NICOTINE DISSOLUTION IN SMOKELESS TOBACCO PRODUCTS

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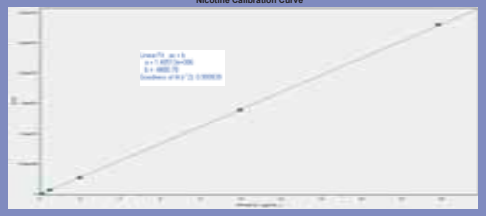
ABSTRACT

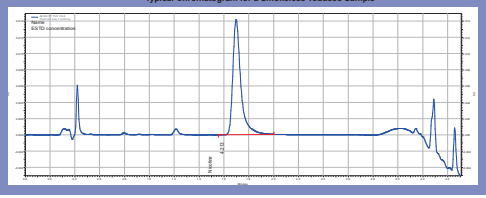
Tobacco products contain nicotine, understanding the bioavailability and absorption of nicotine through oral cavity is important. We developed and validated a method for nicotine dissolution in smokeless tobacco products. Nicotine dissolution is carried out in a dissolution media buffer on a dissolution apparatus 1, which is coupled to an automated sampling station using 8-channel filter plate for sample filtration and direct collection into autosampler vials. Dissolved nicotine at different time points are subsequently analyzed using a Waters Acquity UPLC with TUV detector. The separation uses Waters Atlantis T3 column with 3 µm particle size, 50 mm length x 3.0 mm diameter under isocratic elution. This method is validated to quantitate at an LOQ of 0.1µg/mL for nicotine. The profiles of nicotine dissolution are presented for pouched and loose smokeless products. Both the rate of dissolution (profiles) and final level of dissolved nicotine are determined.

Calibrations, Chromatograms

Nicotine Calibration Curve



Typical Chromatogram for a Smokeless Tobacco Sample



ANALYTICAL FIGURES OF MERIT

Sample Matrix	Pouch 1	Pouch 2	Loose Tobacco
Accuracy	104.3%	90.9%	94.5%
Precision	95.8%	94.6%	99.7%
Standard Stability	1 month at ~6°C	1 month at ~6°C	1 month at ~6°C
Sample Stability	7 days at ~6°C	7 days at ~6°C	7 days at ~6°C
Linearity (R^2)	0.999		
Range	0.1 ~ 20 µg/mL		
LOD	0.03 µg/mL		
LOQ	0.1 µg/mL		

Method

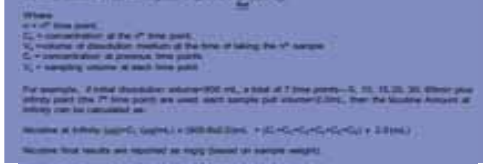
Dissolution Conditions

Parameter	Setting
Apparatus	*Dissolution Apparatus 1 with baskets; Automatic Sampling Station*
Paddle Speed	15 RPM and 250RPM after Infinity
Temperature	37.0±0.5°C
Medium	Ammonium Phosphate at pH 7.4
Dissolution Volume	900 mL
Automated Sampling Time Points	5min, 10min, 15min, 20min, 30min, 60min, 30min @ 250RPM (infinity)
Sample Pull Volume	2 mL
Filter	0.45 µm Nylon membrane

UPLC/UV Parameters

Parameter	Setting
Column	Atlantis T3, 3 µm, 3.0x50mm
Column Temperature	35±1°C
Mobile Phase A	60:40 Phosphate Buffer (25mM, pH 7.2) : MeOH
Mobile Phase B	MeOH
Sample Temperature	6±4°C
Flow Rate	0.75 mL/min
Injection Volume	10 µL
HPLC Mode	Isocratic Elution
Run Time	7.0 min
Wavelength (UV)	259 nm

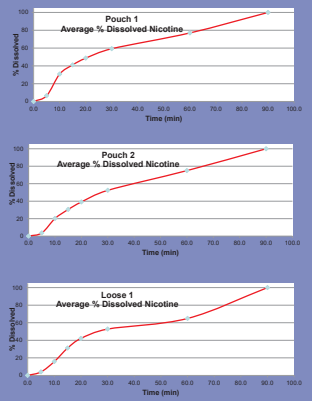
Nicotine Dissolution Calculations



Nicotine Dissolution Profiles

Sample	Time Point (min)	Avg Disso Amt (mg/g)	%Disso at EachTime Point*
Pouch 1	5.0	0.3502	6.5%
	10.0	1.6614	30.8%
	15.0	2.2157	41.1%
	20.0	2.6217	48.7%
	30.0	3.1939	59.3%
	60.0	4.1486	77.0%
	90.0	5.3875	100.0%
Pouch 2	5.0	0.3547	3.6%
	10.0	1.9675	20.2%
	15.0	2.9727	30.5%
	20.0	3.8121	39.1%
	30.0	5.1115	52.4%
	60.0	7.3137	75.0%
	90.0	9.7508	100.0%
Loose 1	5.0	0.5117	4.0%
	10.0	2.0217	15.8%
	15.0	3.9977	31.2%
	20.0	5.4043	42.1%
	30.0	6.7677	52.8%
	60.0	8.3210	64.9%
	90.0	12.8234	100.0%

*Normalized to Infinity at 90 min



CONCLUSIONS

We have developed and validated a dissolution testing method for the quantitation of nicotine in pouched and loose smokeless tobacco products. The method is proven to be linear, precise, accurate, specific, and robust. Compared to the pouched products, the loose tobacco samples generally show slightly slower % dissolution rate in the earlier stage, but higher nicotine results at infinity. This method provides us a new and unique tool in evaluating nicotine dissolution profiles for different smokeless products.

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