Modified QuEChERS Method for the Extraction of Nicotine from Oral Traditional and Innovative Tobacco Products using UPLC-MS/MS

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Introduction

- The tobacco industry continues to embrace tobacco harm reduction
  - As a result, an array of innovative potential reduced-risk products have emerged as alternatives to cigarette smoking

- The wide range of innovative smoke-free products consist of varying matrix types
  - Different and new matrices have led to the development and validation of matrix-specific analytical methods for the quantitation of nicotine content
  - Identified the need for the development of a single analytical method that both extracts and accurately quantitates nicotine in a multitude of differing matrices

Aldeek, F.; Sarkar, M.A. Method Development and Applications for Reduced-Risk Products. Separations 2022, 9, 78.

Gottlieb, S.; Zeller, M. A Nicotine-Focused Framework for Public Health. N. Engl. J. Med. 2017, 377, 1111–1114.

Hatsukami, D.K.; Joseph, A.M.; Lesage, M.; Jensen, J.; Murphy, S.E.; Pentel, P.R.; Kotlyar, M.; Borgida, E.; Le, C.; Hecht, S.S. Developing the science base for reducing tobacco harm. Nicotine Tob. Res. 2007, 9, 537–553.

Zeller, M.; Hatsukami, D. The Strategic Dialogue on Tobacco Harm Reduction: A vision and blueprint for action in the US. Tob. Control 2009, 18, 324–332.

Study Overview

- Liquid-Liquid Extraction and UPLC-MS/MS Method
  - Employed a modified QuEChERS* extraction technique using sodium hydroxide and acetonitrile
  - Used NaCl to improve phase separation
  - Leveraged isotopically labeled internal standard (Nicotine-Methyl-d_3) using UPLC-MS/MS

- Used varying commercially-available Moist Smokeless Tobacco (MST), Oral Tobacco-Derived Nicotine (OTDN) products
  - White pouches
  - Pouched MST
  - Loose MST
  - Gums
  - Lozenges

*QuEChERS: Quick, Easy, Cheap, Effective, Rugged & Safe

S. J. Lehotay et al., J. AOAC. Int. 2003, 86, 412.

S. J. Lehotay et al., J. AOAC. Int. 2002, 85, 1177

Product Matrix Range

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QuEChERS Extraction Process

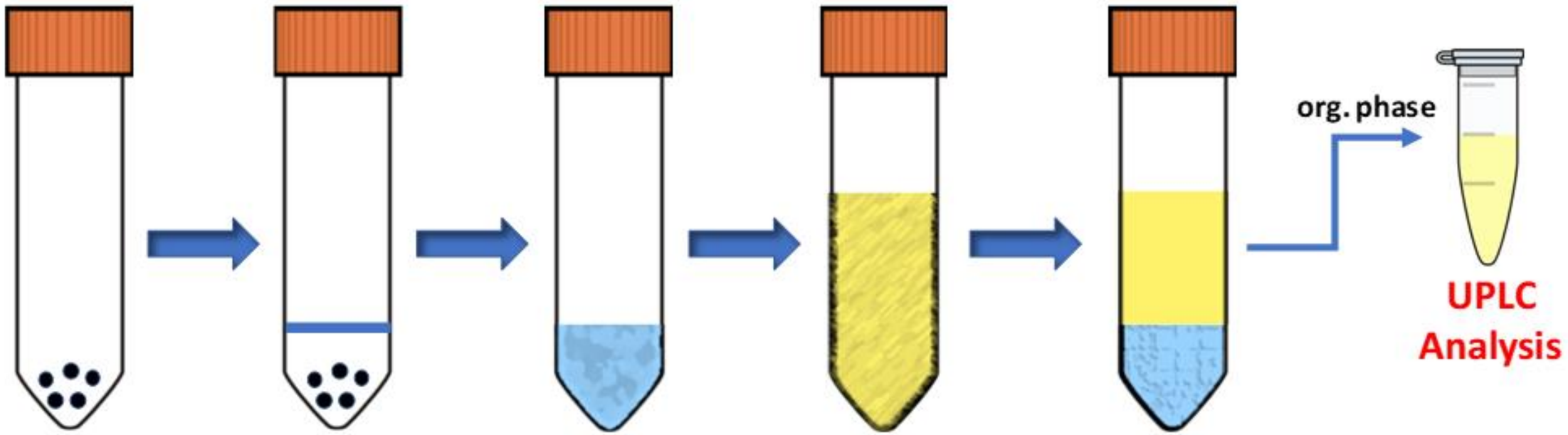
a) Cut/Weigh sample

b) Add 15 mL NaOH (2M)
c) Add 100 µL Nicotine-d_3 (10 mg/mL)

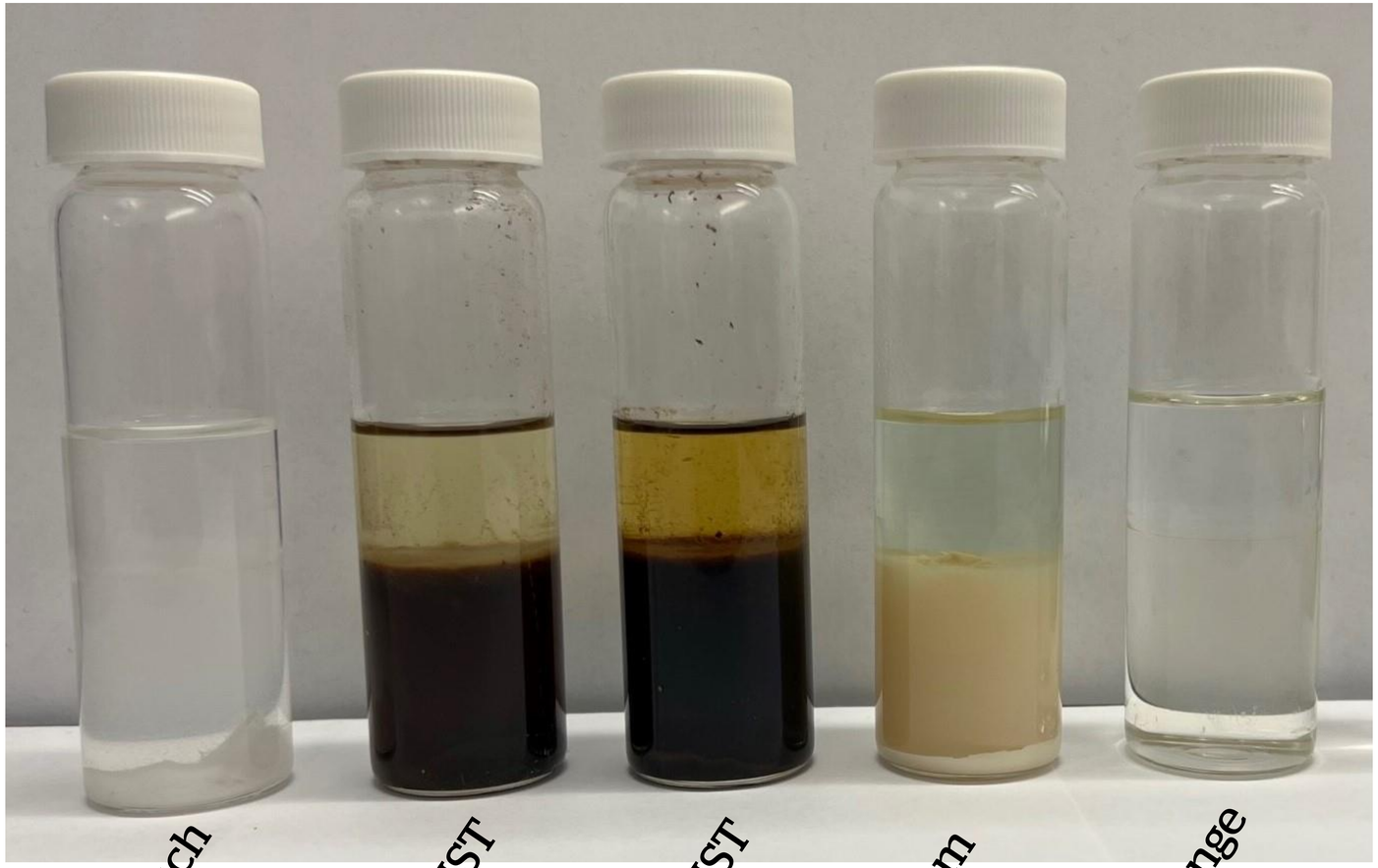
d) Shake (10 min, 1500 RPM)

e) Add 10 mL Acetonitrile
f) Add 1 g NaCl
g) Shake (10 min, 1500 RPM)

h) Centrifuge
i) Dilute/Filter



Example Image of Final Extracted Samples



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Analytical Method

Instrument: Waters Acquity I-Class UPLC coupled to Xevo triple-quadropole mass spectrometry

Column: Acquity BEH C18, 2.1 x 50 mm, 2.5 µm, Waters Corporation

Guard Column: Acquity BEH C18 VanGuard Pre-column, 2.1 x 5 mm, 1.7 µm, Waters Corporation

UPLC Parameter	Setting
Run Time	5.0 min
Injection Volume	1 µL
Autosampler Temperature	10 °C
Column Temperature	45 °C
Mobile Phase A	10 mM Ammonium Acetate, pH 10
Mobile Phase B	Acetonitrile
Pump Program	Gradient Elution
Flow Rate	0.4 mL/min
Ionization	Positive ESI

Compound	Quantitation Trace (m/z)
Nicotine	163.05 > 130.0
Nicotine-Methyl-d_3	166.05 > 132.0

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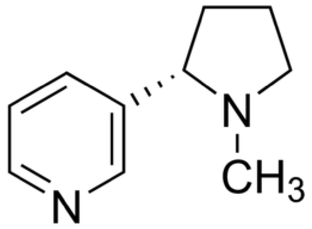
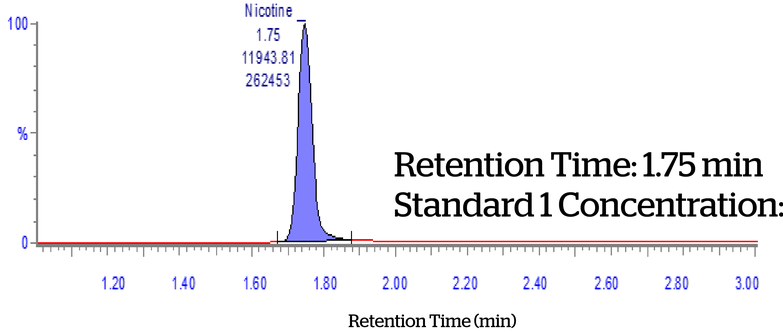
Example Chromatograms for a Calibration Standard and Sample

Nicotine

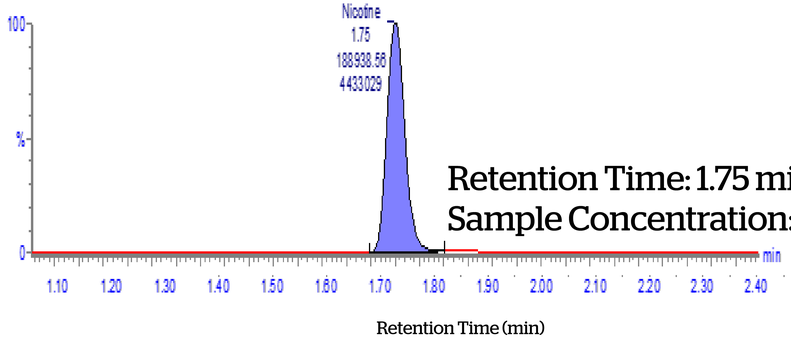


Standard 1



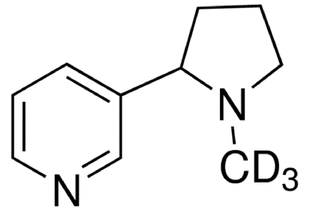
Retention Time: 1.75 min
Standard 1 Concentration: 0.50 µg/mL

Gum Sample

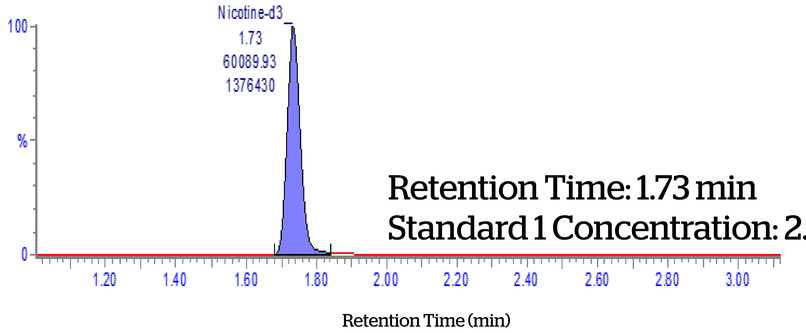


Retention Time: 1.75 min
Sample Concentration: 15.0 µg/mL

Nicotine-methyl-d_3

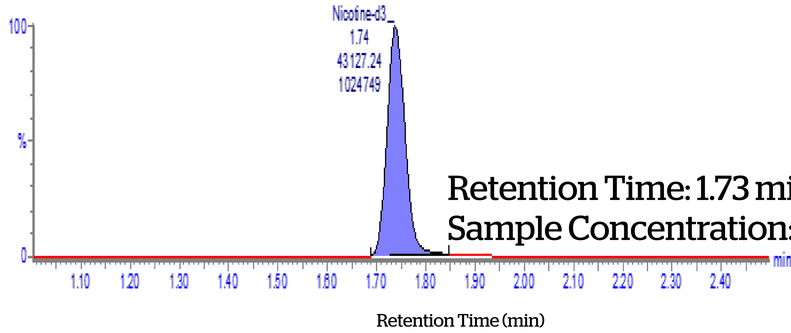


Standard 1



Retention Time: 1.73 min
Standard 1 Concentration: 2.50 µg/mL

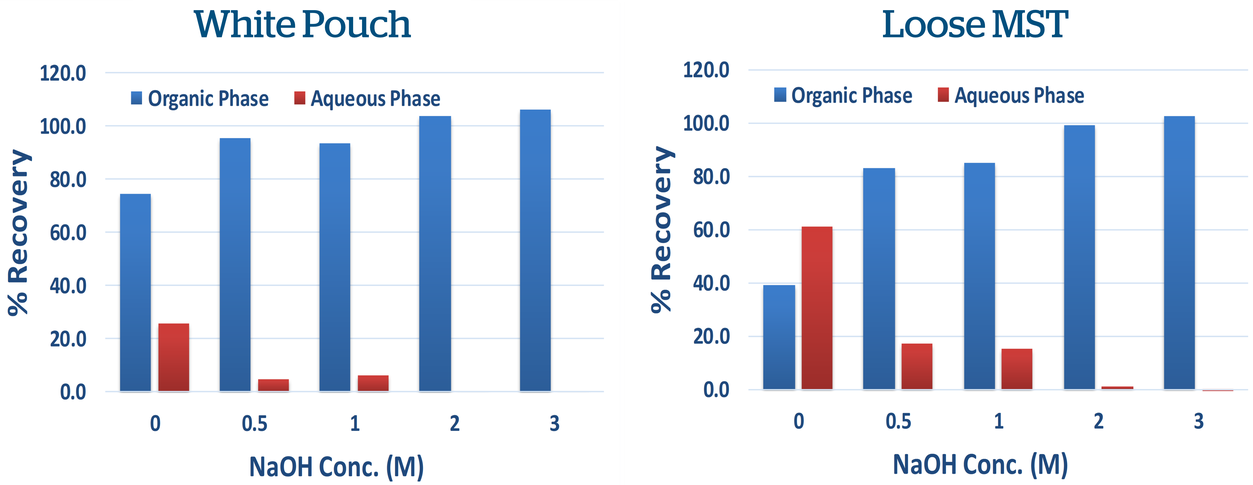
Gum Sample



Retention Time: 1.73 min
Sample Concentration: 2.50 µg/mL

Method Optimization and Robustness

- Evaluated several parameters during development to optimize the method and maximize nicotine recovery
  - Sodium hydroxide concentration (0M - 3M)
    - Higher recoveries using 2M – 3M NaOH
  - Salt type (NaCl, $NaSO_4$, $MgSO_4$)
  - Reversing solvent addition



- Performed robustness experiments during method validation
  - Extraction volume was varied: 5, 10, and 20 milliliters
  - Extraction time was varied: 5, 10, and 15 minutes
  - Final extract filtration/dilution was performed at 0-, 2-, and 4-hours post-extraction at room temperature
  - Robustness experiments demonstrated comparable results for all variables

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Method Validation

Validation Parameter	Outcome
Calibration (0.50-40 µg/mL)	▪ R^2 > 0.997 on all days ▪ %Dev < 4%
Accuracy – One fortification level in triplicate for each product type	89.7% – 107% recovery
Repeatability (Intra-day precision, n=6)	< 7.0 % RSD
Intermediate Precision (Inter-days precision, n=18)	< 7.2 % RSD
Specificity	No interferences observed at the retention time of nicotine or internal standard
Limit of Quantitation (LOQ)	0.50 µg/mL
Stability – samples in extraction vessels and final filtered samples	Stable for up to seven days when stored in refrigerated conditions (0-4°C)

Note: This table summarizes validation results for all five matrices tested

Accuracy Experiment Results

Matrix	Fortification Level (µg/mL)	Mean % Recovery	% RSD (n=3)
White Pouch	7.53	99.8	0.90
Pouched MST	5.02	89.7	4.05
Loose MST	5.02	106	2.32
Gum	14.8	98.6	3.04
Lozenge	5.02	107	1.35

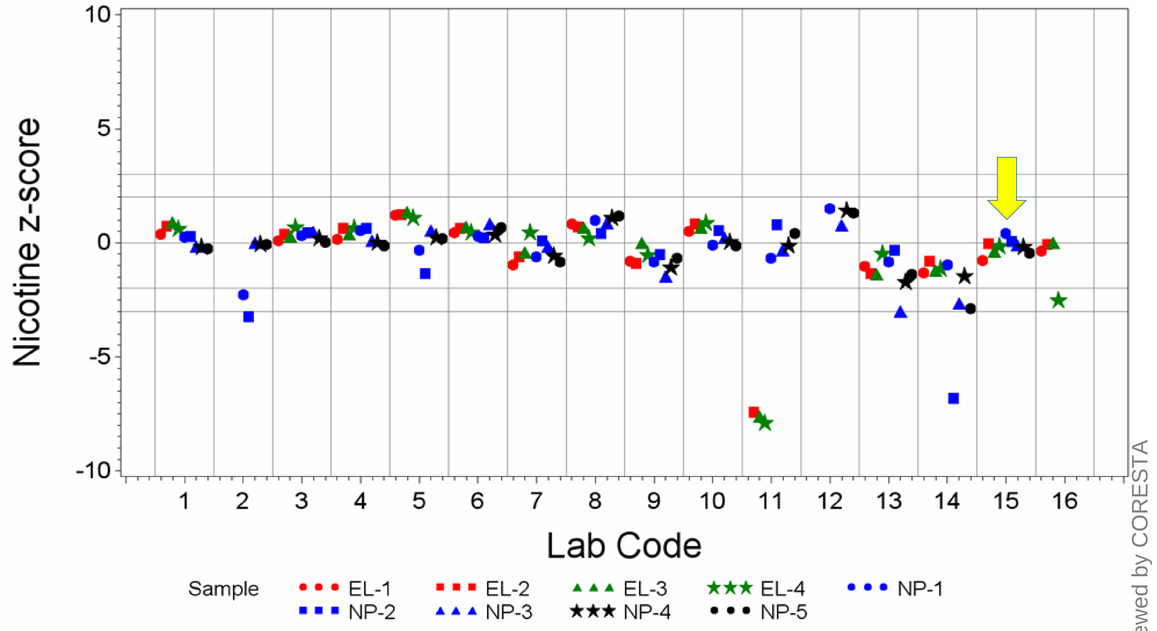
Note: Fortification levels were chosen with the intent to span the nicotine range for all products

Repeatability and Intermediate Precision

Matrix (Target Nicotine, mg/g)	Day 1, (n=6) Mean, mg/g (%RSD)	Day 2 (n=6) Mean, mg/g (%RSD)	Day 3 (n=6) Mean, mg/g (%RSD)	3-Day (n=18) Mean, mg/g (%RSD)
White Pouch (7.30)	7.60 (1.10)	7.80 (1.04)	6.90 (2.23)	7.40 (5.53)
Pouched MST (8.00)	7.50 (6.95)	7.70 (4.51)	8.60 (4.91)	7.90 (7.18)
Loose MST (11.0)	11.0 (1.31)	9.90 (0.80)	11.0 (1.88)	10.0 (4.57)
Gum (2.40)	2.40 (1.15)	2.50 (1.64)	2.50 (2.10)	2.50 (2.47)
Lozenge (1.00)	1.00 (1.59)	1.00 (1.62)	1.00 (0.80)	1.00 (3.93)

CORESTA Proficiency Study 2020

- ALCS Participated in the 2020 Nicotine and Nicotine Degradants CORESTA Proficiency Study
  - Nineteen labs participated in the nicotine analysis study; using different extraction and/or analytical methods
  - Four e-liquid and five white pouch products submitted for testing
  - Results for the QuEChERS analytical method were submitted as Lab # 15
  - QuEChERS' z-scores ranged from –0.84 to 0.31 for pouch and e-liquid products
  - Acceptance criteria: $|z| \leq 2$



Tobacco and Tobacco Products Analytes Sub-Group. "2020 Nicotine and Nicotine Degradants Proficiency Study." Coresta, September 2020, www.coresta.org/sites/default/files/technical_documents/main/TTPA-246-1-CTR_2020-ProfStudy-Nicotine-and-Nicotine-Degradants_Sept2020

Our QuEChERS method demonstrated comparable performance for both matrices

Conclusion

- We developed a single extraction procedure and analytical method that can quantitate nicotine for a wide range of tobacco matrices
  - Reduced extraction and analysis time
  - Easy to implement and maintain
  - Has a potential for standardization

- For all five matrices evaluated:
  - Accuracy results were within 89.7% - 107% Recovery
  - Repeatability (n=6) over three days, < 7.0 % RSD
  - Intermediate precision (n=18), < 7.2 %RSD

- The method was validated internally, following International Council on Harmonization (ICH) guidelines

Thank you!

Questions?

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