Determination of Nicotine in OTDN and Liquid Products by UV-Vis Spectrophotometry

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Introduction

Nicotine Pouch Products

- Non-combustible oral tobacco products currently available in the market
- Tobacco-leaf-free pouches, containing either synthetic or tobacco-derived nicotine
- They come in various nicotine levels and flavors

Quantitative Analysis of Nicotine in OTDN Pouch Products

- Various methods have been developed: GC-FID, GC-MS, UPLC-MS/MS, UPLC-UV
- CORESTA standardized method (CRM #62): GC-FID
- Provide accurate and repeatable methods
- Require laboratory set-up and trained personnel

UV-Vis Spectrophotometry

- Easy-to-implement method
- Rapid analysis

CORESTA=Cooperation Centre for Scientific Research Relative to Tobacco; CRM=CORESTA recommended method; GC-FID=Gas chromatography flame ionization detector; GC-MS=Gas chromatography-mass spectrometry; OTDN=Oral tobacco-derived nicotine; UPLC-MS/MS=ultra-performance liquid chromatography-tandem mass spectrometry; UPLC-UV=ultra-performance liquid chromatography ultraviolet detection; UV=ultraviolet; UV-Vis=ultraviolet-visible spectroscopy.

CRM. No. 62, Determination of Nicotine in Tobacco and Tobacco Products by Gas Chromatographic Analysis, 3rd ed; CORESTA, 2021.

Objective

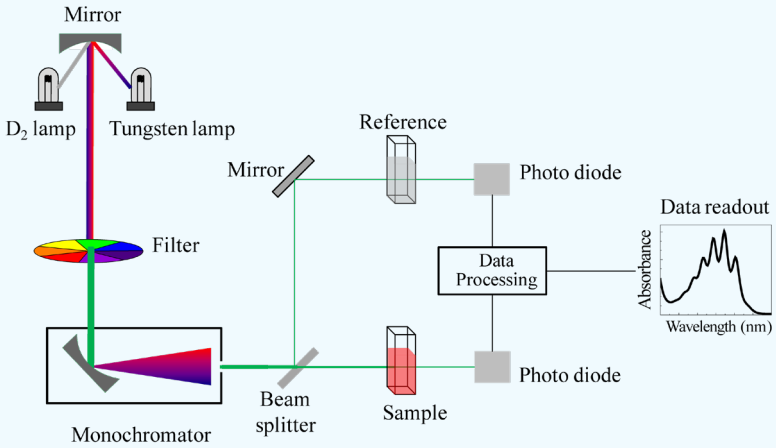
Simple and Rapid Method for Determination of Nicotine

	GC-FID Method	UV-Vis Method
Proximity	Offline analysis in the contract laboratories	Atline analysis at the manufacturing site
Solvent Preparation	Acid/Base, Organic standard reagents	Type 1 water, Acetonitrile (ACN)/Type 1 water
Nicotine Extraction Time[1]	2 hours	1 minute (Extraction) + 5 minutes (Centrifuge)
Run Time[2]	~12 minutes	~1 minute (2 – 3 min between measurement)
Consumables	• Column • Carrier gases • GC autosampler syringes • Inlet septa • Inlet liner • O-ring for inlet liner • GC vials and caps • Gas-tight syringes	• Quartz cuvettes • Deuterium UV lamp • Tungsten Vis lamp

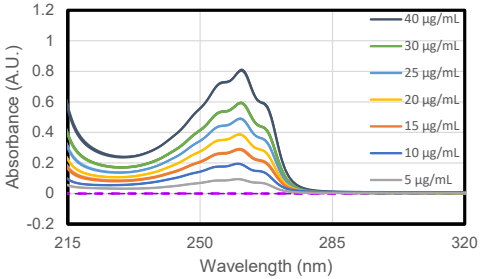
OTDN=Oral tobacco-derived nicotine; UV-Vis=ultraviolet-visible spectroscopy.
[1]Nicotine Extraction time for OTDN pouch product.
[2]Measurement run time per sample.

Nicotine UV Absorption and Background Interference

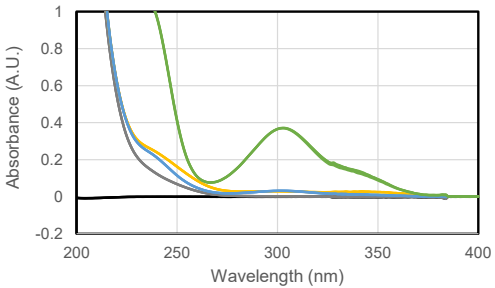
Double Beam UV-Vis Spectrophotometer



Standard Solutions



0mg Fillers (10:90 ACN Reference)



Nicotine UV Absorption

260 nm

Background Interference of Flavor

0 mg fillers were extracted by Type 1 water	Diluted by 10:90 ACN/ Type 1 water	10% ACN was used as reference

ACN=acetonitrile; UV-Vis=ultraviolet-visible spectroscopy.

Method Requirement

Use proper reference

- To provide flavor-specific baseline
- To minimize the background interference
- 0 mg nicotine filler/solution with the same flavor as the sample

Validation

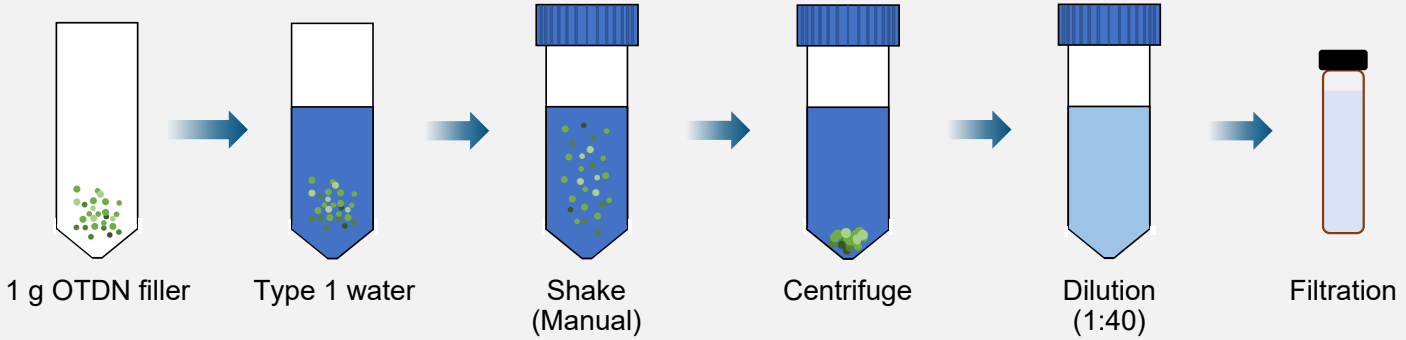
- Linearity
- Accuracy
- Precision/Intermediate Precision
- Robustness
- Stability

Sample		Nicotine Strength	Reference
OTDN Pouch	Original	2 mg	0 mg nicotine filler, No flavor
		4 mg	
		8 mg	
	Citrus	2 mg	0 mg nicotine filler, Citrus
		4 mg	
		8 mg	
	Mint	2 mg	0 mg nicotine filler, Mint
		4 mg	
		8 mg	
	Wintergreen	2 mg	0 mg nicotine filler, Wintergreen
		4 mg	
		8 mg	
Nicotine Solution		40 wt%	0 mg nicotine blank solution

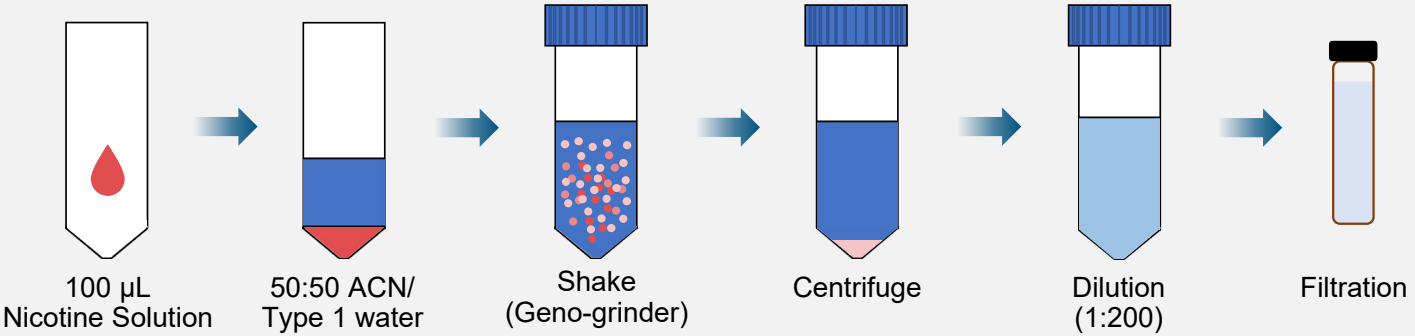
OTDN=Oral tobacco-derived nicotine.

Nicotine Extraction Method

OTDN Pouch Product Extraction

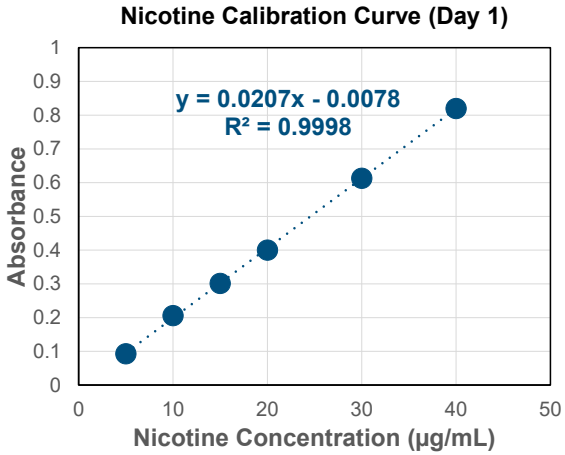


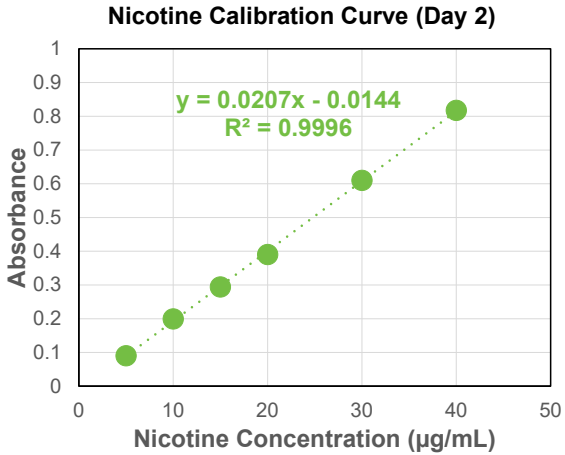
Nicotine Solution Extraction

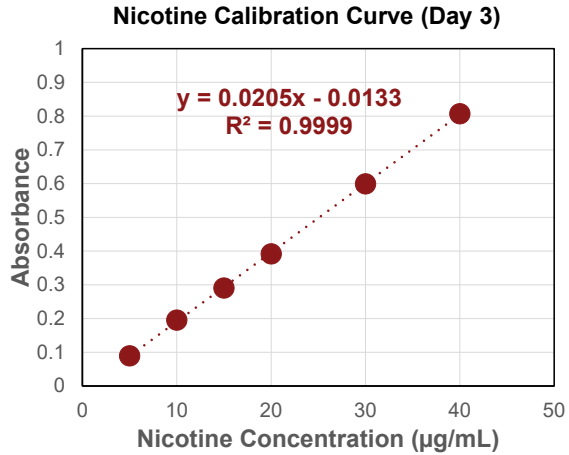


OTDN=Oral tobacco-derived nicotine.

Method Linearity







Evaluation of linear relationship: UV absorption at 260 nm as a function of nicotine concentration

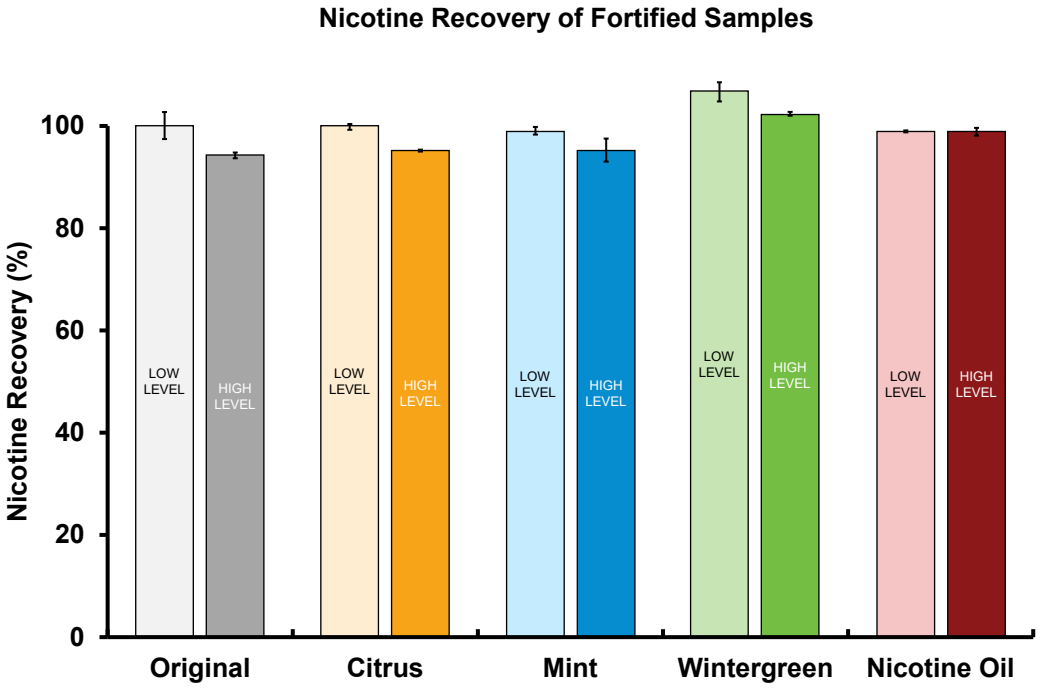
Range: 5 µg/mL - 40 µg/mL

$R^2 \geq 0.999$

Percent deviation from the theoretical value (%Dev) ≤ 3.4%

UV=ultraviolet.

Method Accuracy



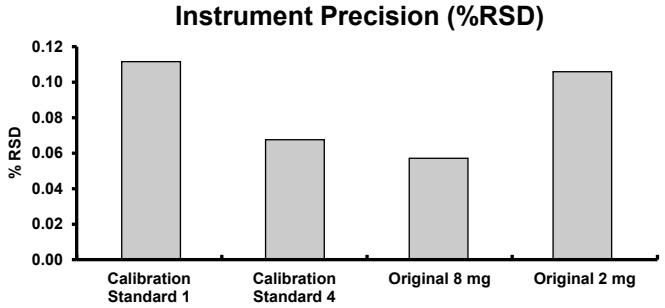
- **Fortification of samples for %Recovery of Nicotine**
  - 0 mg fillers of Original, Citrus, Mint, and Wintergreen flavor
  - 0 mg nicotine solution
  - High- and low-level fortification

	Low Level Fortification	High Level Fortification
OTDN Filler	18 mg/g	36 mg/g
Nicotine Solution	20 mg/g	50 mg/g

- **0 mg nicotine filler of each flavor was utilized as reference**
- **n = 3**
- **%Recovery: 95.2-107.7**

OTDN=Oral tobacco-derived nicotine.

Method Precision and Repeatability



Instrument Precision (n = 6, %RSD ≤ 0.12)

6 replicate spectra from 1 vial of each sample

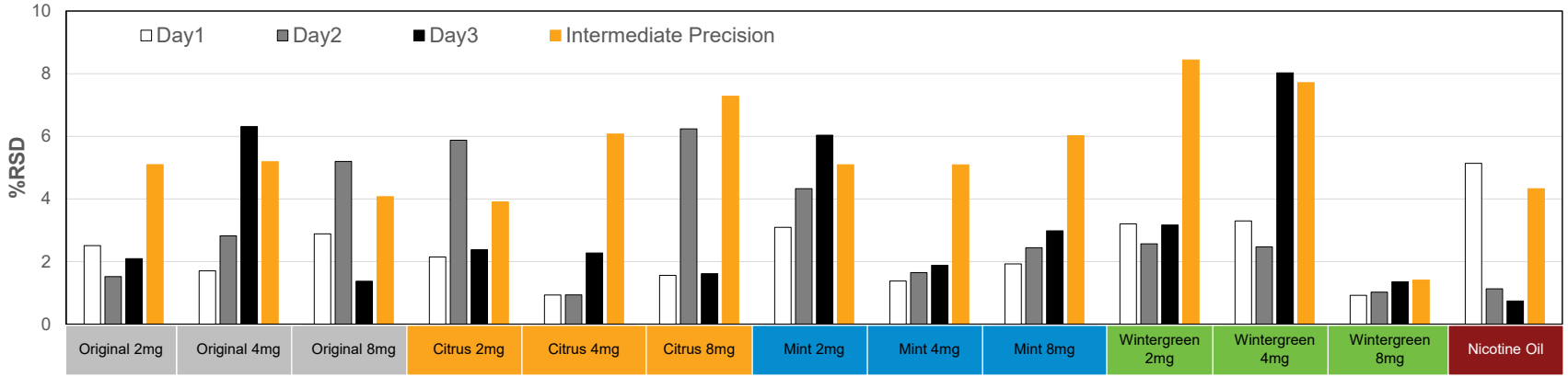
Repeatability (n = 6, %RSD ≤ 8.1)

6 replicate extractions of each sample in one day

Intermediate Precision (n = 18, %RSD ≤ 8.5)

6 replicate extractions of each sample

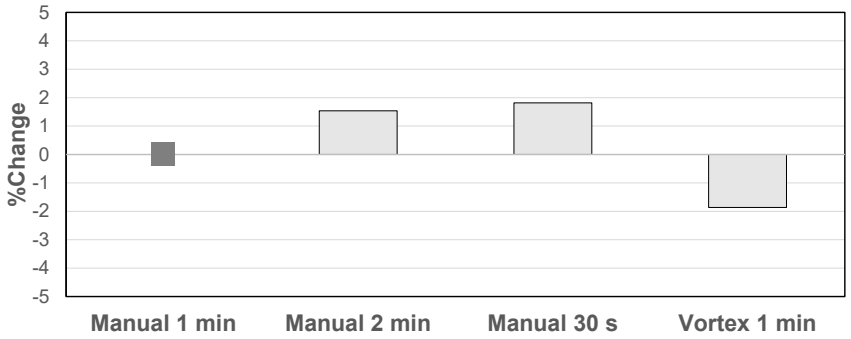
Analyzed over 3 days by 2 analysts



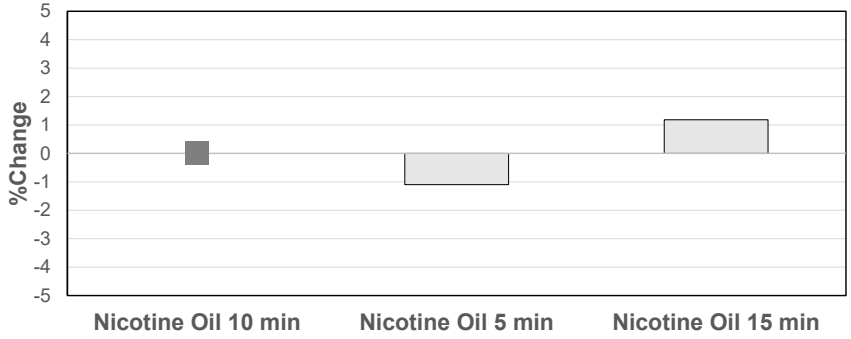
RSD=relative standard deviation.

Robustness

OTDN Pouch Product Extraction
(Time/Device)



Nicotine Solution Extraction
(Time)

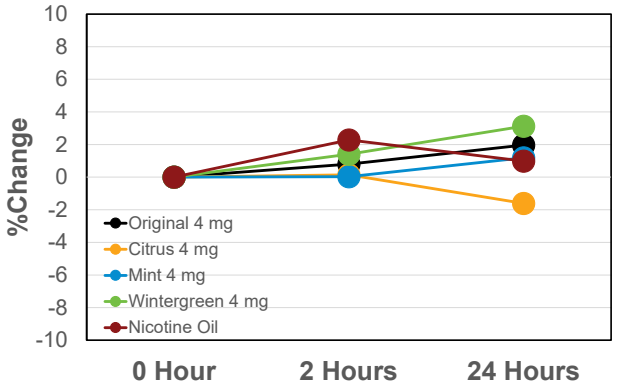


Standard extraction	
Manual shaking for 1 min	**Geno-grinder for 10 min**
Extraction method robustness (% Change)	
≤ 1.9%	**≤ 1.2%**

OTDN=Oral tobacco-derived nicotine.

Sample Extract Stability

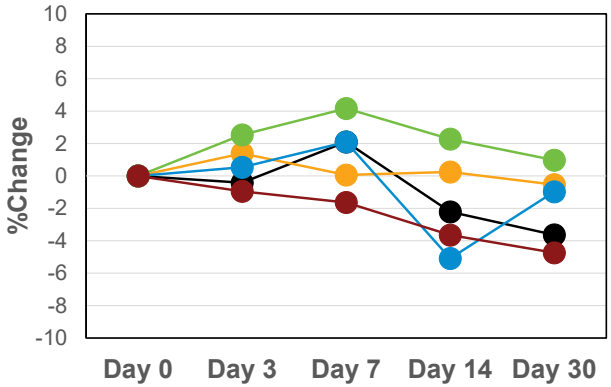
Short-term Stability



- Centrifuged sample stored in the extraction vessels at ambient temperature

% Change ≤ 3.2%

Final Prepared Sample Stability



- Final prepared sample stored under refrigerated (4 ± 3 °C) conditions
- Reference blank with the same age was employed as the reference for data collection

% Change ≤ 5.1%

Conclusion

UV-Vis spectrometry demonstrated an **efficient method for nicotine quantification**

- **Matrix interference due to flavors**
  can affect the nicotine quantification
- **Proper reference is required**
  the same flavor as the sample for each measurement
- **Successful evaluation of analytical method validation parameters**
  - Linearity
  - Accuracy
  - Instrument Precision/Precision/Intermediate Precision
  - Robustness
  - Stability
- **Simple nicotine analysis**
  for routine testing and quick assessment during product development

UV-Vis=ultraviolet-visible spectroscopy..

Q&A