Australian Government
Department of Health, Disability and Ageing
Therapeutic Goods Administration

Screening, identification and content of nicotine in e-cigarette liquids

June 2025

Contents

Chromatographic conditions _____4
System suitability _____4
Buffer preparation ----------4
Mobile phase/Diluent preparation ----------4
Identification and content of nicotine in samples _____5
Standard stock (200 µg/mL) – prepare in duplicate ----------5
Working standard (20 µg/mL)----------5
Sample preparation – prepare in duplicate: ----------5
Acceptance criteria: ----------5
Example chromatograms _____6

Screening, identification and content of nicotine in e-cigarette liquids

This method describes the screening, identification and content of nicotine in e-cigarette liquids by Ultra-Performance Liquid Chromatography (UPLC).

Chromatographic conditions

Table 1. General Chromatographic conditions

Apparatus:	Waters Acquity H-Class UPLC system with PDA detector; or Agilent 1260 with DAD detector.
Column:	Waters Acquity BEH C18, 2.1 × 50 mm, 1.7 µm, with Waters Acquity Column in-Line filter, 0.2 µm
Mobile Phase:	85 : 15, 10mM Ammonium Formate (pH10.1) : Acetonitrile
Diluent:	Mobile Phase
Flow rate:	0.5 mL/min
Injection volume:	1.5 µL
Sample temperature:	20 °C
Column temperature:	35 °C
Detection:	261 nm. PDA detection from 190-400 nm. Resolution 1.2 nm, 20 Hz.
Needle wash:	50 : 50, Methanol : HPLC water
Purge wash:	20 : 80, Acetonitrile : HPLC water
Seal wash:	20 : 80, Acetonitrile : HPLC water
Run time:	5 minutes

System suitability

The system suitability solution will be a standard solution.

For six replicate injections of the system suitability solution, the % RSD on retention time and response (Area, Height or Internal Standard Ratio) is ≤ 1.0 % and ≤ 2.0% respectively. The Symmetry Factor for the nicotine peak is between 0.8 and 2.5.

Buffer preparation

To prepare 1 litre of buffer solution, measure approximately 995mL H_2O, then add 378µL formic acid and 4.98mL 30% ammonium hydroxide solution. Mix, then check the pH of the buffer solution. Adjust to pH 10.1 as necessary with ammonium hydroxide solution or formic acid.

Mobile phase/Diluent preparation

85 : 15, Buffer : Acetonitrile

Identification and content of nicotine in samples

Standard stock (200 µg/mL) – prepare in duplicate

Accurately prepare a standard stock solution in diluent with an equivalent concentration of nicotine (as base) of 200 µg/mL.

Note: Stock standard solution is stable for 4 weeks, stored at 4°C and protected from light.

Working standard (20 µg/mL)

Dilute the Standard Stock 10x in diluent.

Sample preparation – prepare in duplicate:

Accurately dilute 100 µL of sample to 15 mL with diluent and mix thoroughly. Further dilute to obtain a solution with a nicotine concentration of approximately 20 µg/mL. See Table 2 for suggested dilutions.

Note: Dilutions can be performed using a positive displacement piston-operated volumetric apparatus (POVA).

Table 2. Suggested dilutions for varying Nicotine strengths (mg/mL)

Nicotine Strength	Dilution 1 (stock)		Dilution 2 (working sample)		Dilution Factor
	Volume of Sample	Volume of Diluent	Aliquot of Stock Sample	Volume of Diluent added	
1.5-5 mg/mL	100 µL	14,900 µL			150x
5-15 mg/mL			250 µL	500 µL	450x
15-30 mg/mL			225 µL	1275 µL	1000x
30-50 mg/mL			90 µL	1410 µL	2500x

Acceptance criteria:

The retention time for the nicotine peak in the Sample chromatogram should be ± 0.1 minutes of the retention time of the corresponding peak in the Standard chromatogram.

The symmetry factor of the nicotine peak should be between 0.8 and 2.5.

The absorption maxima in the spectrum of nicotine in the sample chromatogram shall be the same wavelength as that of the reference standard within ± 2 nm range.

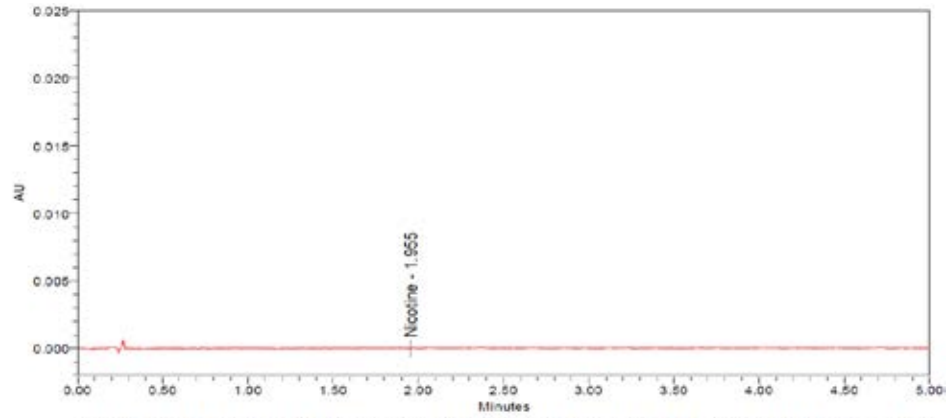
Standard: %RSD ≤ 2.0; Standard correlation 98.0-102.0%.

Sample replicates: %RSD ≤ 2.0

The test is invalid when there is interference with the peak of nicotine.

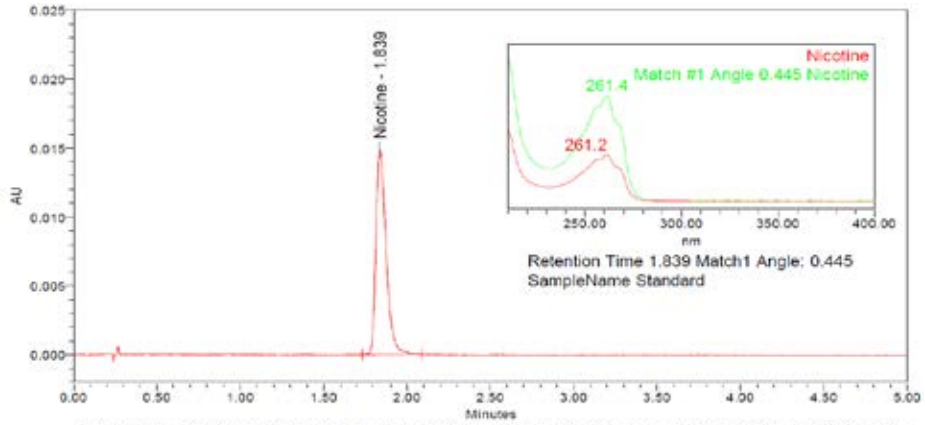
Example chromatograms

Blank:



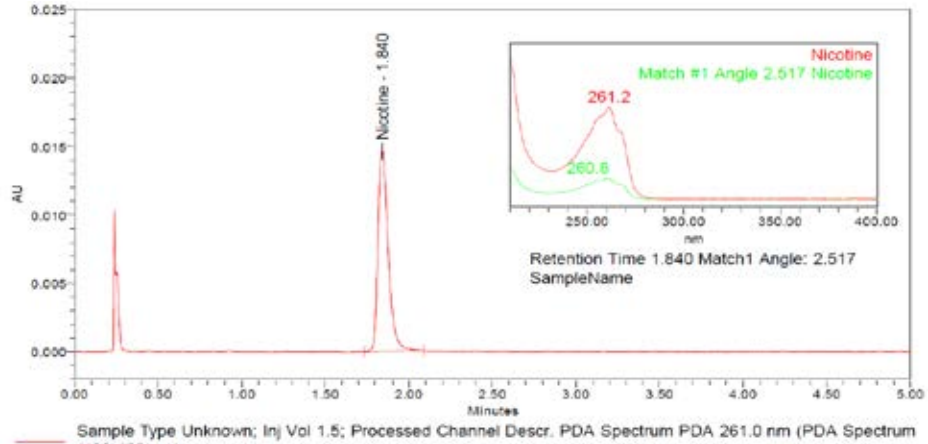
	Sample Name	RT	Area	Symmetry	N (Plates)	Name	Peak Type	Amount
1	Blank	1.96				Nicotine	Missing	

Standard (20 ppm):



	Sample Name	RT	Area	Symmetry	N (Plates)	Name	Peak Type	Amount
1	Standard	1.84	63501	1.47	4551	Nicotine	Found	0.020

Sample:



	Sample Name	RT	Area	Symmetry	N (Plates)	Name	Peak Type	Amount
1		1.84	62833	1.47	4564	Nicotine	Found	19.619

Therapeutic Goods Administration

PO Box 100 Woden ACT 2606 Australia
Email: info@tga.gov.au Phone: 1800 020 653 Fax: 02 6203 1605
Web: tga.gov.au