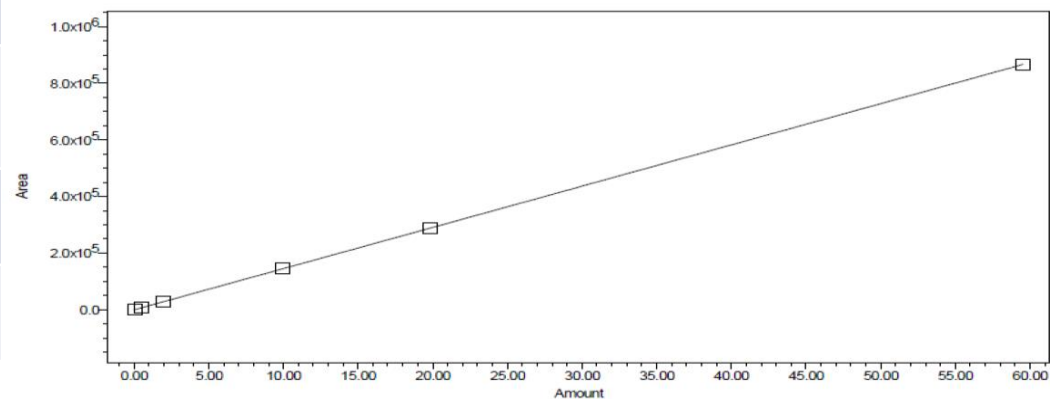
HPLC Analysis

Parameters	Description
Column	Waters Atlantis T3 C18 3mm, 3.0 x 50mm
Mobile phase	60% Phosphate buffer 25mM pH 7.2 / 40% MeOH (v/v)
Flow rate	0.75 mL/min
Injection volume	10 µL
Autosampler temperature	6°C
Column temperature	35°C
Wavelength	259 nm
Run time	7 minutes

Typical Sample Chromatogram



Typical Calibration Curve



Peak Name: Nicotine; RT: 1.681; Fit Type: Linear (1st Order); Cal Curve Id: 24439; R: 0.999999;
 R²: 0.999998; Weighting: None; Equation: Y = 1.46e+004 X - 2.83e+002; Normalized
 Intercept/Slope: -0.000653; RSD(E): 0.214843

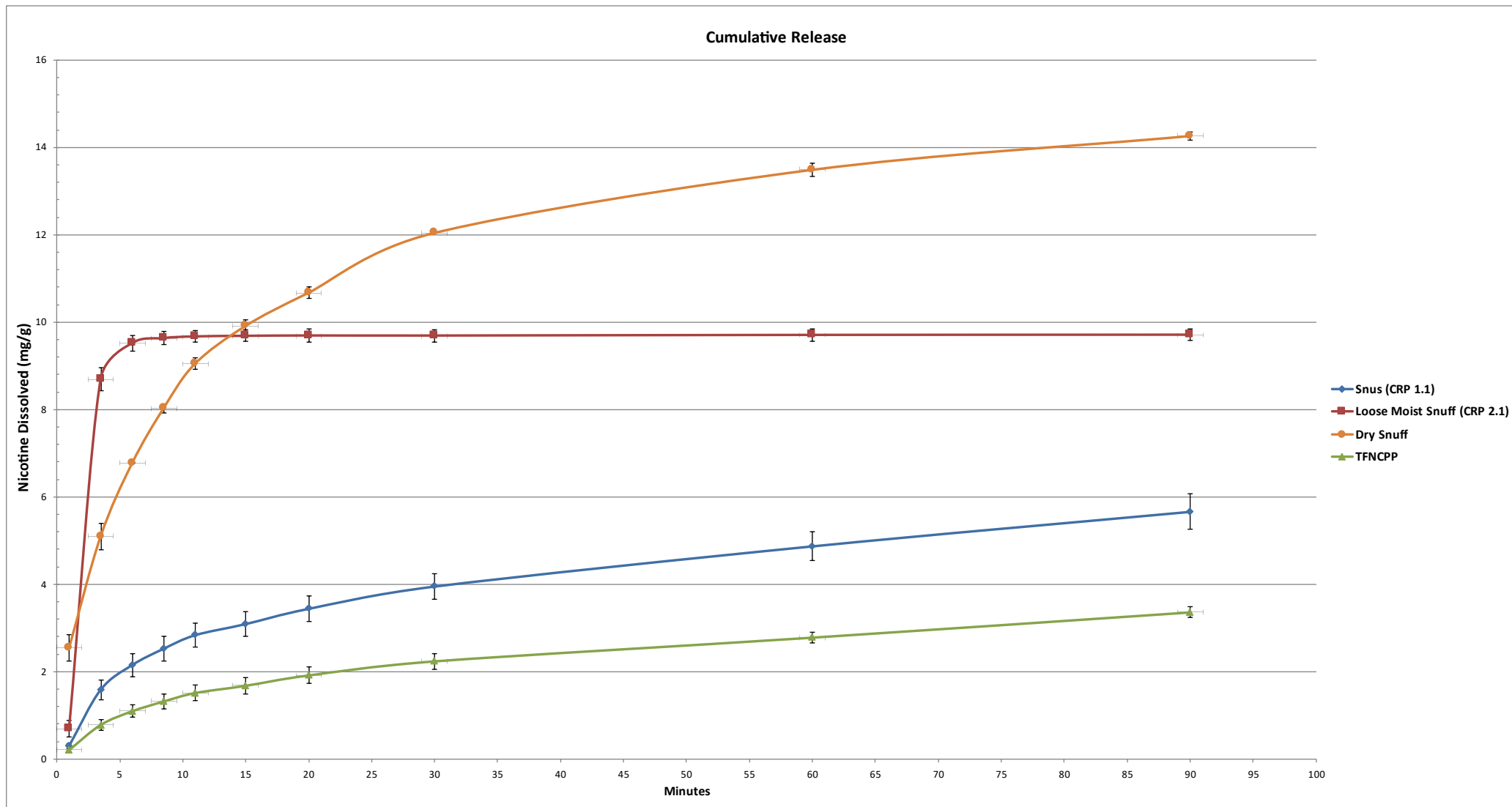
Validation – Apparatus 1

Parameters	Results
Calibration (0.1 to 60 µg/mL)	$R^2 \geq 0.9999$
System Precision (n=6, ~1.9 µg/mL)	SD = 0.012; % RSD = 0.621
Repeatability (n=6, 10 Pulls each)*	% RSD ≤ 15 (for all pulls except 1 min)
Standard Stability	7 days (protected from light at 6°C \pm 2°C)
Sample Stability	7 days (protected from light at 6°C \pm 2°C)

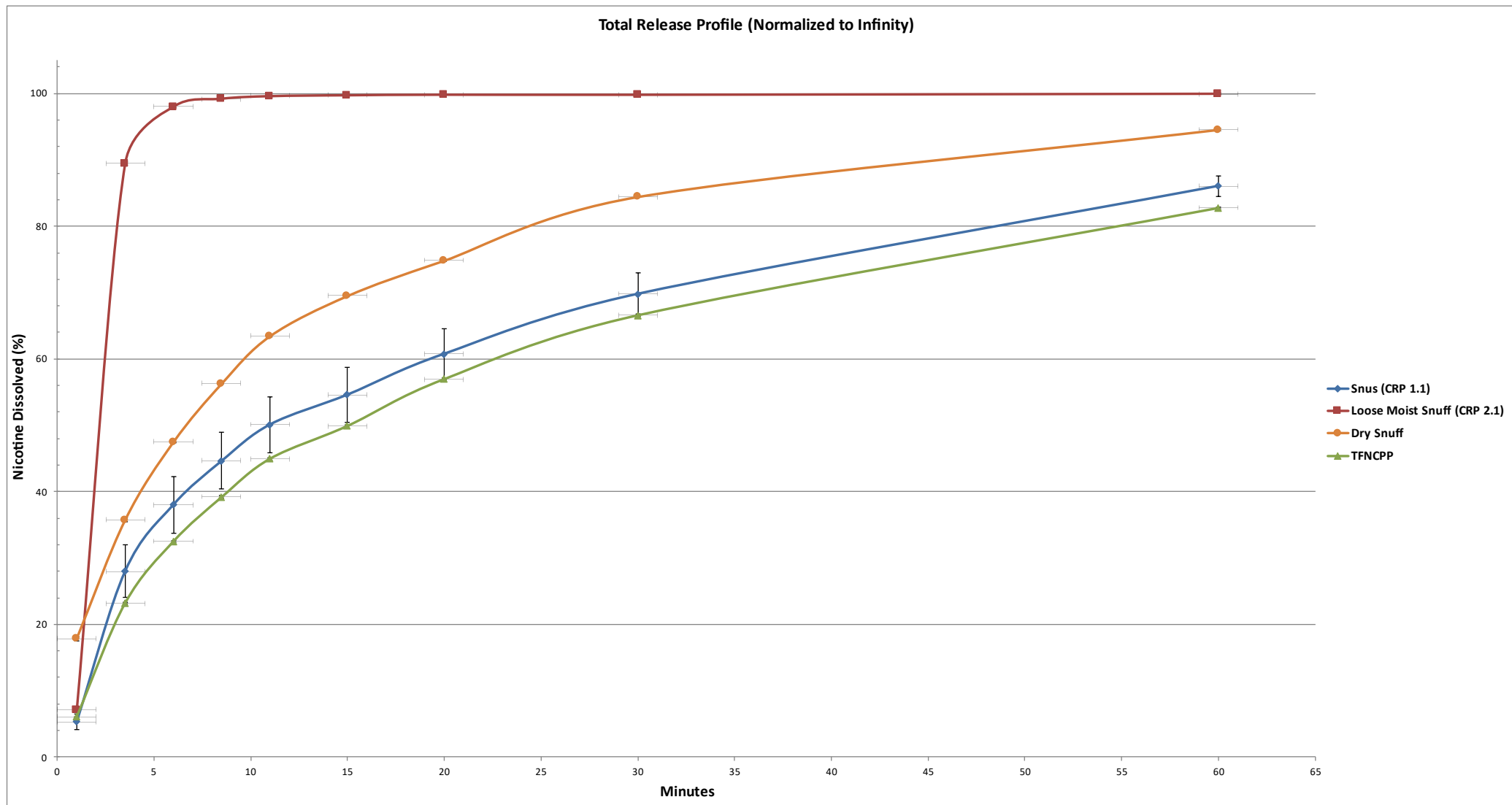
*Dry Snuff uses a paddle over basket method and was validated using n=10

- Accuracy criteria was set based on percent recovery according to results obtained from nicotine analysis by GC-FID

Validation – Apparatus 1



Validation – Apparatus 1



Objectives and Endpoints

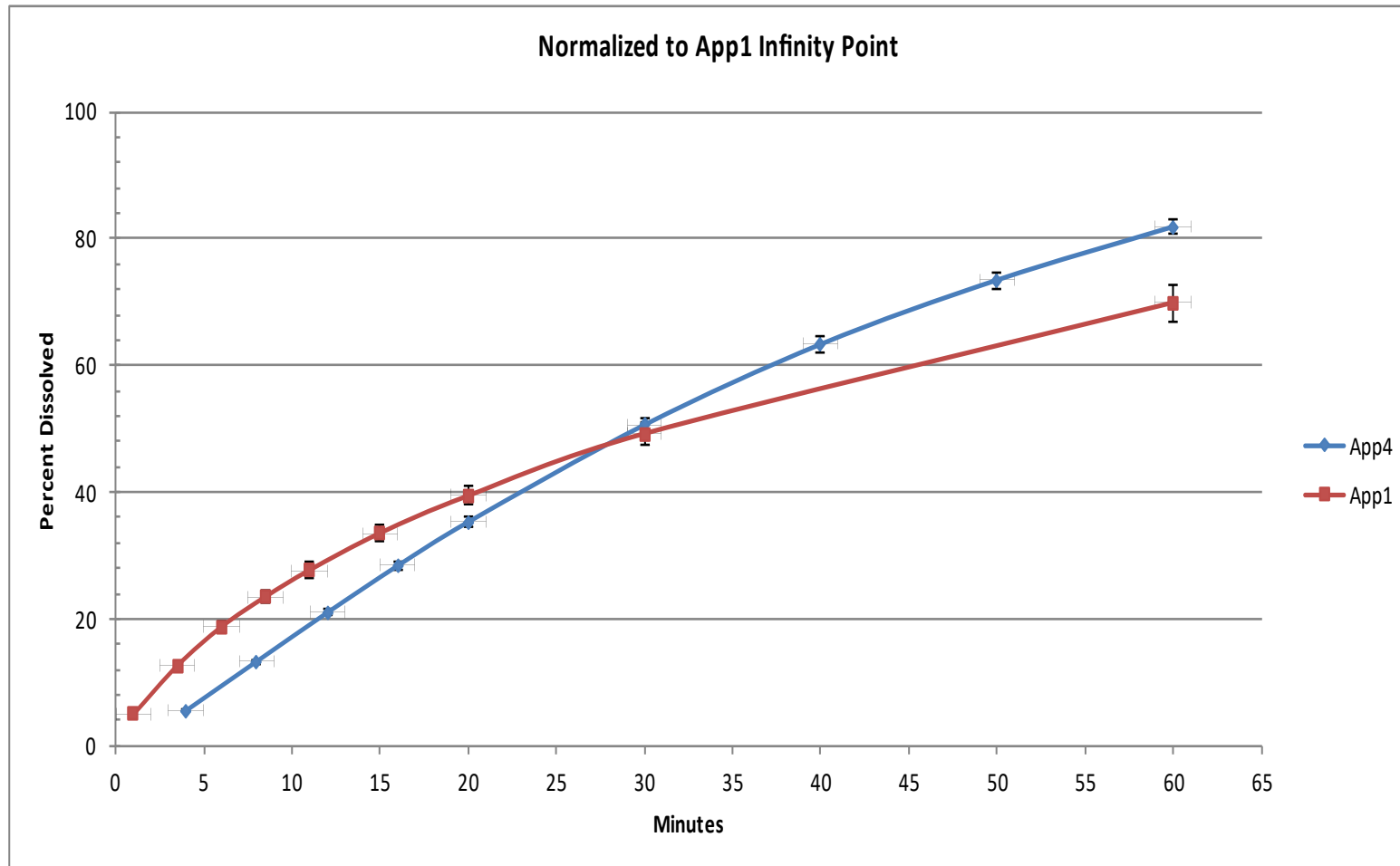
Commercially available products were used for comparison of apparatuses

- Two Snus Products (400 mg/pouch and 1 g/pouch)
- Two Traditional Portioned Moist Snuff Products
- Two Traditional Loose Moist Snuff Products
- Three Tobacco-Free Nicotine Containing Portioned Products (TFNCPP) (2 mg, 4 mg, and 7 mg)

- 15 replicates of each product were analyzed utilizing the Apparatus 1 for nicotine dissolution
- 18 replicates of each product were analyzed utilizing the Apparatus 4 for nicotine dissolution

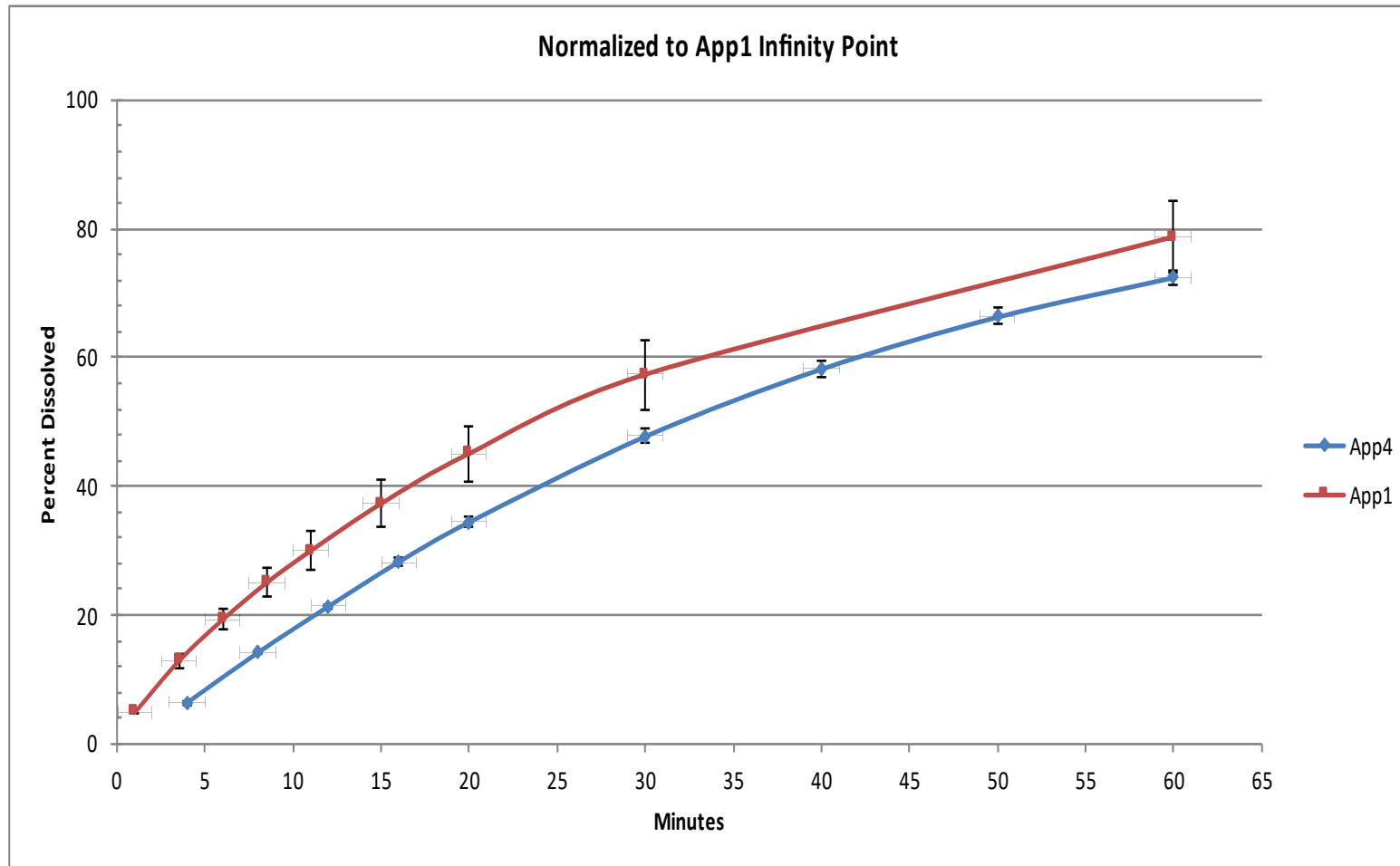
- Mean nicotine dissolution values from each apparatus (at the initial fraction pull, and fraction pulls at 20, 30 and 60 minutes) were used to calculate the difference factor (f_1) and similarity factor (f_2).
 - Difference factor values from 0-15 and similarity factor values of 50-100 ensure equivalence between two products.

Results – Snus Product 1 (1 g Pouch)



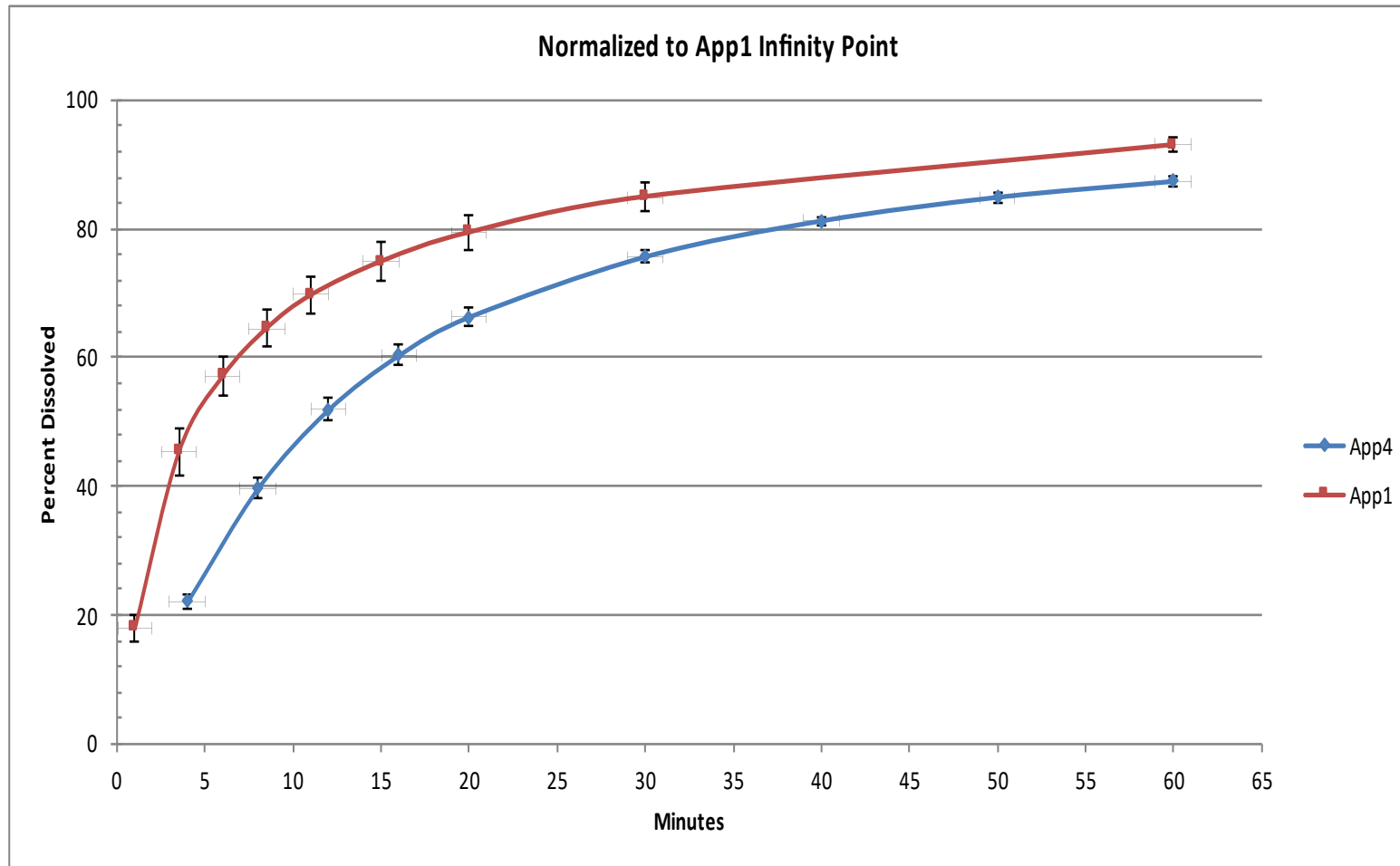
- $f_1 = 10.5$
- $f_2 = 79.3$
- Result = Equivalent

Results – Snus Product 2 (400 mg Pouch)



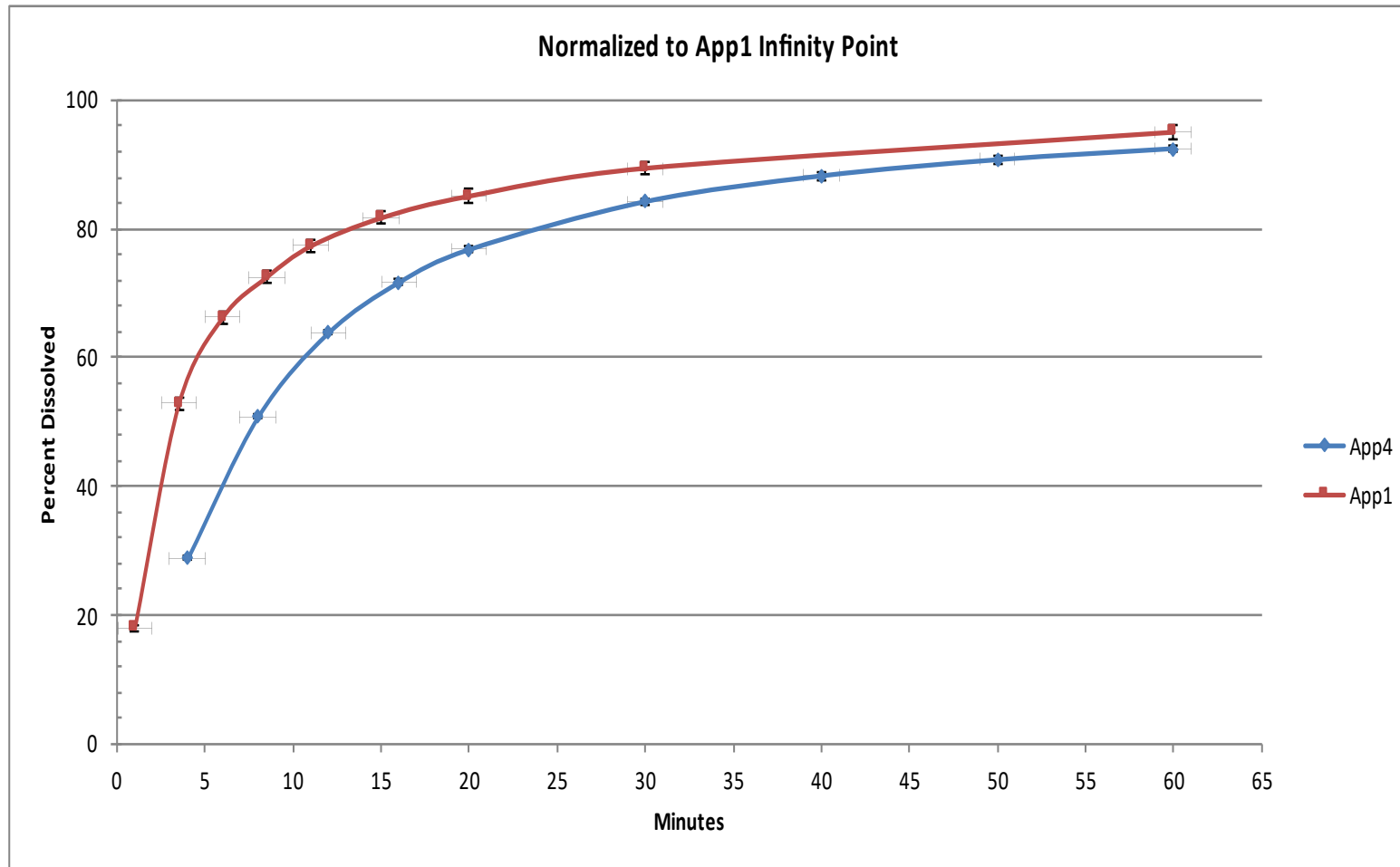
- $f_1 = 15.6$
- $f_2 = 68.6$
- Result = Not Equivalent*

Results – Traditional Portioned Moist Snuff Product 1



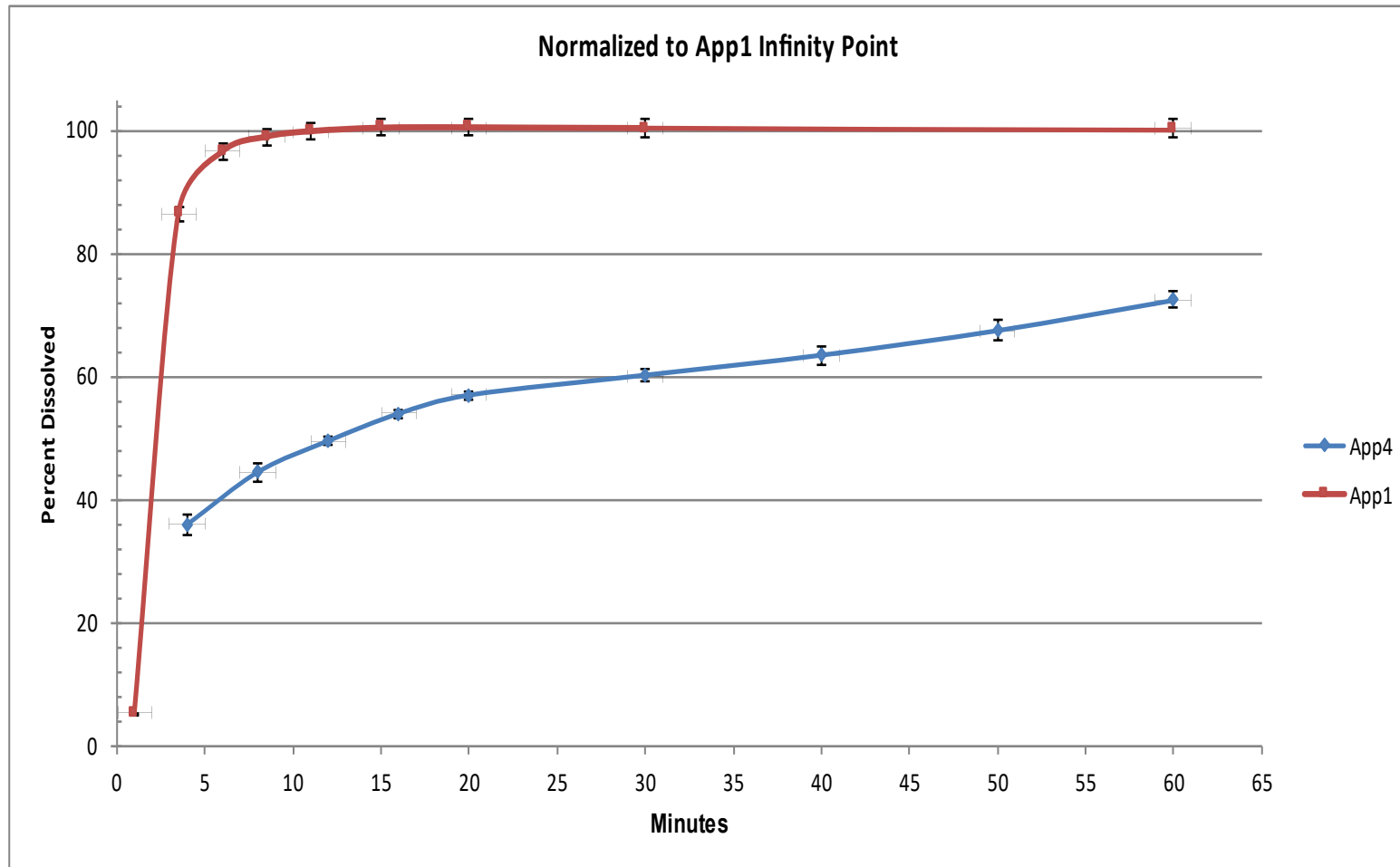
- $f_1 = 12.8$
- $f_2 = 68.3$
- Result = Equivalent

Results – Traditional Portioned Moist Snuff Product 2



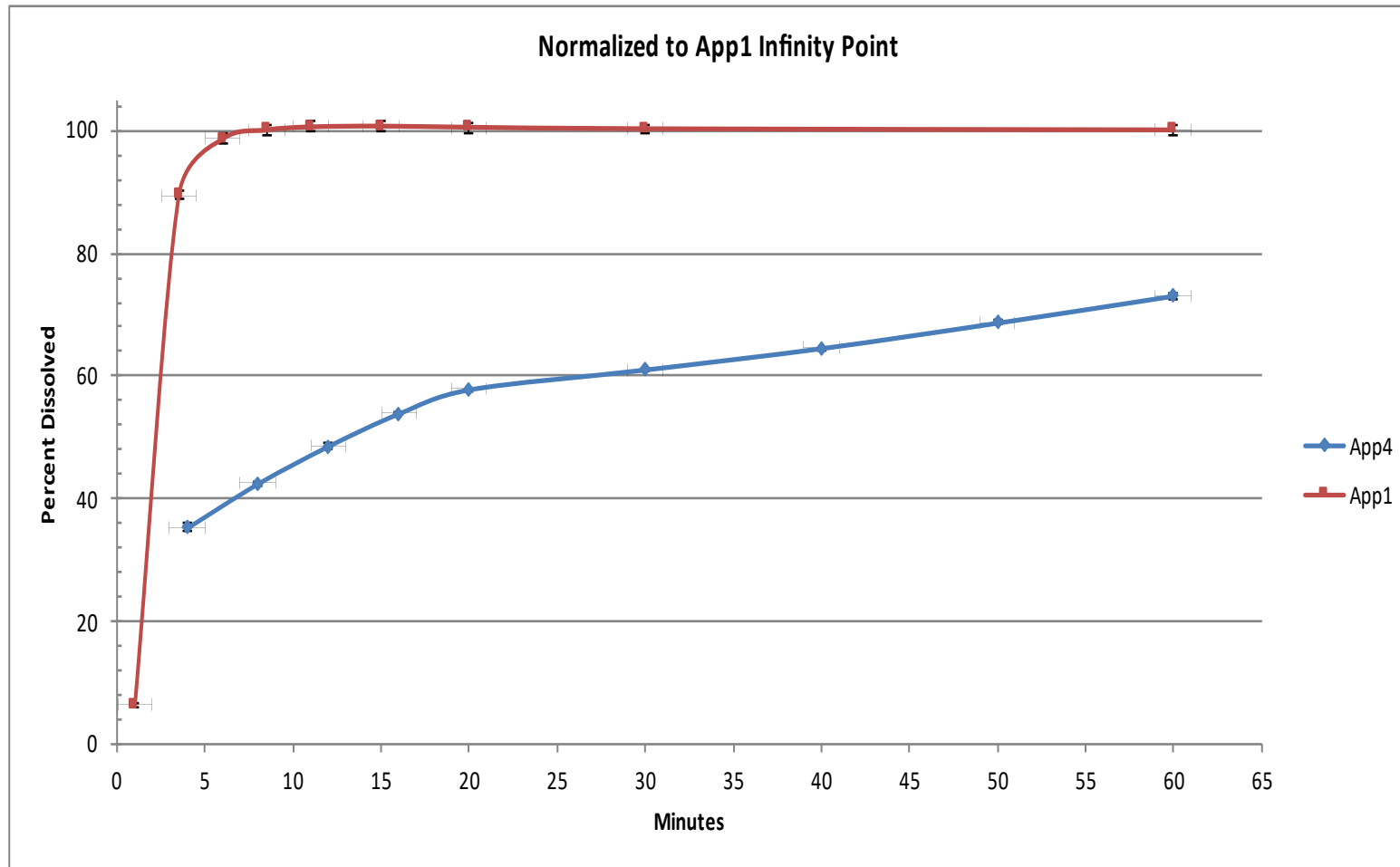
- $f_1 = 9.6$
- $f_2 = 73.8$
- Result = Equivalent

Results – Traditional Loose Moist Snuff Product 1



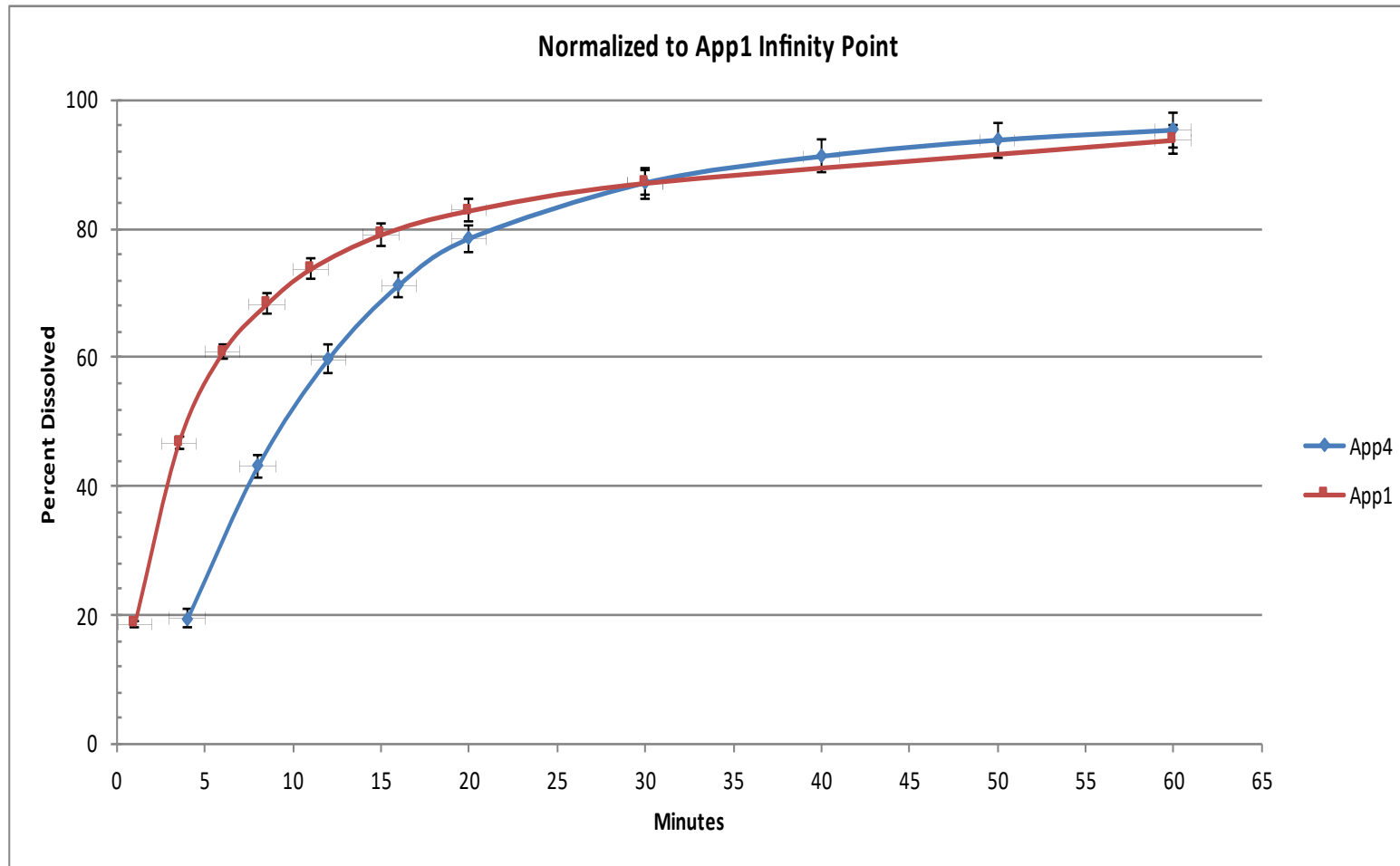
- $f_1 = 62.7$
- $f_2 = 45.8$
- Result = Not Equivalent

Results – Traditional Loose Moist Snuff Product 2



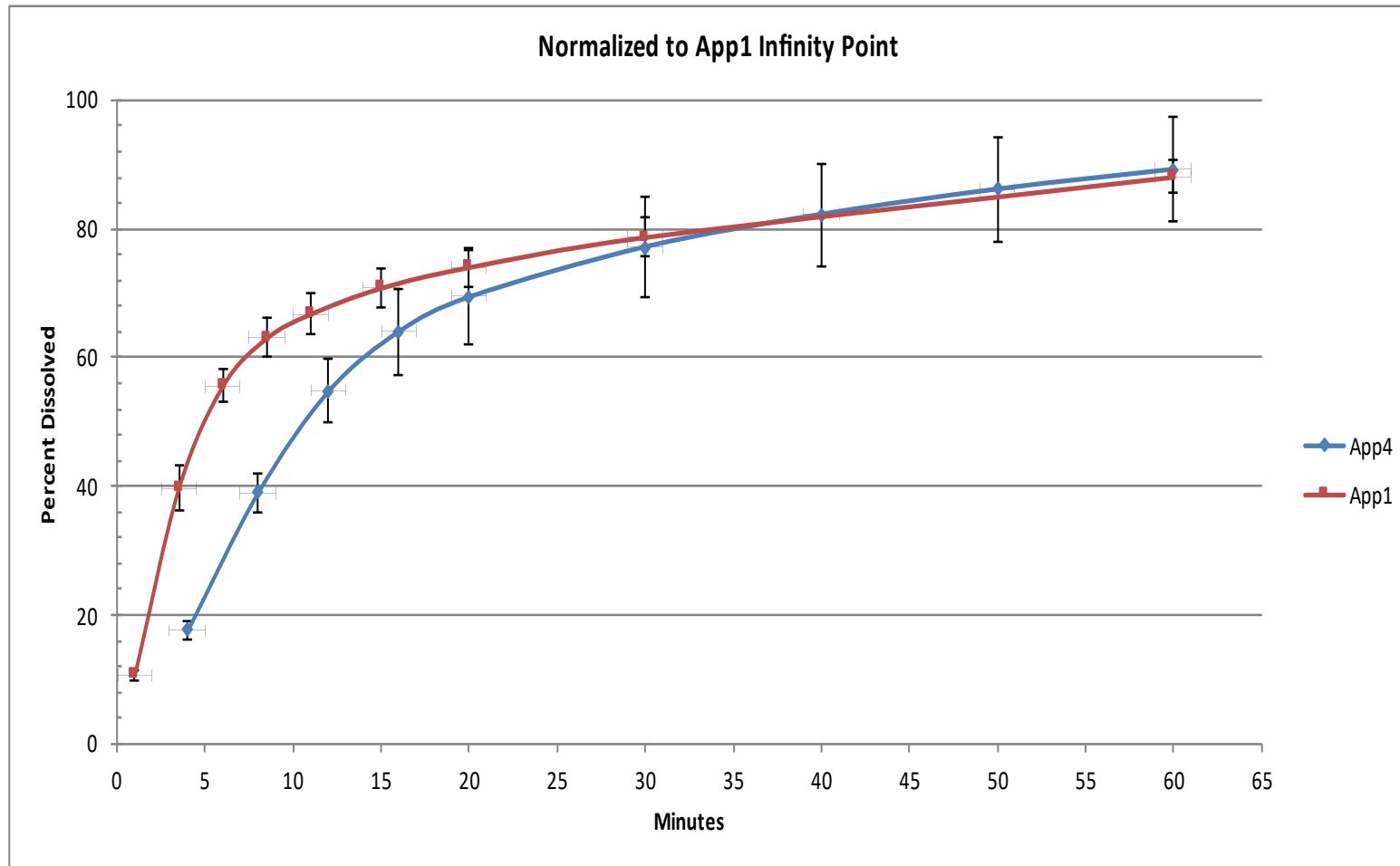
- $f_1 = 60.7$
- $f_2 = 45.8$
- Result = Not Equivalent

Results – TFNCPP 2 mg



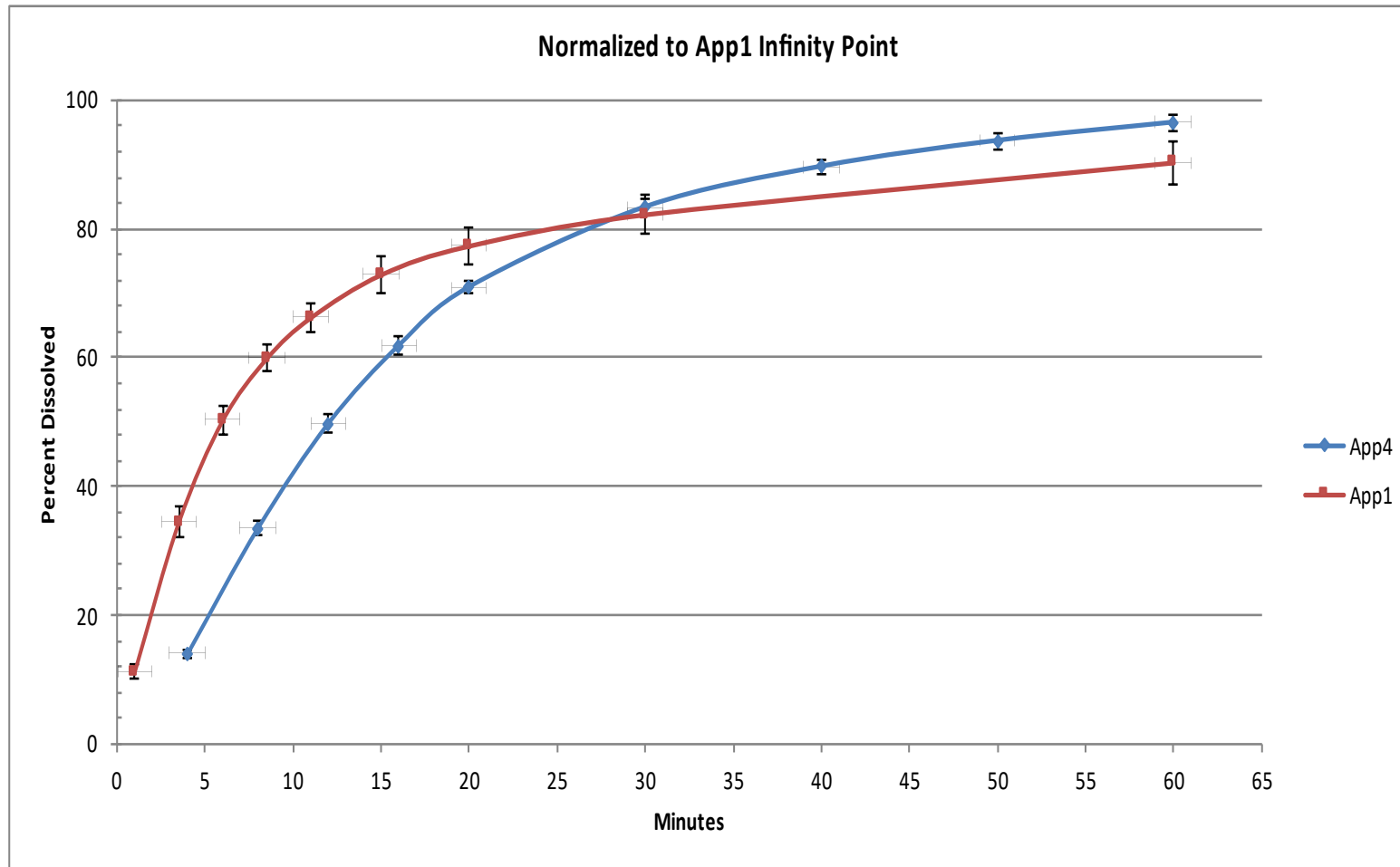
- $f_1 = 2.4$
- $f_2 = 94.2$
- Result = Equivalent

Results – TFNCPP 4 mg



- $f_1 = 5.7$
- $f_2 = 85.6$
- Result = Equivalent

Results – TFNCPP 7 mg



- $f_1 = 6.7$
- $f_2 = 79.9$
- Result = Equivalent

Conclusions

1

USP1 and USP4 are equivalent for 1 g Snus products, Traditional Portioned Moist Snuff Products and TFNCP.

2

Additional testing is required to determine if USP4 and USP1 are equivalent for 400 mg Snus Products

3

USP1 and USP4 are not equivalent for Traditional Loose Moist Snuff Products

4

Either Apparatus is suitable for determination of nicotine release rates in any of the matrices included in this study.



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