# Nicotine-Related Impurities in E-Cigarette Cartridges and Refill Formulations

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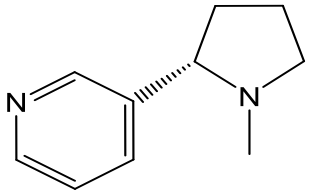
# Background

- The nicotine used in e-cigarette formulations is extracted from tobacco, and the purity of the nicotine can vary depending upon manufacturer and grade (e.g., US Pharmacopeia grade)

- The US and European Pharmacopeia make recommendations for the purity of nicotine intended for pharmaceutical products

- Recommendations are also made in the ICH Guidelines “Impurities in New Drug Products Q3B(R2)”*

Nicotine
3-[(2S)-1-methylpyrrolidin-2-yl]pyridine




**Q3B(R2): International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Impurities in New Drug Products. International Conference on Harmonisation. 2006.*

# Background

- No official purity recommendation for the nicotine used in e-cigarettes has been made

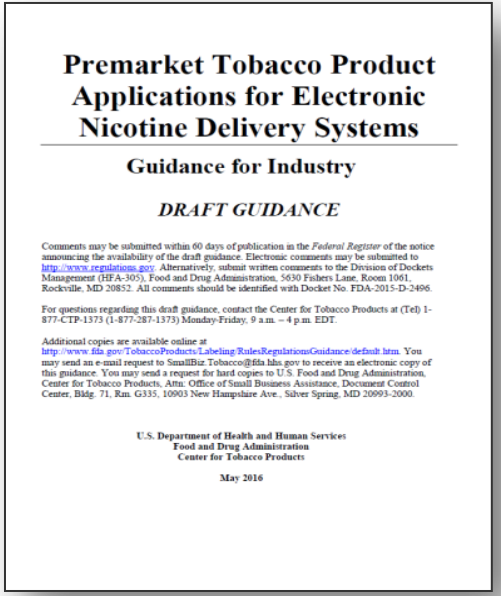
- Only a few publications have evaluated the nicotine-related impurities in e-cigarette formulations and none have evaluated these e-liquids during long-term storage


- Etter, J. F., Zather, E., Svensson, S., Analysis of refill liquids for electronic cigarettes. *Addiction* **2013**, 108 (9), 1671-1679.
- Trehy, M. L., Ye, W., Hadwiger, M. E., Moore, T. W. *et al.* Analysis of Electronic Cigarette Cartridges, Refill Solutions, and Smoke for Nicotine and Nicotine Related Impurities. *Journal of Liquid Chromatography & Related Technologies* **2011**, 34 (14), 1442-1459.
- Westenberger, B., Evaluation of e-cigarettes. Washington, DC: US Food and Drug Administration, **2009**.
- Westenberger, B., Evaluation of Johnson Creek Liquids for E-cigarette Fills. Washington, DC: US Food and Drug Administration, **2009**.
- Flora J.W., Meruva N., Huang C.B, Wilkinson C.T., Ballentine R., Smith D.C. , Werley M.S., McKinney W.J, Characterization of potential impurities and degradation products in electronic cigarette formulations and aerosols, Regul. Toxicol. Pharmacol. **2016**, 74, 1-11.

# Background

- FDA recommends a list of constituents to be measured in e-vapor products and e-liquids

- “FDA also recommends that you include a complete list of uniquely identified constituents, including those listed below, as appropriate for your product, and other toxic chemicals contained within the product or delivered by the product, such as a reaction product from leaching or **aging** and aerosol generated through the heating of the product.”*



**Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems**

**Guidance for Industry**

***DRAFT GUIDANCE***

Comments may be submitted within 60 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Electronic comments may be submitted to http://www.regulations.gov. Alternatively, submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Room 1061, Rockville, MD 20852. All comments should be identified with Docket No. FDA-2015-D-2496.

For questions regarding this draft guidance, contact the Center for Tobacco Products at (Tel) 1-877-CTP-1373 (1-877-287-1373) Monday-Friday, 9 a.m. – 4 p.m. EDT.

Additional copies are available online at http://www.fda.gov/TobaccoProducts/Labeling/RulesRegulationsGuidance/default.htm. You may send an e-mail request to SmallBiz.Tobacco@fda.hhs.gov to receive an electronic copy of this guidance. You may send a request for hard copies to U.S. Food and Drug Administration, Center for Tobacco Products, Attn: Office of Small Business Assistance, Document Control Center, Bldg. 71, Rm. G335, 10903 New Hampshire Ave., Silver Spring, MD 20993-2000.

**U.S. Department of Health and Human Services**
**Food and Drug Administration**
**Center for Tobacco Products**

**May 2016**

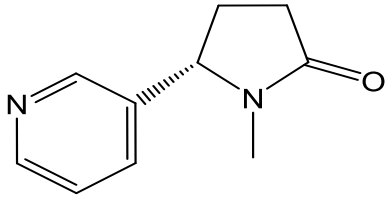
*Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems Guidance for Industry – Draft – CTP-FDA - 2016

# Objectives

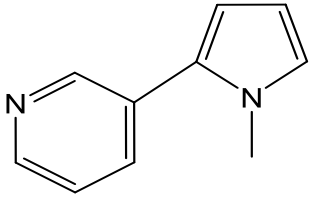
- To develop a sensitive, selective, and robust analytical method (LC-MS) for the quantitation of nicotine-related impurities and degradation products in e-vapor products
- To evaluate these impurities in a variety of commercial e-cigarette cartridges and refill solutions (e-liquids).
- To quantify changes in these impurities during long-term storage of commercial e-cigarettes
- To apply this method to estimate the transfer efficiency of the nicotine-related impurities to the aerosol

# Specified Nicotine Impurities from European Pharmacopoeia (monograph 1452)

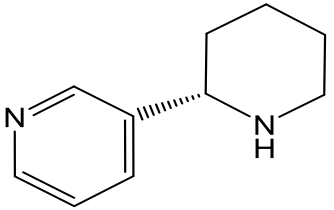
Cotinine
(5S)-1-methyl-5-(pyridin-3-yl)pyrrolidin-2-one



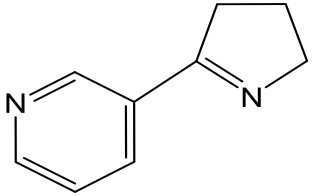

Beta-Nicotyrine
3-(1-methyl-1H-pyrrol-2-yl)pyridine



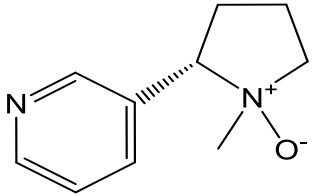

Anabasine
3-[(2S)-piperidin-2-yl]pyridine



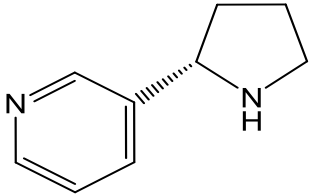

Myosmine
3-(4,5-dihydro-3H-pyrrol-2-yl)pyridine



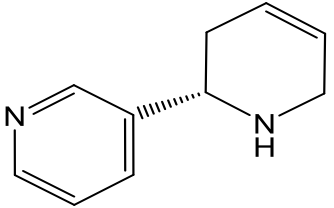

Nicotine-N'-Oxides
(1RS,2S)-1-methyl-2-(pyridin-3-yl)pyrrolidine 1-oxide (epimer at N+)




Nornicotine
3-[(2S)-pyrrolidin-2-yl]pyridine




Anatabine
(2S)-1,2,3,6-tetrahydro-2,3'-bipyridyl



# Analytical Methodologies

| Analyte | Analytical Platform |
|---|---|
| Nicotine | GC-FID |
| Nicotine-Related Impurities | LC-MS/MS |

GC-FID = Gas Chromatograph with Flame Ionization Detector
LC-MS = Liquid Chromatography with Mass Spectrometry

- Formulations from disposable e-cigarette cartridges were removed by centrifugation at 4800 rpm for 30 seconds
- Sealed tank models were disassembled and formulation was removed
- Refill e-liquids were pipetted directly for analysis
- Method validations were conducted consistent with ICH guidelines “Validation of Analytical Procedures: Test and Methodology Q2(R1)”*

**Q2(R1): International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Validation of Analytical Procedures. International Conference on Harmonisation. 2005.*

# Nicotine Method

- For all samples, formulations were diluted in *n*-propanol containing quinoline as the internal standard
- All data were collected on either an Agilent 7890 or 6890 GC with FID detection
- The analytical column used was a DB-ALC1 fused silica capillary column, 30 m × 0.320 mm ID, 1.8 m film thickness (Agilent Technologies), and the carrier gas was helium

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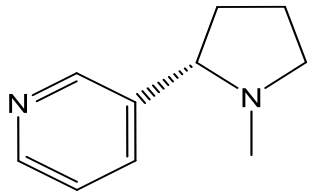


# Nicotine-Related Impurities Method

- For all samples, formulation was diluted in 70/30 methanol/water containing deuterium labeled internal standards
- Chromatography - Waters ACQUITY UPLC with X-Bridge C18 (2.5um) 2.1 x 50 mm column
- Mobile phase A is 10 mM ammonium acetate (pH 10) and mobile phase B is optima grade methanol (gradient run)
- MS detection using Multiple Reaction Monitoring (MRM)

| Analyte | Internal Standard |
|---|---|
| Myosmine | Myosmine-d4 |
| Nornicotine | Nornicotine-d4 |
| β-nicotyrine | Myosmine-d4 |
| Anatabine | Myosmine-d4 |
| Anabasine | Anabasine-d4 |
| Cotinine | Cotinine-d3 |
| Nicotine-N-oxide | Cotinine-d3 |

# Commercial Refill E-Liquids Tested (Jan 2016)

| product code | type | percent nicotine by weight | production date | "expiration", "sell by", or "do not use after" Date | Package |
|---|---|---|---|---|---|
| 1 | refill e-liquid | 1.2 | - | - | glass bottle with plastic overwrap |
| 2 | refill e-liquid | 1.2 | - | - | glass bottle with plastic overwrap |
| 3 | refill e-liquid | 1.2 | - | 5/19/2016 | plastic bottle with plastic overwrap |
| 4 | refill e-liquid | 1.2 | - | 5/15/2016 | plastic bottle with plastic overwrap |
| 5 | refill e-liquid | 1.2 | - | 6/23/2016 | glass bottle |
| 6 | refill e-liquid | 1.2 | - | 10/8/2016 | glass bottle |
| 7 | refill e-liquid | 1.2 | - | - | glass bottle with plastic overwrap |
| 8 | refill e-liquid | 1.2 | - | - | glass bottle with plastic overwrap |
| 9 | refill e-liquid | 1.6 | 8/1/2014 | 8/1/2016 | plastic bottle with plastic overwrap |
| 10 | refill e-liquid | 1.6 | - | 5/23/2015 | plastic bottle with plastic overwrap |

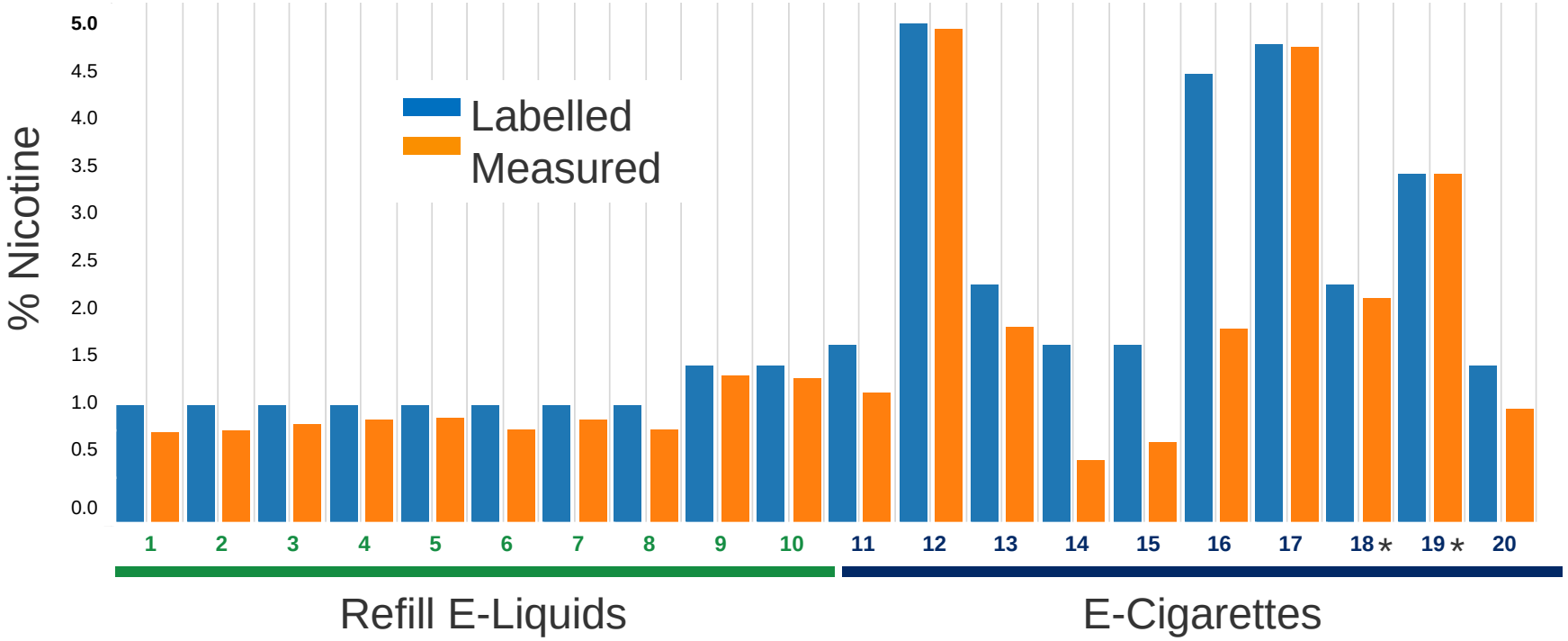
- Limited production and shelf life data provided from manufacturers
- Variety of packaging configurations and product ages

# Commercial E-Cigarettes Tested (Jan 2016)

| product code | type | percent nicotine by weight | production date | "expiration", "sell by", or "do not use after" Date | Package |
|---|---|---|---|---|---|
| 11 | disposable prefilled tank | 1.8 | 12/3/2014 | 12/3/2015 | blister pack with rubber mouthpiece cover |
| 12 | disposable prefilled tank | 5.0 | - | - | blister pack with plastic backing |
| 13 | disposable prefilled tank | 2.4 | - | 12/18/2016 | plastic box with rubber or plastic endcaps |
| 14 | disposable device | 1.8 | - | 2/10/2013 | plastic box with rubber or plastic endcaps |
| 15 | disposable device | 1.8 | - | 2/10/2013 | plastic box with rubber or plastic endcaps |
| 16 | disposable device | 4.5 | - | - | Plastic box with plastic overwrap |
| 17 | disposable cartridge | 4.8 | - | - | blister pack with foil backing |
| 18 | disposable cartridge | 2.4 | - | 8/1/2016 | blister pack with foil backing |
| 19 | disposable cartridge | 3.5 | - | 5/9/2016 | blister pack with foil backing |
| 20 | disposable cartridge | 1.6 | - | - | plastic overwrap, blister with foil backing |

- Limited production and shelf life data provided from manufacturers
- Variety of packaging configurations and product ages

# Nicotine Level: Labelled vs. Measured




*18 and 19 are MarkTen® e-cigarettes manufactured by NuMark LLC

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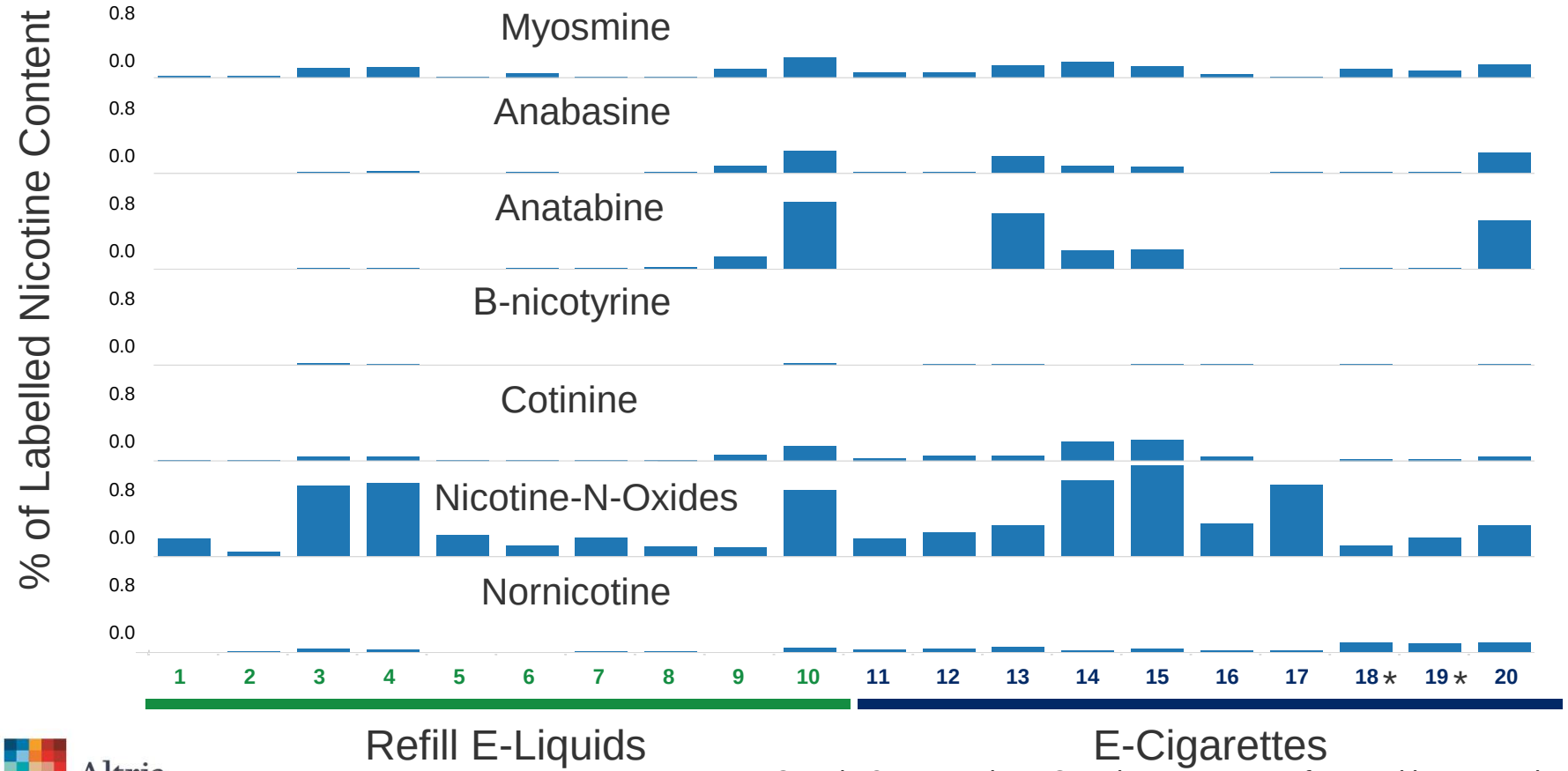
# Nicotine Level: Labelled vs. Measured

- 6 products contained nicotine between 90 and 100% of label claim
  - 4 had shelf life information
  - 1 was expired
- 11 products contained nicotine between 70 and 90% of the label claim
  - 6 had shelf life information
  - 1 was expired
- 3 products contained nicotine less than 50% of the nicotine label claim
  - 2 had shelf life information
  - 2 were expired

# Nicotine-Related Impurities

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# Nicotine-Related Impurities

- Myosmine, anabasine, β-nicotyrine, cotinine, and nornicotine, all had values below 0.2% of the labelled nicotine content where many were well below 0.1%

- Products 10, 13, and 20 had relatively high average anatabine levels at 0.57, 0.47, and 0.41%

- 6 of the 20 products contained nicotine-N-oxide levels greater than 0.5% of the labelled nicotine content
  - 3 were expired
  - 2 were within labelled shelf life
  - 1 had no shelf life information

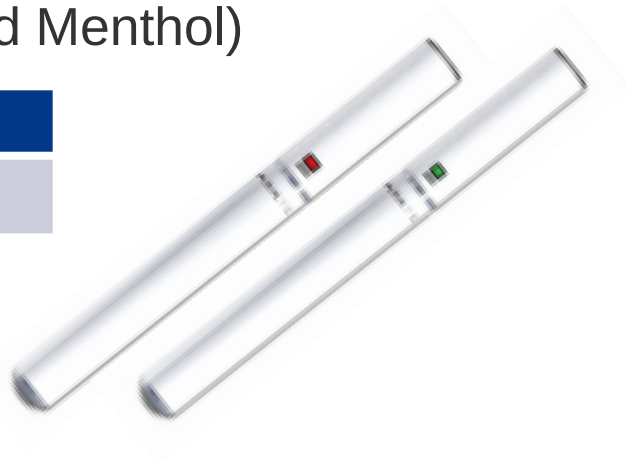
# Nicotine-Related Impurities During Shelf-life

- To quantify changes in these impurities during long-term storage of commercial e-cigarettes

- MarkTen® XL e-cigarettes manufactured by NuMark LLC were evaluated during long-term storage (Classic and Menthol)

| Storage Description | Storage Conditions |
|---|---|
| Long-term (Q1A(R2))* | $25^0$C ± $2^0$C / 60% RH ± 5% |

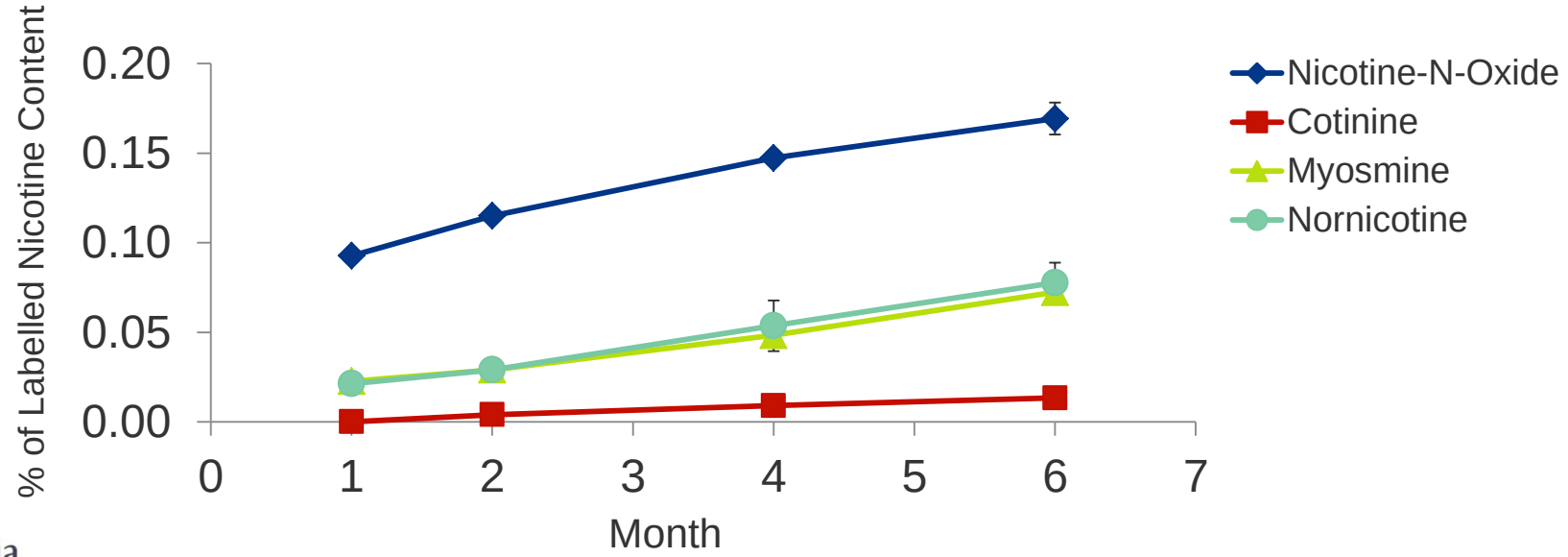
- Tested at 1, 2, 4 and 6 months



* ICH Guidance for Industry Q1A(R2) Stability Testing of New Drug Substances and Products

# Nicotine-Related Impurities During Shelf-life

- All specified impurities are <0.2% of the total nicotine concentration after 6 months at long-term conditions
- No measurable increase was observed for anabasine, β-nicotyrine, and anatabine

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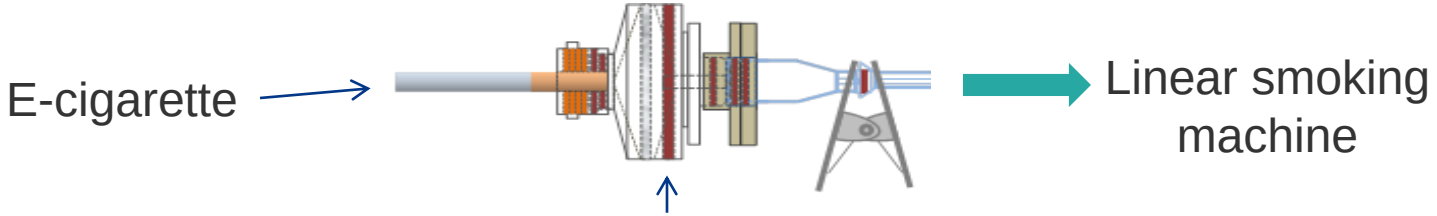
# Nicotine-Related Impurities: Estimated Transfer Efficiency




- Estimate the % transfer efficiency to the aerosol (n=6)

| Transfer % in aerosol | Myosmine | Nornicotine | Cotinine | Anabasine | Nicotine-N-Oxide | Anatabine | B-nicotyrine |
|---|---|---|---|---|---|---|---|
| MarkTen® Classic | 97 | 95 | 103 | 80 | 5.4 | 89 | 141 |
| MarkTen® Menthol | 97 | 110 | 103 | 91 | 4.9 | 96 | 164 |

Non-validated method

- GC-MS analysis of nicotine-N-oxides showed primary thermal decomposition pathways were to nicotine and β-nicotyrine

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# Conclusions

- Shelf-life information on e-cigarettes and refill e-liquids should be established through rigorous stability testing measuring a variety of constituents appropriate for the products

- This selective and sensitive method is suitable to provide quantitative data for risk assessment analysis and for use in e-vapor product and refill solution stability studies as one of the stability indicating measures

- Nicotine-N-oxide, nornicotine, mysomine, and cotinine have been observed to increase with respect to time during stability studies*

- Transfer efficiency of nicotine-N-oxide is low (<10%) due to thermal degradation during the aerosol formation process

* ICH Guidance for Industry Q1A(R2) Stability Testing of New Drug Substances and Products

Acknowledgements:
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