Quantification of Nicotine Related Impurities in Novel, Oral Tobacco-Derived Nicotine Products

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Abstract

VERVE® Discs and Chews are oral, non-dissolvable, tobacco-derived nicotine products. All tobacco products sold in the US are regulated by the FDA and this category of products will ultimately require a market authorization through the premarket tobacco application (PMTA) pathway. This regulatory pathway requires "Established shelf life of the product to include data establishing the stability of the product through the stated shelf life." The US and European Pharmacopeias recommend purity specifications for nicotine intended for pharmaceutical products; however, there are no official purity specifications recommended for the tobacco-derived nicotine added to novel tobacco products. We developed a sensitive, selective, and robust liquid chromatography-mass spectrometry (LC-MS) method utilizing an Agilent® 1260 HPLC/6150 Mass Spectrometer with a Waters® C18 column to quantify nicotine impurities. The method accuracy and precision was found to be acceptable at 88-103% and 1.6-6.7%, respectively. The Limit of Detection (LOD) was found to be 0.070 wt% of the nicotine concentration for VERVE® Discs and Chews which was sufficient to quantify nicotine-related impurities listed in Pharmacopeia guidelines. An overview of the challenges and solutions that transpired during method validation for these unique matrices is provided. In addition, we provide nicotine impurity results in VERVE® Discs and Chews and monitored these impurities over time. This selective and sensitive method provides data suitable for quantitative risk assessments and for stability studies.

Methodological Relevance

- In the absence of specific guidance from the FDA, we validated a method to measure 8 nicotine and nicotine-related impurities based on specifications in the European Pharmacopeia Council of Europe.[1]
- The U.S. Pharmacopeia (USP) recommends the measurement of nicotine degradants and impurities in nicotine intended for pharmaceuticals.[2]
- USP-grade nicotine has no single impurities greater than 0.3% (3 mg/g) and total impurities less than 0.8% (8 mg/g) on a weight percent basis relative to nicotine at the time of manufacture.
- An acceptance limit approach consistent with the International Conference on Harmonization (ICH) Guidance Q3B(R2) – Impurities in New Drug Products, July 2006 Revision 2, provides a reasonable and appropriate approach for evaluating nicotine stability.[3]
- Considering that nicotine-N´-oxide is a metabolite of nicotine and is also less acutely toxic than nicotine, we determine a higher threshold level of 3.0% for nicotine-N´-oxide as acceptable because it would not result in additional toxicological concern.
- 4% to 7% of metabolized nicotine is excreted as nicotine-N´-oxide [4,5] and nicotine-N´-oxide is a less acutely toxic metabolite compared to nicotine.[6,7]

Impurity	CAS#	Acceptance Limit	LOQ	LOD
Anabasine	532-12-7	0.5%	0.175	0.070
Anatabine	5746-86-1	0.5%	0.175	0.070
Nornicotine	2743-90-0	0.5%	0.175	0.070
β-Nicotyrine	13078-04-1	0.5%	0.175	0.070
Cotinine	487-19-4	0.5%	0.175	0.070
Myosmine	486-56-6	0.5%	0.175	0.070
Nicotine-N'Oxide	51095-86-4	0.5% at manufacture and 3.0% at end of shelf-life	0.175	0.070

All units are wt % relative to the nicotine concentration.

Methods

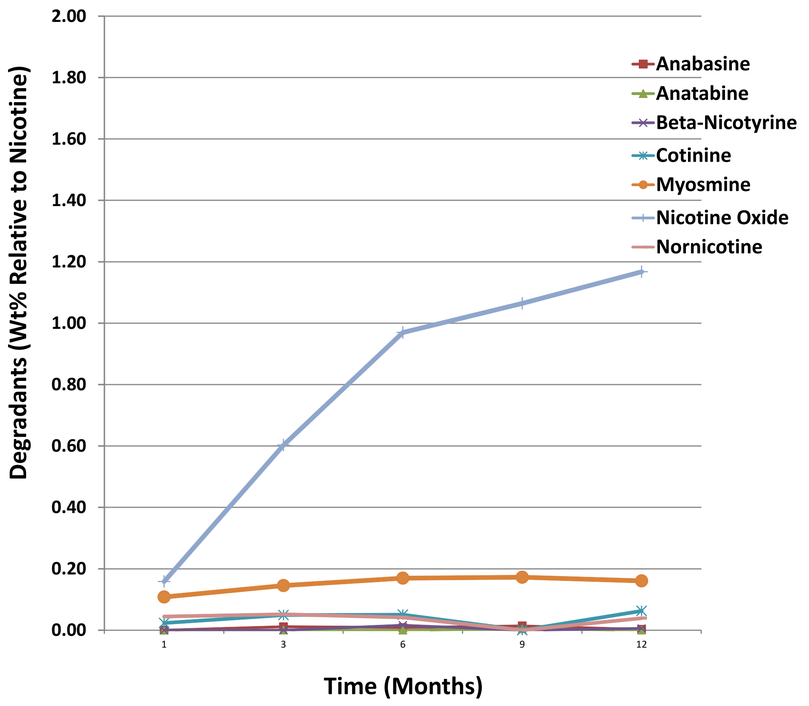
- VERVE® Discs are milled to <1.25 mm using a Wiley® Mill. The particle reduction is necessary in order to adequately extract the analytes of interest using solvents that are amenable to LC-MS analysis. An aliquot is extracted by vortexing for 1 hour in a methanol solution containing internal standard.
- VERVE® Chews are sonicated in water. This sonication step is necessary in order for the organic solvent to adequately disintegrate the polymer matrix and extract the analytes of interest. Methanol is then added along with internal standard before vortexing for 1 hour to extract. After centrifuging, an aliquot of the extracts is prepared for analysis.
- Extracts are analyzed on an Agilent® 1260/6150 LC/MS using a Waters® Xbridge™ BEH C18 column with caffeine as the internal standard.
- Nicotine degradants are reported in units of weight percent relative to the nicotine target value.

Validation Summary

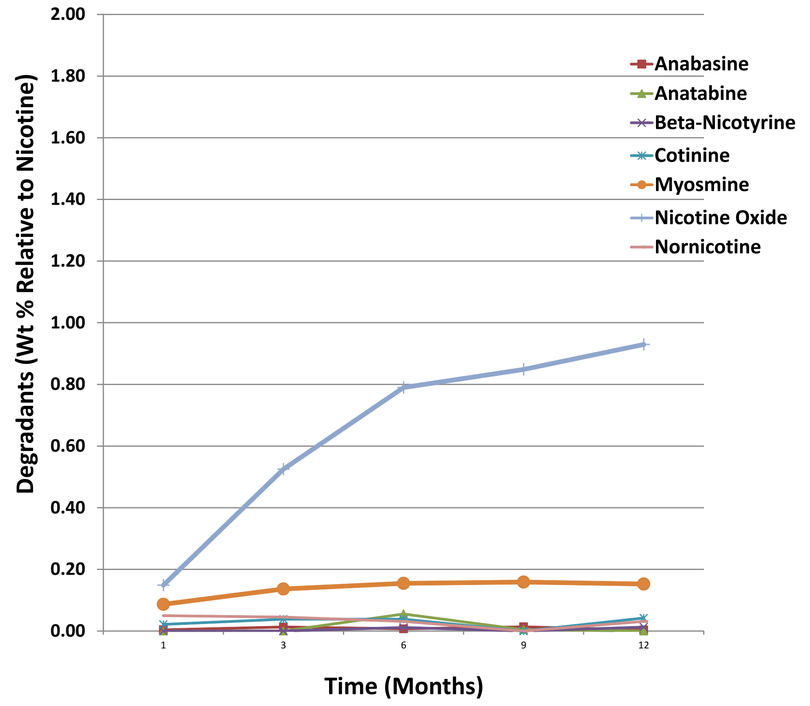
Parameter	Established Criteria
Calibration	R^2 = 0.995
Accuracy	Discs Mean Recovery = 95% Chews Mean Recovery = 93%
Intermediate Precision	Discs %RSD < 5% Chews %RSD < 6%
Extract Stability	72 hours (ambient or 4 °C)

Results

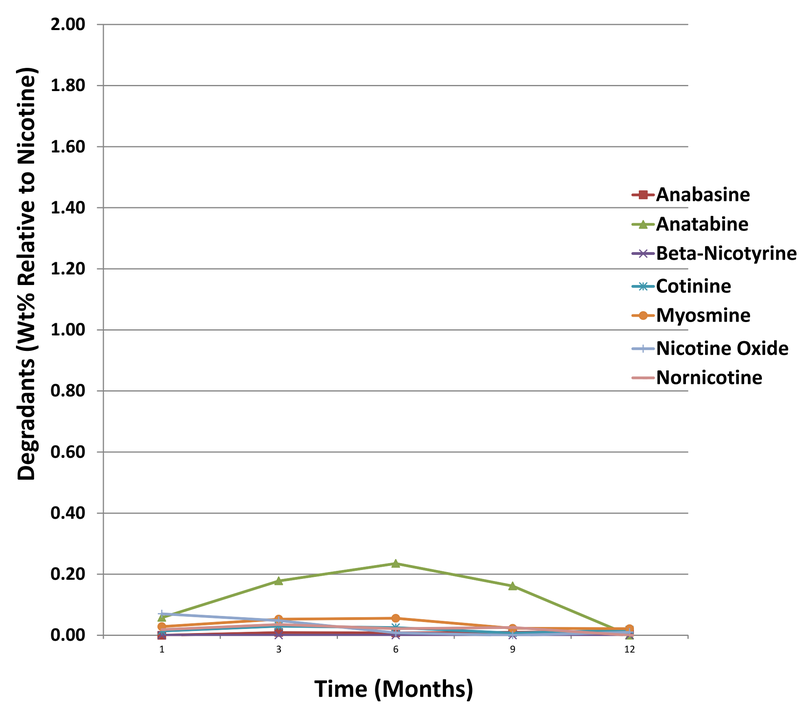
VERVE® Blue Mint Discs



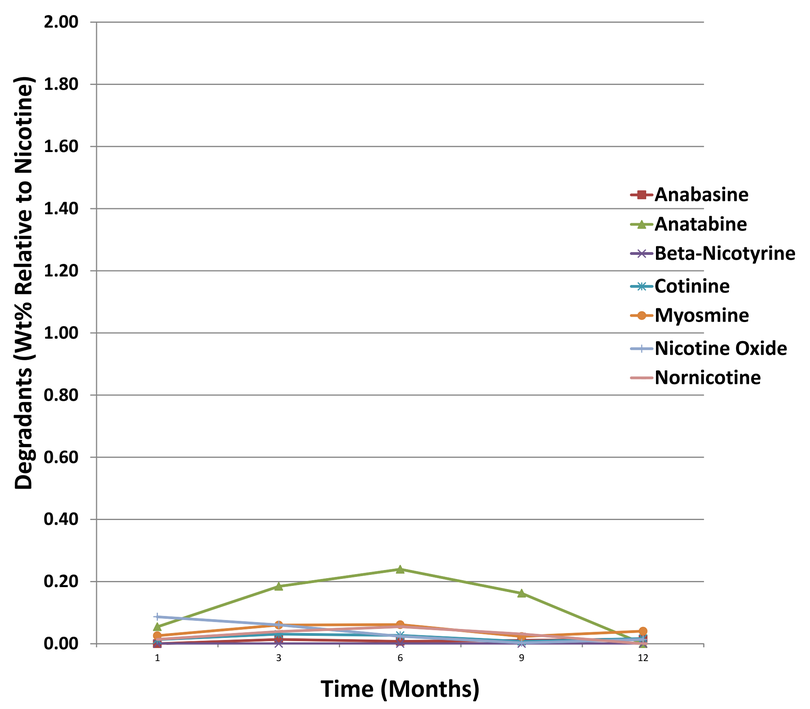
VERVE® Green Mint Discs



VERVE® Blue Mint Chews



VERVE® Green Mint Chews



Data shown are the mean values of 3 production lots. Product was stored in commercial packaging at 25°C ± 2°C/60%RH ± 5%RH environmental conditions for 12 months duration. Each lot of product was tested at N=3 or 7.

Observations and Conclusions

- 1-year shelf-life studies showed minimal variations in nicotine related degradants and impurities over time. At the end of one year, all nicotine impurities measured were <0.5% (expressed as % nicotine) with the exception of nicotine N-oxide in VERVE® Discs which was <3.0% (expressed as % nicotine).
- Within the targeted shelf life of one year, the nicotine-N'-oxide levels in the Discs products did not exceed the ALCS acceptance threshold of 3.0% wt/wt of USP grade tobacco-derived nicotine added to the product.
- The method adequately measures the nicotine degradants and impurities with the specificity and sensitivity necessary to meet the acceptance thresholds we have established.
- During stability studies for Chews, an interfering compound was discovered coeluting with anatabine. This was subsequently mitigated by adjusting the mobile phase gradient on the LC and the fragmenter voltage on the MS. The data at 3, 6 and 9 months are indicative of the coelution which was resolved by the method improvements by the 12-month time point.
- With the exception of nicotine N'-oxide in Discs, the majority of nicotine degradants and impurities measured were either not detected (below the limit of detection, BLOD) or below the limit of quantitation (BLOQ).
- In the absence of Guidance from FDA regarding acceptable levels of nicotine degradants over the shelf-life of tobacco products containing USP grade tobacco-derived nicotine, we have developed an approach adapted from ICH Guidelines Q3B(R2) and based on sound scientific principles.

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