APPLICATION NOTE

Quantification of nicotine in liquids for electronic cigarettes

A-106.1

Keywords

Tobacco, e-cigarettes, regulation, limit test, legislation

Introduction

The increasing availability of novel and emerging tobacco and related nicotine containing products, particularly liquids for electronic cigarettes (e-liquids), will prompt authorities to create and implement new regulations. E-liquids are usually composed of nicotine in propylene glycol, glycerol, and water with added flavorings. In Europe consumers can buy e-liquids containing nicotine up to 20 mg/mL.

Scope

An HPTLC method was developed for the quantification of nicotine by image-based evaluation or scanning densitometry. The absence or presence of nicotine may be confirmed by hR_F values, UV and MS spectra compared to those obtained with the reference substance. To check compliance with the European limit of 20 mg/mL for nicotine in e-liquids, a limit test for controlling the maximum authorized content is proposed. Furthermore, the actual nicotine content can either be determined based on a single-point or a five-point calibration.

Required or recommended devices

Automatic TLC Sampler 4, Automatic Developing Chamber ADC 2, TLC Visualizer 2, TLC Scanner 4, visionCATS, UV Cabinet 4, TLC-MS Interface 2, Waters ACQUITY QDa Detector (Performance), Empower® or MassLynx® software.

Standards and Samples

Stock solutions of nicotine were prepared in propylene glycol/glycerol solution (1:1) at different levels from 0.135 mg/mL to 20 mg/mL. 0.3 g of each stock solution or sample was dissolved in methanol (final volume 10 mL).

Chromatography

Stationary phase	HPTLC Si 60 F_{254}, 20 x 10 cm (Merck)
Sample application	Bandwise application, 15 tracks, band length 8 mm, track distance 11.4 mm, distance from left edge 20 mm, distance from lower edge 8 mm, application volume 2 µL.
Developing solvent	Toluene - acetone - diethylamine 10:10:1 (v/v/v)
Development	In the ADC 2 with 20 min chamber saturation (with filter paper); after conditioning at 33% relative humidity for 10 min using a saturated solution of magnesium chloride
Developing distance	70 mm (from the lower edge)

Plate drying	Drying 5 min in the ADC 2
Documentation	With the TLC Visualizer 2 under UV 254 nm.
Densitometry	Densitometric analyses are performed at 260 nm in absorption mode, at a scanning speed of 50 mm/s using a slit of 5.0 × 0.2 mm
MS confirmation	Localizing the nicotine zone on the HPTLC plate: Marking zones to be eluted by using TLC Visualizer or UV Cabinet 4 under UV 254 nm or based on R_F values of reference substances obtained by scanning densitometry. Target zones are directly eluted using the TLC-MS Interface 2 with oval elution head into the ACQUITY QDa Detector at a flow rate of 0.5 mL/min with methanol + 0.1% formic acid. For a full scan spectrum it is recommended to first elute a blank, which can be subtracted from the spectra of the target zones.
MS parameter	The ACQUITY QDa Detector is operated in ESI^+ mode using default settings. The ESI capillary is set to 0.8 kV, cone voltage to 15 V, and desolvation temperature at 600 °C. A full scan mass spectrum between m/z 50 - 500 is acquired at a sampling rate of 10.0 points/sec (continuum). Data processing and evaluation of mass spectra are performed with Empower. For routine use in quality control Single Ion Recording (SIR) can be performed.

Results

System Suitability Test (SST) under UV at 254 nm:
Nicotine zone at RF ~ 0.56

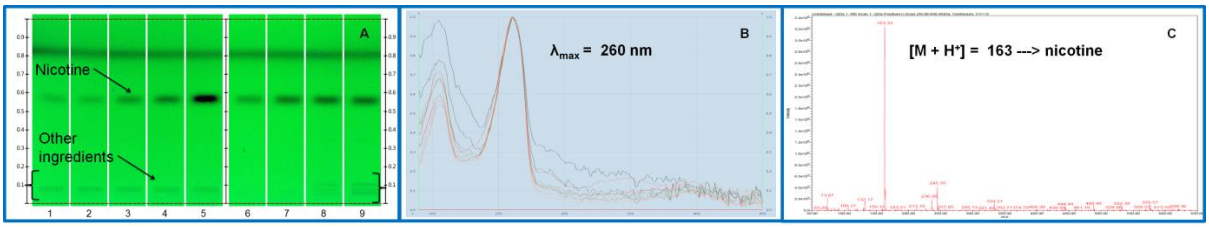

Fig. 1 Left: Chromatogram under UV 254 nm (tracks 1–5: reference solutions, tracks 6–7: samples without flavor; tracks 8–9: samples with flavor); center: UV spectra of all reference and sample solutions; right: mass spectrum of the eluted nicotine zone in a sample at m/z 163 $[M+H]^+$

a) Limit test for samples at 20 mg/mL

A reference solution with 20 mg/mL nicotine (in propylene glycol/glycerol) and 9 e-liquid samples were applied. All samples show values below that of the reference solution.

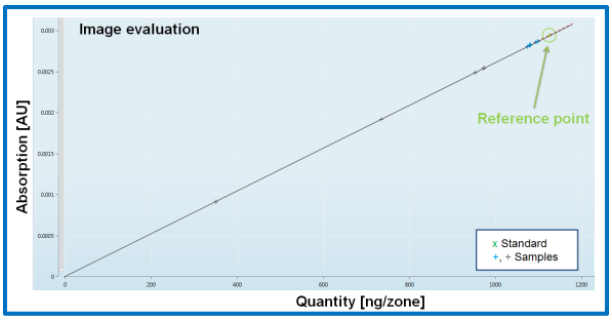

Fig. 2 Calibration curve (image evaluation at UV 254 nm)

NOTE: The presented results are to be regarded as examples only!

Please contact CAMAG for more application notes and products!

b) Single-point calibration

The consistency of the nicotine labeling of 3 samples, claimed to contain 18 mg/mL was tested. The measured concentrations were slightly higher, but below the European limit.

Sample	Claimed concentration (mg/mL)	Measured concentration (mg/mL)	
		Scan	Image
1	18.00	18.50	19.11
2	18.00	19.01	19.79
3	18.00	19.45	19.88

**extrapolation range of 10%*
***extrapolation range of 15%*

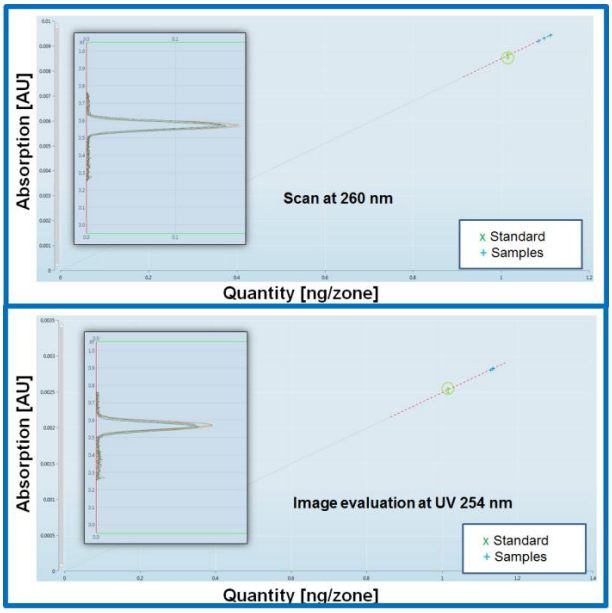

Fig. 3 Single-point calibration curves of image-based anddensitometric determination

c) Five-point calibration

For quantification of e-liquids containing different concentrations of nicotine a five-point calibration was used.

Sample	Claimed concentration (mg/mL)	Measured concentration (mg/mL)	
		Scan	Image
1	6.00	6.50	5.74
2	12.00	12.33	12.56
3	18.00	17.67	17.75
4	18.00	17.25	17.90
5	18.00	17.88	18.13
6	18.00	16.59	16.72

7	18.00	16.36	16.20
8	18.00	16.51	16.36
9	18.00	16.64	17.57

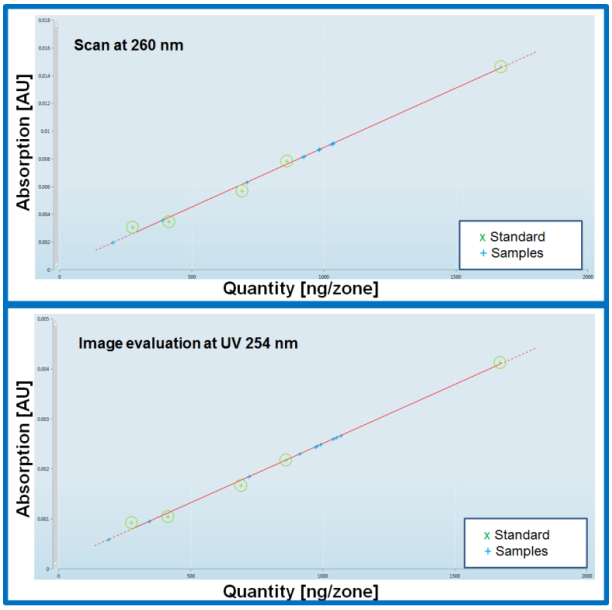

Fig. 4 Five-point calibration curves of image-based and densitometric determination

Conclusion

The proposed method is applicable to e-liquid samples containing different concentrations of nicotine. No interference with excipients and flavor ingredients was found. Depending on the regulatory requirements to be met, analytical approaches can be adopted to either include image-based evaluation or densitometric determination of nicotine.

Contact

Tiên Do, CAMAG, tien.do@camag.com

Literature

[1] Summary of Results: Laboratory Analysis of Electronic Cigarettes Conducted by the FDA: http://www.fda.gov/ NewsEvents/PublicHealthFocus/ucm173146.htm

[2] Kenyon et al., J AOAC Int 81 (1998) 44–50

[3] Dr. Tiên Do, CAMAG Bibliography Service CBS 116 (2016) 13-15