SIMULTANEOUS DETERMINATION OF NICOTINE AND RELATED IMPURITIES IN E-LIQUIDS USING UPLC-UV-MS

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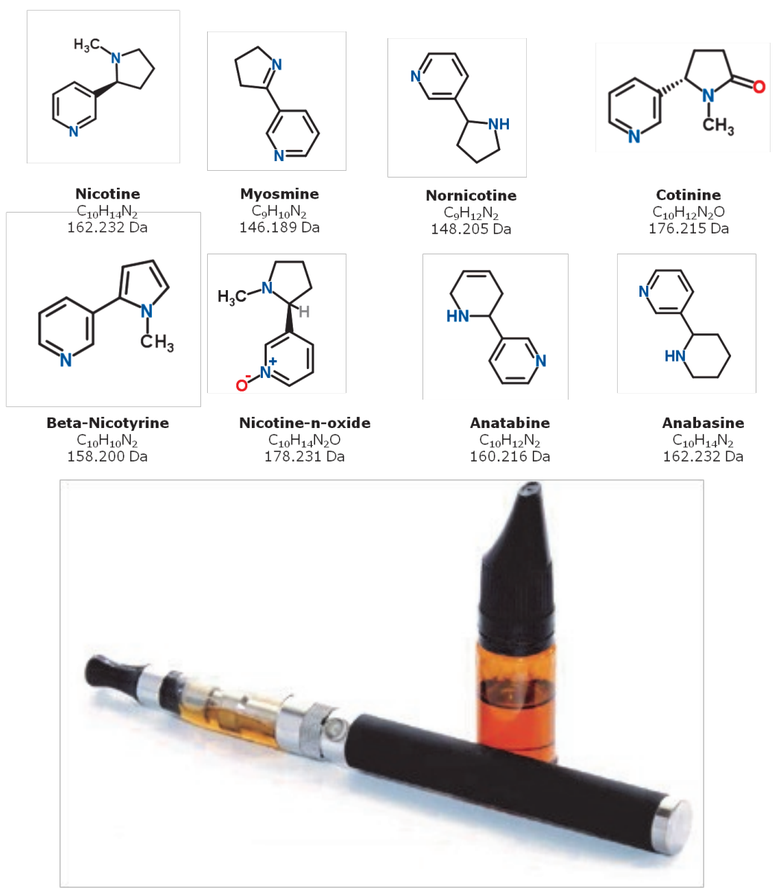
INTRODUCTION

A dilute and shoot method for simultaneous determination of nicotine and 7 related impurities in e-liquid formulations by UPLC-UV-MS has been developed for routine QC testing of e-liquids and e-cigarette products.

E-liquid manufacturing standards are currently being established by regulatory agencies [1]. American E-liquid Manufacturing Standards Association (AEMSA) recommends using USP or certified nicotine with purity greater than or equal to 99%, with nicotine-N-oxide less than or equal to 1% and total contaminants less than or equal to 1% [2].

Simultaneous determination of nicotine and related impurities in e-liquids was achieved using a 7 minute UPLC separation coupled to UV and MS detections. A wide linear dynamic range for nicotine (2.5-500 µg/mL) using PDA detector and for related impurities (0.005-0.5 µg/mL) using MS detector was applied. Six different commercially available e-liquids and e-cigarette cartridges were analyzed following a 100-fold dilution to eliminate the need for multiple methods and

Figure 1. *Structure and chemical properties of nicotine and related impurities.*



METHODS

UPLC CONDITIONS:

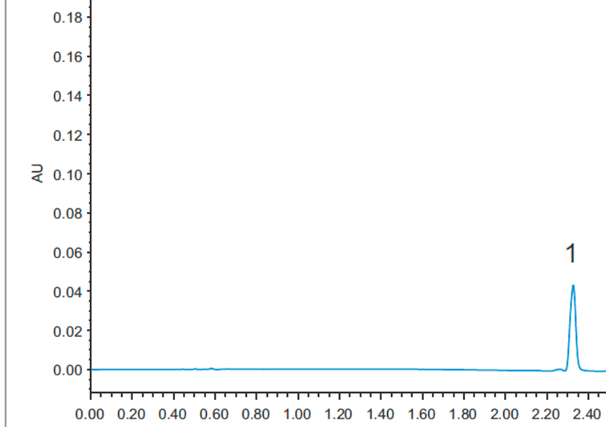
LC system:	Waters ACQUITY UPLC H-Class
Column:	HSS T3 2.1 X 100 mm, 1.7 µm
Column temp:	45 °C
Injection volume:	5 µL
Flow rate:	0.45 mL/min
Mobile phase A:	10 mM Amm acetate in water (pH = 8.9)
Mobile phase B:	10 mM Amm acetate in MeOH
Needle wash:	80/20 methanol/water (v/v)

Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	0.45	98	2	6
0.25	0.45	98	2	6
5.25	0.45	2	98	6
6.25	0.45	2	98	6
6.50	0.45	98	2	6
7.00	0.45	98	2	6

Table 1. UPLC gradient for the separation of nicotine and related impurities.

Detector 1:	**ACQUITY PDA**
Mode:	Absorbance
Wavelength range:	200-350 nm
Nicotine detection:	260 nm
Resolution:	1.2 nm

Detector 2:	**ACQUITY QDa Performance**
Ionization mode:	ESI+
Mass range:	50-250 Daltons
Probe:	600 °C
Capillary voltage:	0.8 kV
Sampling rate:	2 Hz
Cone voltage:	15 V
SIR channels:	See Table 2



RESULTS AND DISCUSSION

Analyte	Detection	Retention time (min)	SIR (*m/z*)	Capillary voltage (kV)	Cone voltage (V)
Nicotine	UV	4.20	N/A	N/A	N/A
Quinoline	UV/MS	4.40	130.2	0.8	15
Nicotine-n-oxide	MS	2.30	179.2	0.8	15
Nornicotine	MS	3.00	149.2	0.8	15
Cotinine	MS	3.10	177.2	0.8	15
Anabasine	MS	3.45	163.2	0.8	15
Anatabine	MS	3.65	161.2	0.8	15
Myosmine	MS	3.85	147.2	0.8	15
Beta-nicotyrine	MS	4.60	159.2	0.8	15

Table 2. Chromatographic retention times and detection parameters for nicotine and related impurities.

Figure 2. UPLC H-Class with ACQUITY PDA and ACQUITY QDa detectors.

Figure 3. UV chromatogram of 10 µg/mL standard mixture of nicotine, related impurities and quinoline using HSS T3 column.

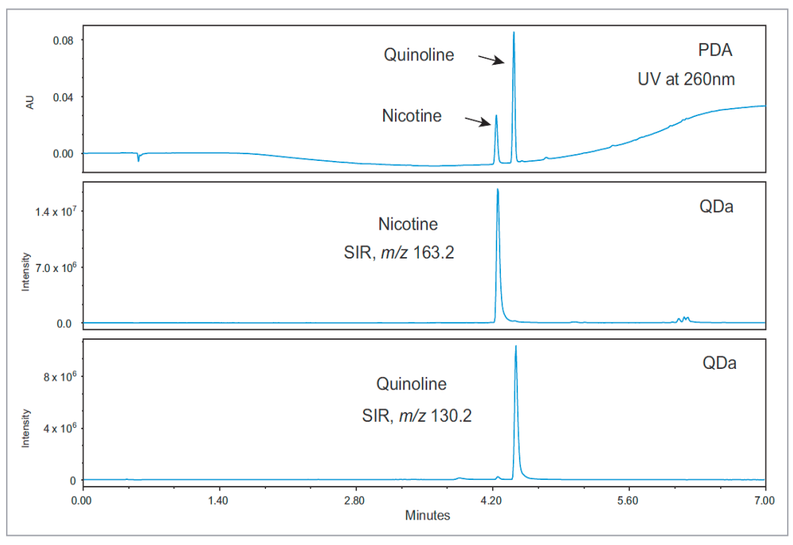


Figure 4. PDA (top) and QDa chromatograms of nicotine and quinoline (ISTD).

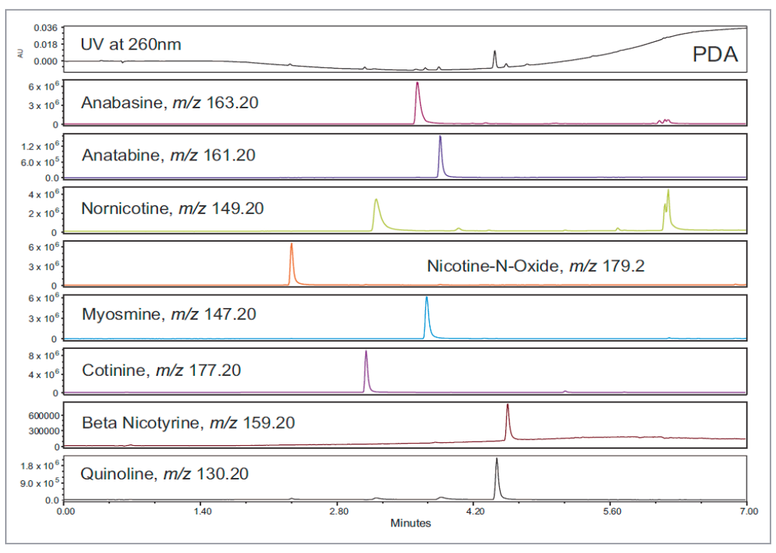


Figure 5. PDA (top) and QDa chromatograms of nicotine impurities.

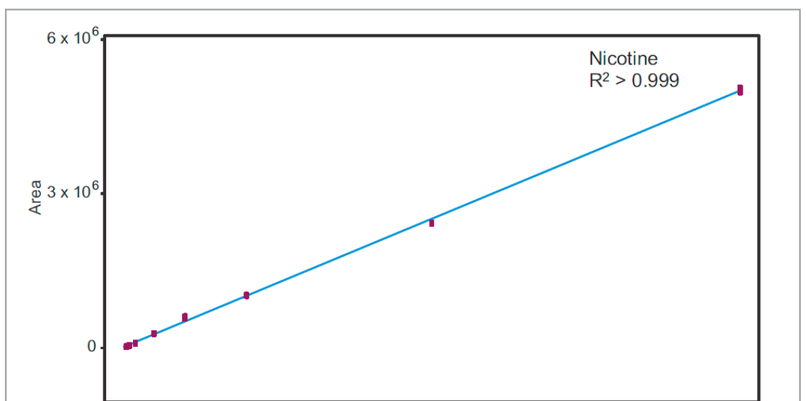


Figure 6. Calibration plot for nicotine using the ACQUITY PDA Detector (range: 2.5 to 500 µg/mL).

Sample ID	% Nicotine labeled	% Nicotine measured
1	2.4%	2.2%
2	2.4%	2.1%
3	2.4%	2.3%
4	4.8%	4.6%
5	4.8%	4.4%
6	2.4%	2.1%

Table 3. Comparison of labeled and measured nicotine concentrations in e-liquid and e-cigarette cartridge extracts (average, n=3).

Analyte	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Nicotine-n-oxide, %	2.74%	1.42%	2.79%	1.08%	1.89%	0.31%
Nornicotine, %	1.17%	1.28%	nd	0.98%	nd	0.09%
Cotinine, %	0.59%	0.09%	nd	nd	nd	1.14%
Anabasine, %	0.22%	0.26%	0.03%	0.15%	0.24%	0.05%
Anatabine, %	0.03%	0.07%	nd	nd	0.11%	nd
Myosmine, %	2.27%	1.03%	0.67%	0.15%	0.42%	1.27%
Beta-nicotyrine, %	1.60%	0.65%	nd	nd	nd	0.09%

Table 4. Percent impurity relative to nicotine in e-liquids and e-cigarette cartridge extracts (average, n=3, nd = not detected).

CONCLUSIONS

- **Simultaneous determination of nicotine and 7 related impurities in e-cigarette products in less than 7 minutes.**
- **Sensitive and selective detection of nicotine (mg) using UV detection and trace impurities (µg) using MS on a single instrument platform.**
- **Simplified workflow using single dilution of e-liquid samples eliminates the need for multiple methods and analyses.**
- **The measured nicotine levels in e-liquids were observed to be within 15% of labeled claims.**
- **The levels of impurities in e-liquids ranged from not detected to 2.79% of nicotine concentration.**
- **Single quadrupole MS detection provides orthogonal detection to UV for existing LC workflows.**
- **Benefits of QDa over optical detectors:**
  - **Detection of compounds with no or poor UV chromophores**
  - **Low level analytes in complex samples**
  - **Peak tracking and peak purity during method development and validation**
  - **Developing multi-component methods**

References

1. Trehy et al, Analysis of Electronic Cigarette Cartridges, Refill Solutions, and Smoke for Nicotine and Nicotine Related Impurities, Journal of Liquid Chromatography and Related Technologies, 34:14, 1442-1458, 2011.

2. 2012 E-liquid Manufacturing Standards, American E-liquid Manufacturing Standards Association.

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