Analysis of Nicotine and Nicotine Related Compounds in Electronic Cigarette Refill Solutions and Aerosols by Liquid Chromatography-Tandem Mass Spectrometry

Xinyu LIU, Peter JOZA, Bill RICKERT

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LABSTAT INTERNATIONAL ULC.
262 Manitou Drive
Kitchener, Ontario, Canada N2C 1L3
Phone: (519) 748-5409 Fax: (519) 748-1654 Web: www.labstat.com

Objectives

- To develop and validate a reliable HPLC-MS/MS method for the quantitative analysis of nicotine and nicotine related compounds in e-cigarette solutions and aerosols.

- To demonstrate the applicability of the method to the determination of nicotine stability in e-cigarette liquids.

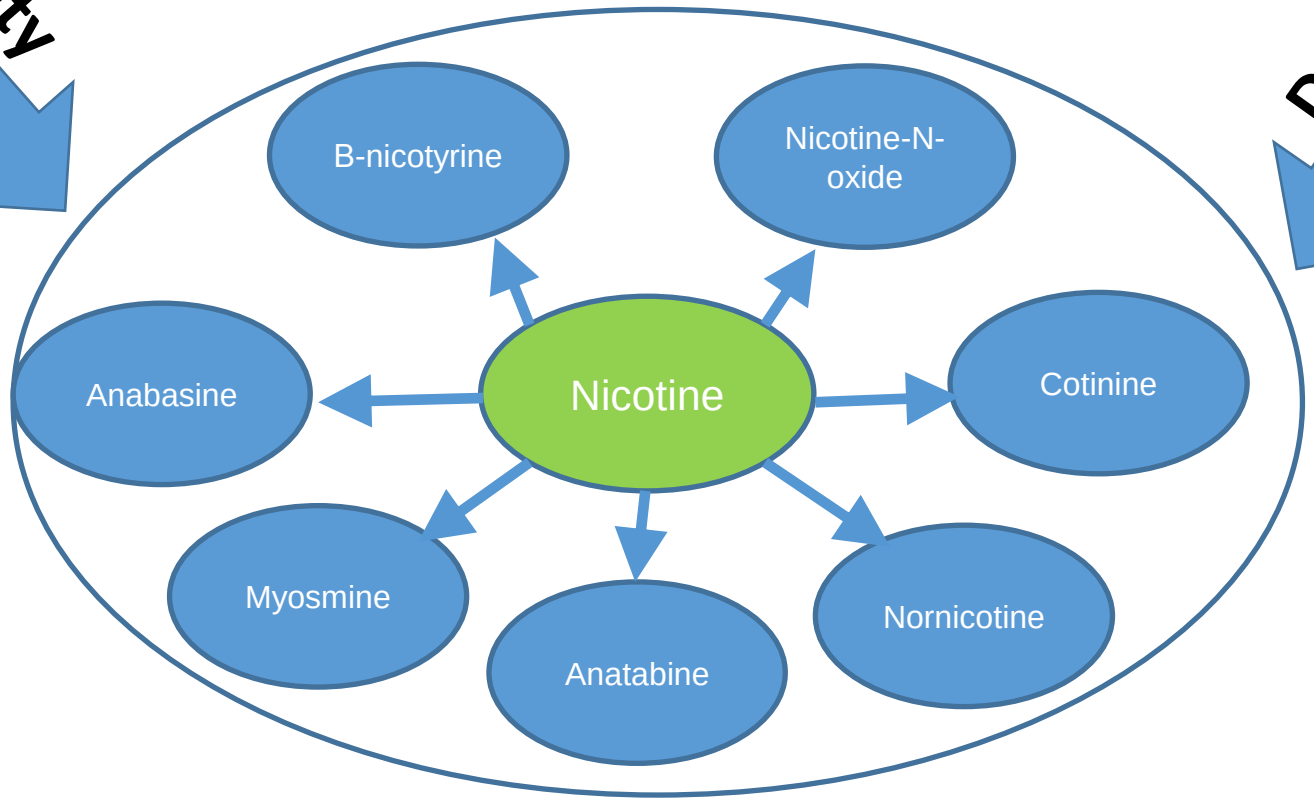
Possible Sources of Nicotine Related Compounds

Nicotine Source

Nicotine is decomposed by various chemical reactions and /or microorganisms

Impurity

Degradation



Method Choice

➢GC or GC-MS Analysis

- “Hot” injection(>200°C)
- Nicotine-N-oxide is thermally unstable

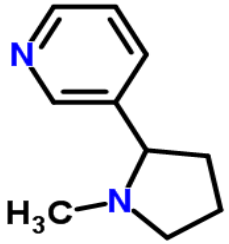
➢HPLC-UV Analysis

- “Cold ” injection (4°C-room temperature)
- United States Pharmacopeia method (USP32/NF27)
- The result is susceptible to bias due to flavoring or/and coloring

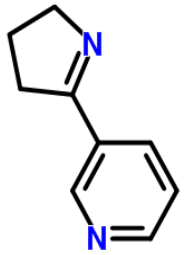
➢LC-MS/MS Analysis

- “Cold ” injection (4°C-room temperature)
- High selectivity and sensitivity

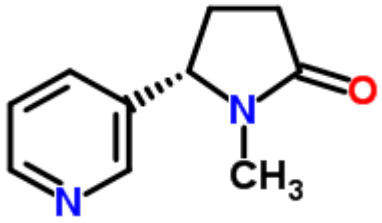
Structures of Nicotine and Related Compounds



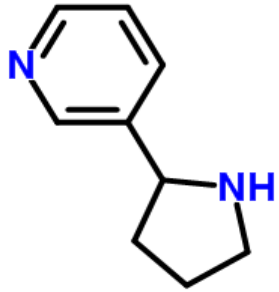
Nicotine
$C_{10}H_{14}N_2$
Cas: 54-11-5



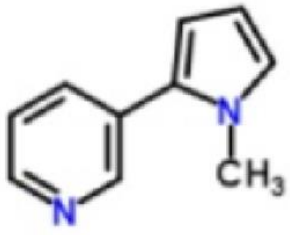
Myosmine
$C_9H_{10}N_2$
Cas: 532-12-7



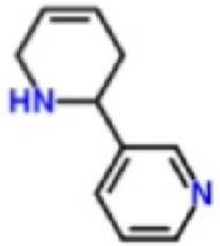
Cotinine
$C_{10}H_{12}N_2O$
Cas: 15569-85-4



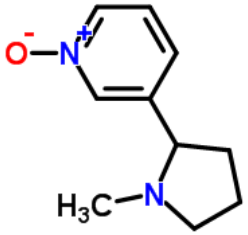
Nornicotine
$C_9H_{12}N_2$
Cas: 5746-86-1



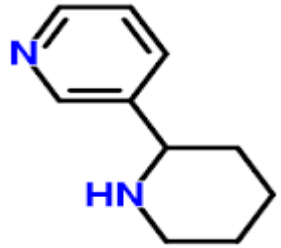
β-Nicotyrine
$C_{10}H_{10}N_2$
Cas: 487-19-4



Anatabine
$C_{10}H_{12}N_2$
Cas: 581-49-7

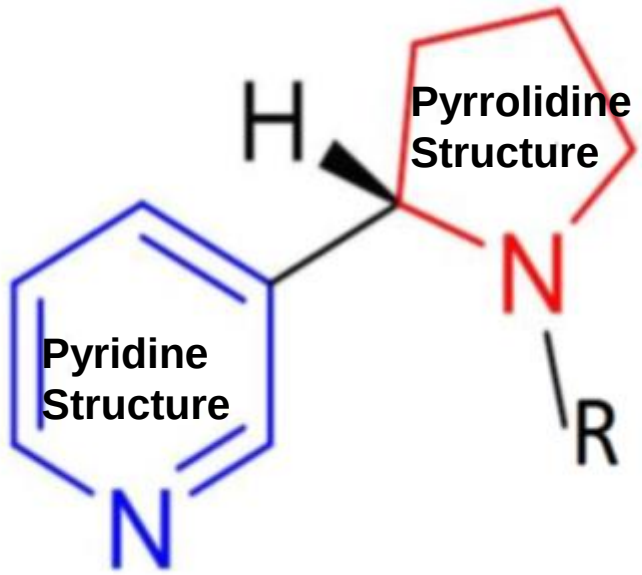


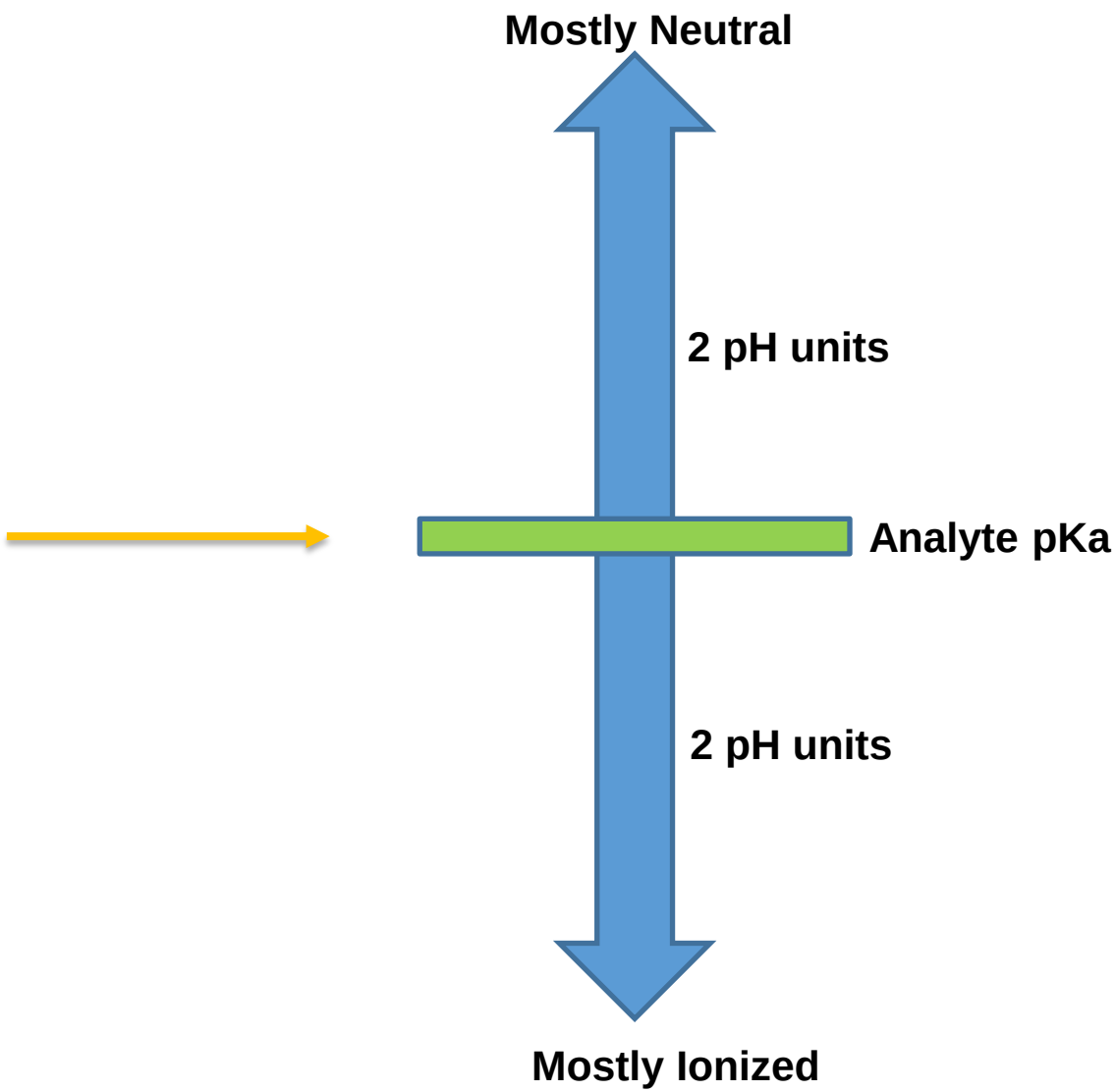
Nicotine-n-oxide
$C_{10}H_{14}N_2O$
Cas: 63551-14-4



Anabasine
$C_{10}H_{14}N_2$
Cas: 13078-04-1

Effect of Mobile Phase pH (1)





Effect of Mobile Phase pH (2)

Analyte	pKa	logP	pH=6.52	pH=7.68	pH=9.00	pH=9.33	pH=10.0	pH=10.6
			RT time (min)					
Nicotine-N-oxide	4.63	na	1.07	1.06	1.04	1.04	0.99	0.95
Cotinine	4.72	0.04	2.94	2.88	2.78	2.75	2.73	2.81
Myosmine	7.81	0.70	5.55	5.51	5.39	5.34	5.22	5.41
β-nicotyrine	na	1.00	11.5	11.5	11.3	11.3	11.2	11.3
Nicotine	9.13	0.93	3.04	5.98	7.14	7.24	7.25	7.43
Nornicotine	9.83	0.50	1.30	2.16	2.82	3.93	4.41	4.58
Anatabine	8.77	0.93	2.01	3.97	4.99	5.09	5.08	5.26
Anabasine	9.86	0.96	1.87	3.42	5.30	5.85	6.45	6.69

Analytical Conditions (LC Parameters)

Instrument: Waters ACQUITY UPLC-XEVO-TQMS

Column: Gemini NX-C_{18} (3μm,150X3 mm)

Mobile Phase: A: Acetonitrile
B: 10% acetonitrile in 10mM ammonium bicarbonate (pH=8.0)

Gradient Program:

Time (min)	Mobile phase B (%)	Flow rate (mL/min)	Curve
0.00	90	0.5	Initial
8.00	75	0.5	6
15.0	40	0.5	6
17.0	90	0.5	6
18.0	90	0.5	6

Analytical Conditions (MS Parameters)

Instrument	Parameters
Ionization Mode:	ESI+
Capillary Voltage (Kv):	3.0
Source Temp (°C) :	150
Desolvation Temp (°C):	350
Cone Gas Flow(L/Hr):	20 (Nitrogen)
Desolvation Gas Flow (L/Hr)	800 (Nitrogen)
Collision Gas Flow (mL/min):	0.15 (Argon)
Data Acquisition:	MRM

MRM Transitions

Analyte	Retention time (min)	Precursor ion (m/z)	Quantitation ion (m/z)	Confirmation ion (m/z)
Nicotine-N-Oxide	1.41	179	132	130
Nicotine-N-Oxide-d_3	1.41	182	132	130
Cotinine	2.37	177	80	98
Cotinine-d3	2.38	180	80	101
Nornicotine	2.97	149	117	149
Nornicotine-d4	2.99	153	121	153
Anatabine	3.82	161	144	161
Anatabine-d4	3.85	165	111	165
Myosmine	4.11	147	117	147
Myosmine-d4	4.18	151	81	151
Anabasine	4.14	163	94	163
Anabasine-d4	4.20	167	96	167
Nicotine	5.52	163	132	130
Nicotine-d3	5.54	166	117	130
β-nicotyrine	9.91	159	144	159
β-nicotyrine-d3	9.97	162	135	162

Experimental

E-liquid Samples

Accurately weigh 0.1g solution

↓

Add 0.5 mL ISTD

↓

Add 100 mM NH_4AC buffer to 10 mL mark

↓

Ultrasonic extract 1 minute

↓

Filter through 0.22 μm syringe filter

↓

Filtrate for LC-MS/MS analysis

Aerosol Samples

Transfer Pad into Erlenmeyer flask

↓

Add accurately 2.0 mL ISTD

↓

Add 38 mL 100 mM NH_4AC buffer

↓

Extract via Shaker 30 minutes

↓

Filter through 0.22 μm syringe filter

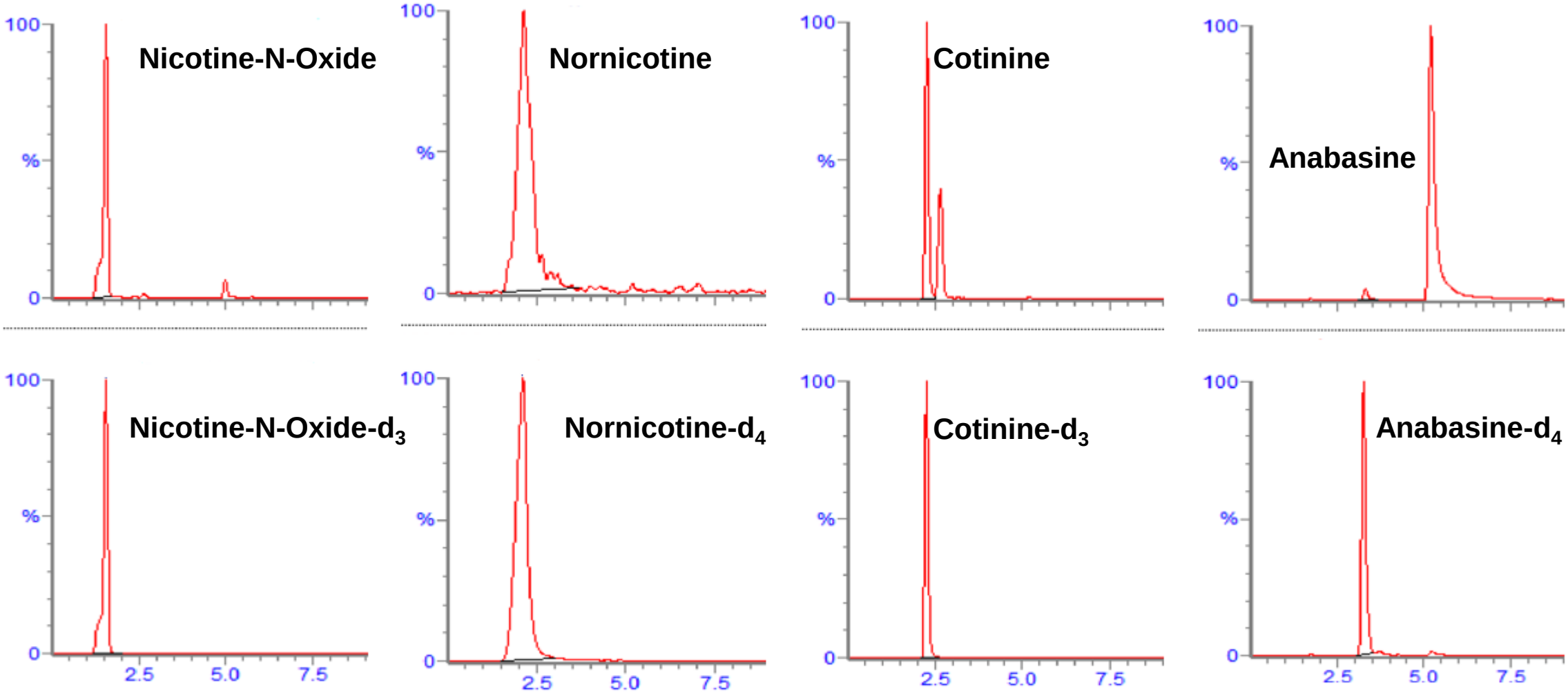
↓

Filtrate for LC-MS/MS analysis

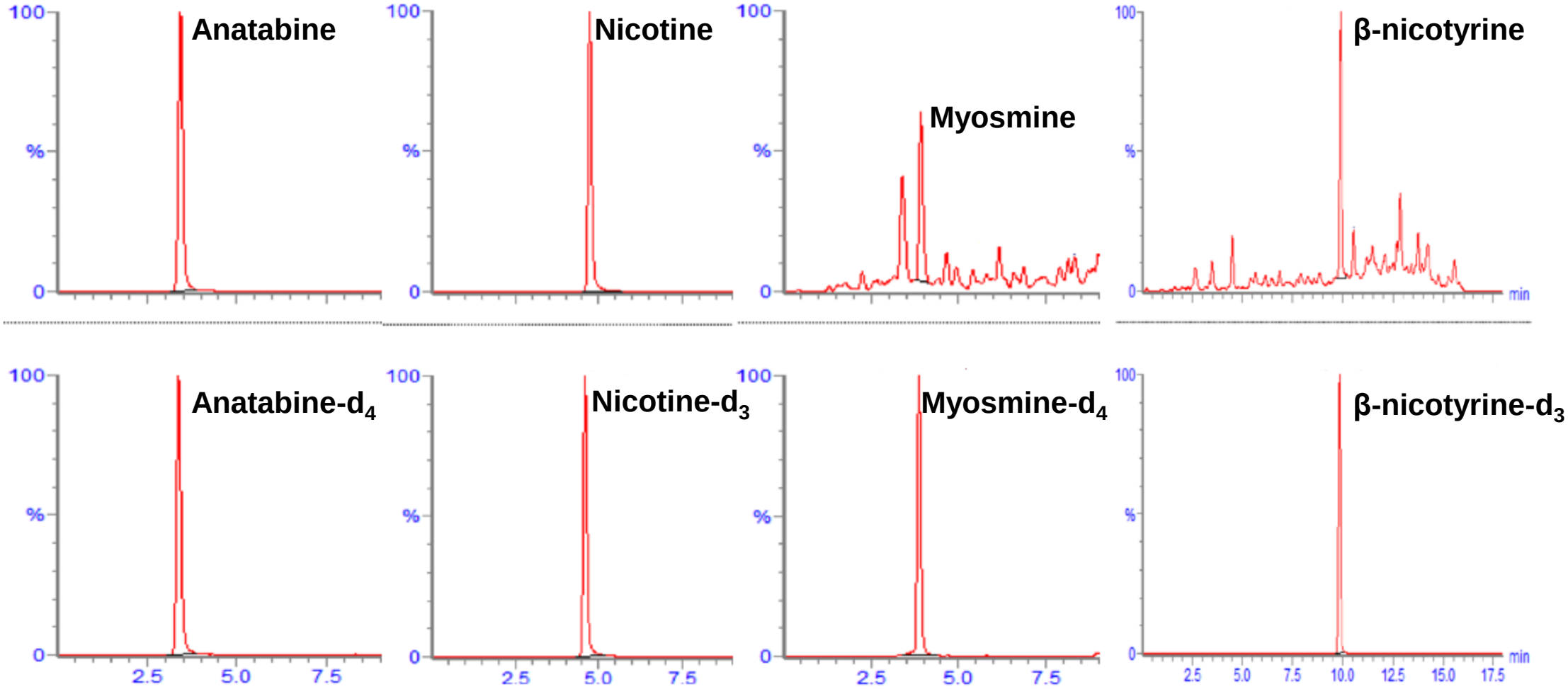
Method Characteristics

	E-Liquid	E-Aerosol
Linearity		
Linear Range (µg/ml)	0.05-5.00	0.05-5.00
Correlation Coefficient (R^2)	>0.995	>0.995
Recovery (Accuracy)		
Laboratory Reagent Blank (LRB)	Not determined to <LOQ	Not determined to <LOQ
Laboratory Fortified Blank (LFB) (%)	85.7-110	83.2-113
Laboratory Fortified Matrix (LFM) (%)	85.2-110	87.8-111
Detection Limit		
Limit of Detection (LOD)	0.234 -1.66 µg/g	0.094-0.872 µg/collection
Limit of Quantitation (LOQ)	0.781 -5.48 µg/g	0.312-2.87 µg/collection

Example of MRM Chromatograms of E-liquid (1)



Example of MRM Chromatograms of E-liquid (2)



Example of E-Liquid Analysis

Analyte	Mean [µg/g]	Std. Dev. [µg/g]	RSD [%]
Nicotine-N-Oxide	9.06	0.164	1.81
Cotinine	30.8	0.181	0.59
Nornicotine	4.83	0.148	3.05
Anatabine	22.7	0.845	3.72
Myosmine	6.24	0.742	11.9
Anabasine	<LOD	NA	NA
Nicotine	8350	173	2.10
β-nicotyrine	<LOD	NA	NA

Aerosol Analysis-Functionality of E-cigarette Device

Applied Power	Accumulated Mass	Nornicotine	Anatabine	Anabasine	Myosmine
(W)	(mg)	(n=7) (µg/collection)	(n=7) (µg/collection)	(n=7) (µg/collection)	(n=7) (µg/collection)
5*	126±34	0.91±0.27	6.04±1.96	2.07±0.60	<LOQ
20*	780±45	2.85±0.38	11.7±3.28	4.10±0.97	56.6±16.1
5**	198±57	1.33±0.30	11.4±3.40	3.37±0.91	1.02±0.57
20**	835±173	3.81±0.87	12.1±3.79	4.10±1.34	70.5±8.74

* 55/30/3 " square wave" profile puffing regimen (volume/interval/duration) with a 50 puff collection
**80/30/4 " square wave" profile puffing regimen (volume/interval/duration) with a 50 puff collection

Factors Affecting Nicotine Stability

➢Formulations

- Flavors (e.g. Mint, Vanilla) oxidation of nicotine.
- Microorganisms

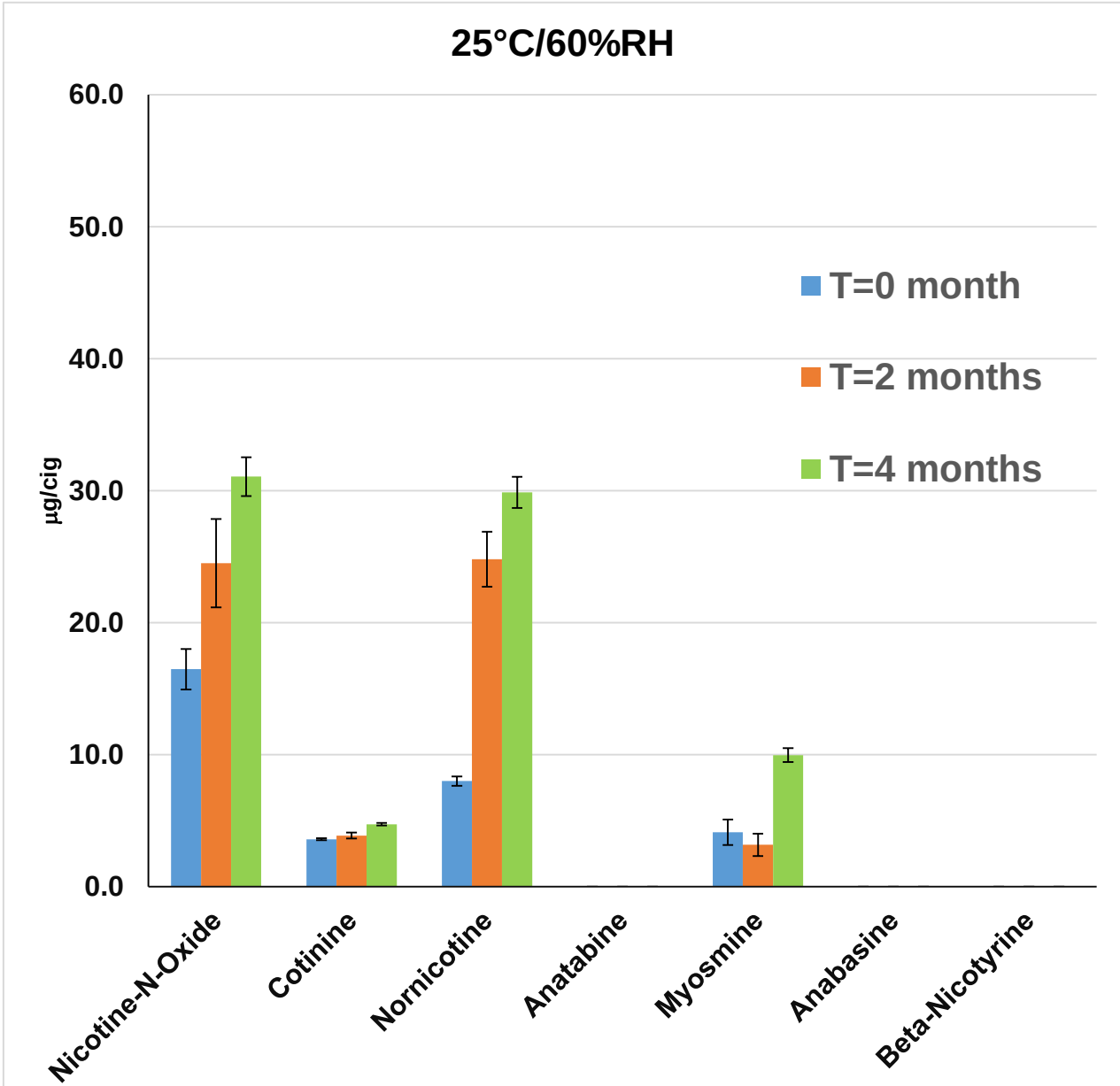
➢Package Materials

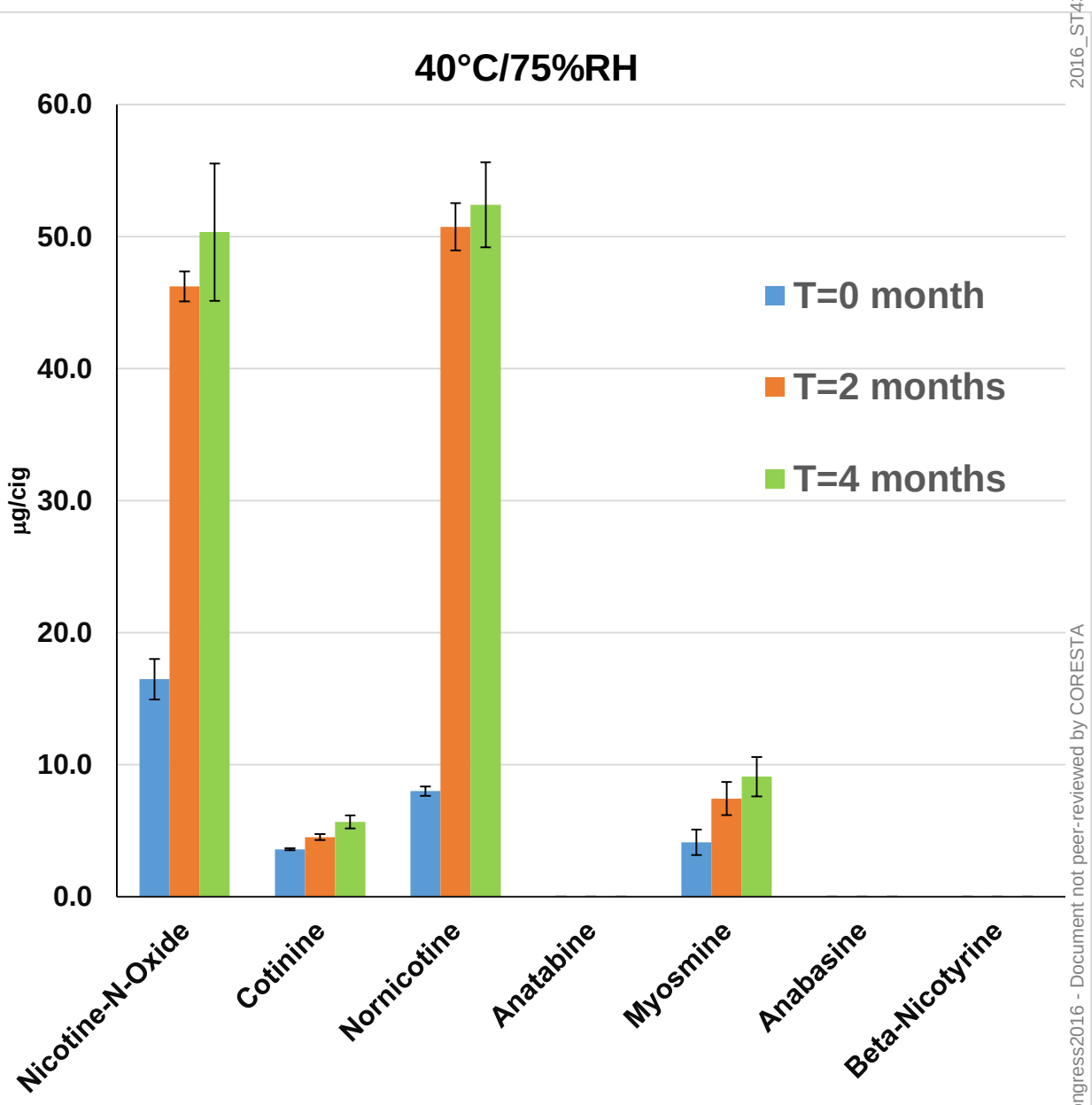
- Interaction with the packing material
- Trace metals as catalysts

➢Storage, Shipment, Handling Conditions

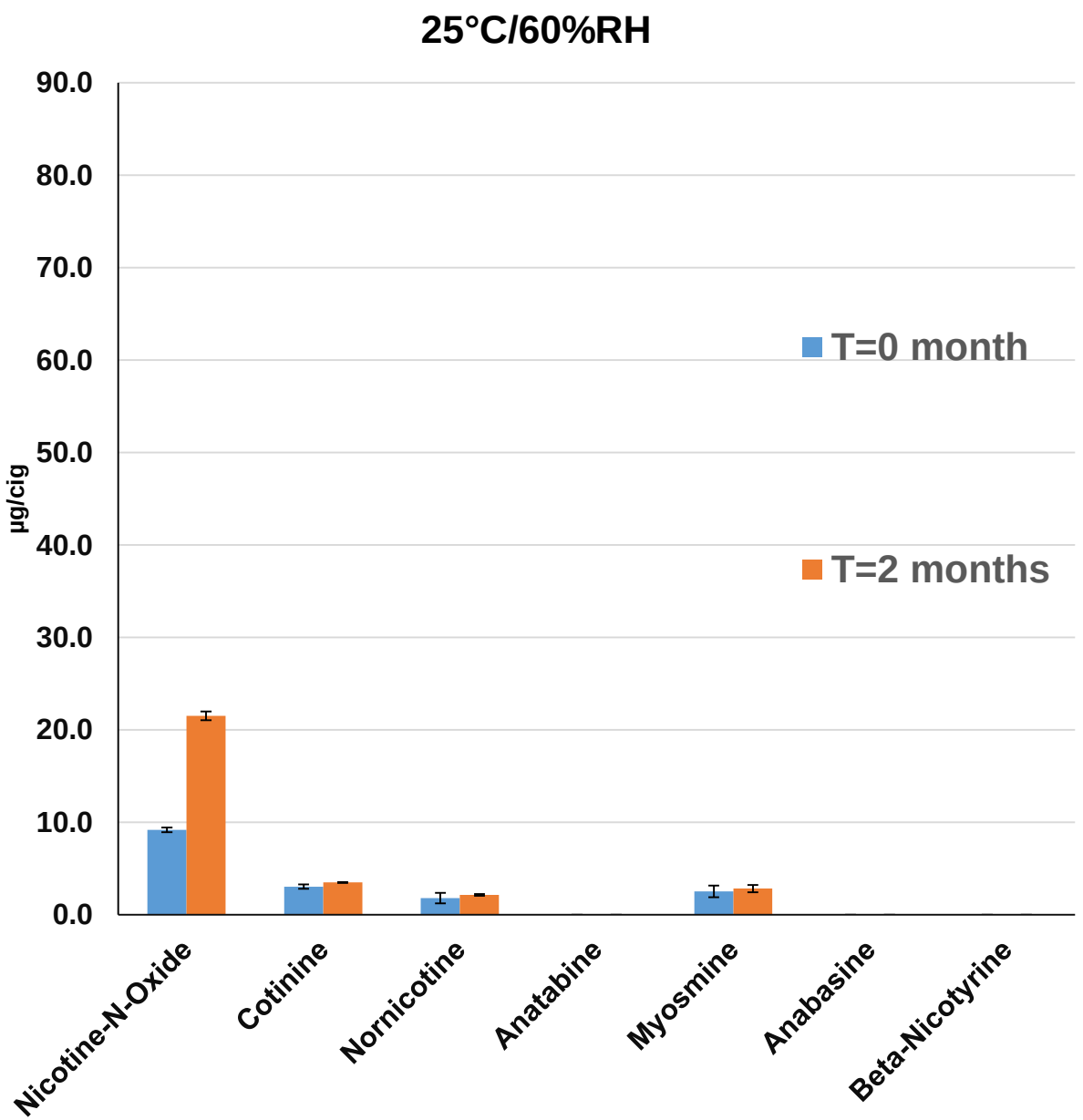
- Temperature
- Moisture
- Light

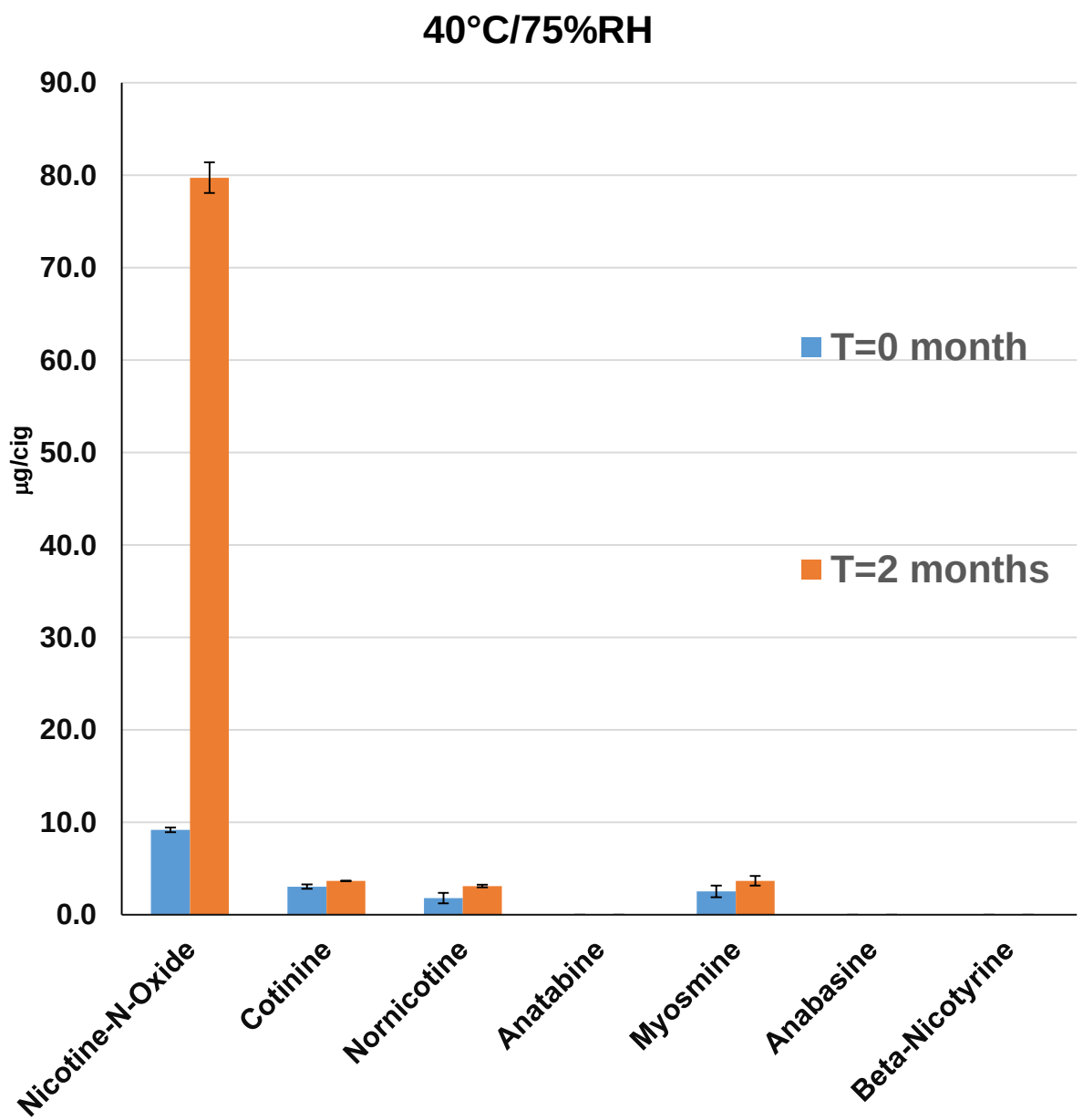
Nicotine Stability – Effect of Storage Conditions





Nicotine Stability – Highly Flavoring E-liquid





Summary

- A specific and reliable HPLC-MS/MS method was developed, validated and successfully applied to the analysis of nicotine and nicotine related compounds in e-cigarette aerosol and e-liquids.

- The method has been successfully applied to studies of nicotine stability, e-cigarette device performance.

- This method can be applied to other studies such as the photo degradation of e-liquids.

Acknowledgements

Labstat International ULC Scientists, Sample Preparation Technicians and Analysts.