CORESTA RECOMMENDED METHOD Nº 80

USE OF THE PART-FILTER METHOD FOR THE ESTIMATION OF SMOKERS' EXPOSURE TO NICOTINE AND NICOTINE-FREE DRY PARTICULATE MATTER

(January 2016)

0. INTRODUCTION

The CORESTA Smoking Behaviour Sub-Group (TSB) conducted two Part-Filter Method (PFM) ring trials (collaborative studies) involving 12 laboratories from 8 countries in 2011 and 10 laboratories from 10 countries in 2013. The objective of the ring trials was to gain a measure of repeatability and reproducibility of the PFM across the participating laboratories and to ascertain whether changes introduced to the protocol resulted in an improved performance. The conclusion of the second ring trial characterized the PFM suitable for a Recommended Method.

1. FIELD OF APPLICATION

This Recommended Method is applicable to the estimation of human smoke exposure to nicotine and NFDPM (nicotine-free dry particulate matter, also known as tar) using linear calibration regression equations between mainstream smoke yields and corresponding analytes from part-filter extracts. The method is referred to as the Part-Filter Method (PFM). The cigarettes under investigation must contain a cellulose acetate filter element at the mouth-end. The method has been successfully applied to population of smokers smoking cigarettes between 1 and 15 mg ISO NFDPM yields as determined by ISO 4387.

2. NORMATIVE REFERENCES

ISO 10315:2013

Cigarettes – Determination of nicotine in smoke condensates – Gas-chromatographic method

ISO 10362-1:1999

Cigarettes – Determination of water in smoke condensates – Part 1: Gas-chromatographic method

ISO 3308:2012

Routine analytical cigarette-smoking machine – Definitions and standard conditions

ISO 4387:2000

Cigarettes – Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine

Automotive Industry Action Group (AIAG) (2010)

Measurement Systems Analysis, 4th edition. Chrysler Group LLC, Ford Motor Company and General Motors Supplier Quality Requirements Task Force

CORESTA Recommended Method N° 9

Determination of Nicotine in Cigarette Filters by Gas Chromatographic Analysis

3. METHOD SUMMARY

The cigarettes under investigation are smoked using a routine analytical smoking engine which can be of rotary or linear design. The cigarettes should not be conditioned prior to calibration smoking and must be of the same batch which is distributed to human subjects. It is recommended that cigarettes are taken from freshly opened packs prior to machine smoking.

Estimated human exposure to nicotine and NFDPM are determined by constructing linear calibration regression equations. Calibration regressions are generated by determining mainstream nicotine and NFDPM yields against filter tip nicotine and filter tip solanesol from analysis of part-filters. The derived linear regressions are used to estimate human yields from part-filters collected from the subjects, it is necessary to construct regression equations of each cigarette product investigated.

Nicotine and water captured on a Cambridge Filter Pad (CFP) are analysed following the general methodology listed in ISO 10315:2013, ISO 10362-1:1999, ISO 3308:2012 and ISO 4387:2000 (or their equivalents) allowing the calculation of the nicotine and NFDPM yields at each of the smoking regimes used in calibration smoking detailed in this CRM.

Immediately following the completion of each smoking regime each cigarette is extinguished intact and the cigarette butt is removed from the smoking machine. The whole smoked filter is then cut either using a purpose made filter cutter or with a blade, the cut filter is now referred to as a part-filter. The analysis for nicotine ~~on~~ in a part-filter is different from the methodology in CRM N° 9. The length of each part-filter is then measured with calibrated callipers prior to solvent extraction. Part-filters are extracted in methanol containing an internal standard, followed by gas chromatographic (GC) analysis with flame ionisation detection (FID) and high performance liquid chromatography (HPLC) with UV detection. The suggested internal standard (IS) for nicotine analysis is n-heptadecane, however an equivalent compound may be substituted; the solanesol analysis does not make use of an internal standard. Methanol must be used as the extracting solvent since possible alternatives such as propan-2-ol or ethanol do not extract part-filters with sufficient efficiency. Extracted nicotine from part-filters is used to estimate human nicotine yields whereas solanesol is used to estimate NFDPM yields. However it is also possible to obtain estimated nicotine and estimated NFDPM yields from solanesol and nicotine extracted from part-filters respectively. It is not necessary to complete *both* solanesol and nicotine analyses on the tip extract solutions, however, nicotine is invariably determined.

It is important to appreciate that the method is applicable to carbon-containing filters, however, the part-filter element cut from the whole filter must not contain carbon. This is because the presence of carbon in the portion of the filter which is extracted in TES (tip extraction solution) can interfere with the added Internal Standard (IS).

4. APPARATUS

Normal laboratory apparatus, in particular, the glassware should be cleaned, rinsed with deionised water and dried prior to use

Analytical balance; capable of reading to 4 decimal places

Solvent dispenser system able to deliver 20 ± 0.1 mL of TES

Orbital flask shaker capable 160 rpm

5 L volumetric flask for preparation of TES (or similar)

150 mL flat-bottomed flasks

200, 100, 50 and 20 mL amber volumetric flasks and stoppers

Calibrated pipettes of suitable volumes

Amber GC vials and caps

Calibrated digital calipers (for measuring lengths to ± 0.1 mm)

Filter cutters or suitable cutting device

Gas chromatograph, capable of flame ionisation detection (FID), with a suitable column installed

HPLC system with UV detector with data handling software, column heater and auto sampler cooler, with suitable column installed

Ultra Sonic Bath (for solubilisation of solanesol)

5. REAGENTS AND SUPPLIES

Use only reagents of recognized analytical grade. Reagents specific to each analytical approach are identified as either for nicotine or solanesol analyses

Helium GC carrier gas, air for FID detector and hydrogen fuel gas for FID detector (for nicotine analysis)

n-heptadecane, minimum purity 99 %, CAS number 629-78-7 (IS used in nicotine analysis)

Nicotine, minimum purity greater or equal to 99 %, CAS number 54-11-5. A further source of nicotine of the same purity but of a different batch can be used to prepare a calibration check standard. (Care must be taken when handling pure nicotine which is a poison, please observe all relevant local safety instructions)

Solanesol, purity > 95 % CAS number 13190-97-1

Propan-2-ol – AR Grade, CAS number 67-63-0 (for preparation of IS stock solution)

Methanol – AR Grade, CAS number 67-56-1 (for preparation of TES)

Methanol, HPLC Grade, CAS number 67-56-1 (mobile phase in solanesol analysis)

Acetonitrile HPLC Grade, CAS number 75-05-8 (mobile phase in solanesol analysis)

6. PREPARATION OF STANDARDS AND SOLUTIONS

6.1 Extracting solution

n-heptadecane stock standard

Weigh 25.0 ± 0.05 g of n-heptadecane into a 200 mL volumetric flask and dilute to volume with propan-2-ol. Sonicate until dissolved, the solution remains stable for 12 months at room temperature.

Tip extraction solution (TES)

Add 2 mL of n-heptadecane Stock Standard to 2.5 L methanol (AR grade) in a 5 L volumetric flask and make up to the mark with methanol. Cap and invert several times to ensure thorough mixing before pouring into the dispenser reservoir. This gives an internal standard concentration of approximately 0.05 mg/mL. TES remains stable for 3 months at room temperature.

6.2 Preparation of nicotine standards

Amber glassware is used for the preparation and storage of the standards.

Nicotine calibration stock standard

Weigh 0.4000 g (± 0.005 g) nicotine into a 200 mL amber volumetric flask and make up to volume with TES. The exact weight of nicotine used is recorded and adjusted for purity. The concentration is then calculated using the adjusted weight as shown below in Equations 1 and 2. This gives a concentration of approximately 2 mg/mL.

The nicotine calibration stock standard remains stable for 3 months when stored at 4 °C.

Equation 1

Calculation for weight of nicotine adjusted for purity = (weight of nicotine (g) / 100) * purity

Equation 2

Calculation for nicotine concentration in stock (mg/mL) = (purity adjusted weight of nicotine used (g) * 1000) / volume of stock standard (mL)

Nicotine calibration working standards

Make the standards up to volume with TES as detailed in Table 1. The standards remain stable for 1 month when stored at 4 °C.

Table 1 - Preparation of Working Nicotine Standards

Stock Standard (mL)	Final volume (mL)	Nominal concentration (mg/mL)
0.05	20	0.005
0.40	20	0.040
1.00	20	0.100
2.00	20	0.200
3.00	20	0.300

The preparation of an *independent* nicotine quality check standard is described below.

6.3 Nicotine calibration check stock standard

Weigh 0.4000 (± 0.005 g) nicotine into a 200 mL amber volumetric flask and make up to volume with TES as detailed above for the nicotine calibration stock standard. This gives a nominal concentration of 2 mg/mL. The calibration check stock standard remains stable for one month when stored at 4 °C.

Nicotine calibration check standard - 0.05 mg/mL

Pipette 5 mL of the nicotine calibration check stock standard into a 200 mL amber volumetric flask and make up to the mark with TES. The independent calibration check standard has a nominal concentration of 0.05 mg/mL.

The calibration check standard should be run at regular intervals within the analysis sequence, for instance every 10 samples. The acceptance criterion of the calibration check standard is ±10% of its nominal concentration value.

6.4 Preparation of solanesol standards

Solanesol calibration stock standard

Weigh out 0.015 ± 0.0001 g solanesol in a weighing boat and quantitatively transfer into an amber 50 mL volumetric flask. Dissolve the solanesol in HPLC grade methanol with the assistance of an ultrasonic bath, then make up to 50 mL with the methanol.

Solanesol calibration working standards

Prepare calibration standards in amber flasks using calibration stock standard, detailed in Table 2:

Table 2 - Preparation of Working Solanesol Standards

Stock Standard (mL)	Final Volume (mL)	Nominal concentration (µg/mL)
10.000	50	60.00
5.000	50	30.00
2.000	50	12.00
1.000	50	6.00
0.150	50	0.90
0.075	50	0.45

6.5 Solanesol calibration check standard – 15 µg/mL

The preparation of an independent solanesol quality check standard is described below. Weigh 0.015 ±0.0001 g solanesol into a 50 mL amber volumetric flask following the method for the calibration stock. This gives a concentration of 300 µg/mL.

Pipette 2.5 mL of this stock standard into a 50 mL amber volumetric flask, make up to the mark with HPLC grade methanol. This calibration check standard will contain 15µg/mL solanesol. All stock solutions, calibration standards and the calibration check standard should be stored in a -20 °C freezer. All standard and stock solutions are stable for 2 months when stored at -20 °C.

The calibration check standard should be run at regular intervals within the analysis sequence, for instance every 10 samples. The acceptance criterion of the solanesol calibration check standard is ±10% of its nominal concentration value.

7. PROCEDURE

7.1 Calibration smoking

Calibration smoking must be undertaken at approximately the same time as the human subjects are performing their smoking to ensure similar aging on both sets of tips – calibration smoked tips and human smoked tips.

Laboratories should construct linear regression calibration equations using the smoking regimes detailed in Table 3. The purpose of machine-smoking cigarettes for calibration is to relate the amount of 'tar' or nicotine that exits the filter to the amount filtered by the tip. This removes the constraints required by standardized smoking methods to obtain an absolute yield at controlled conditions. These constraints include pre-conditioning the cigarettes, smoking

the cigarettes at a controlled temperature and humidity or maintaining a fixed air-flow rate around the cigarettes while smoking (ISO 3308). Therefore, the test cigarettes are smoked according to ISO 3308:2012 with the following exceptions:

- The smoking machine air flow is not specified
- The cigarettes are not preconditioned prior to smoking
- Quarter portions of CFPs are not used to wipe out the CFP holder
- CFPs are weighed outside of the pad holder as detailed below

Table 3 - Calibration Smoking Regimes

Smoking Regime	Puff Volume	Puff Duration	Puff Interval	CFP diameter	Smoked length/puffs	Number of cigarettes
A	35 mL	2.0 s	60 s	44 mm	Over Tipping+3 mm	5
B	60 mL	2.0 s	30 s	44 mm	4 puffs	5
C	60 mL	2.0 s	30 s	44 mm	Over Tipping+3 mm	3
D	70 mL	2.0 s	30 s	44 mm	Over Tipping+3 mm	3
E	40 mL	2.0 s	30 s	44 mm	Over Tipping+3 mm	5
F	70 mL	1.5 s	20 s	44 mm	Over Tipping+3 mm	3
0					Unsmoked Blank	5

CFPs are weighed *outside* the CFP holder[1]. To to minimise evaporative losses, the time interval between the removal of the CFP from the holder, the weighing of the CFP and placing the CFP in a sealed vessel is kept to the minimum. CFPs must be extracted soon after smoking and analysed for nicotine and water on the same day as smoking, following ISO 10315:2013, ISO 10362-1:1999, ISO 3308:2012 and ISO 4387:2000. Three cigarettes are smoked for certain regimes to avoid breakthrough of the smoke condensate. Not all smoking machines are able to perform Regime F, in which case this regime is not run.

Following the calculation of total particulate matter (TPM), smoke yields are determined by extracting each CFP in 20 mL pad extraction solution, the preparation of which is detailed in ISO 3308:2012. The measurements of water and nicotine yields are subtracted from the TPM to calculate NFDPM (in mg/cig) at each of the smoking regimes. For Regime 0 nicotine and NFDPM yields are set to zero. The regimes in Table 3 must be repeated on two different days to account for daily variation.

[1] CFPs are weighed *outside* the CFP holder for the following reason: combustion water increases disproportionately to the amount of 'tar' as puff volumes used in calibration smoking are increased resulting in the deposition of moisture in inaccessible parts of the CFP holder which are not incorporated into the extract solution even after wiping with the quartered pad specified in ISO 3308:2012. The under-reporting of water and consequential over-reporting of NFDPM would produce a systematic error in the part-filter method that is avoided by weighing the CFP only which contains the overwhelming proportion of NFDPM emitted from the cigarettes. The weight of the CFP gives the pad TPM which is used to derive the NFDPM after subtracting for water and nicotine.

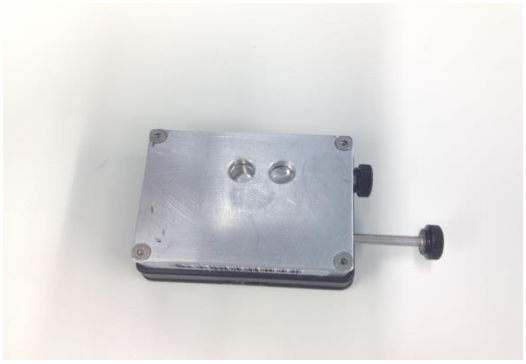
7.2 Treatment of part-filters

The length of part filter cut down stream of ventilation depends on the filter design of the test product. Smoked cigarette whole filters obtained from calibration smoking are usually cut to 10 mm, 7 mm or 5 mm mouth-end portions. Filter segments containing carbon must be excluded from the part-filter. The tips are usually cut using a specially designed filter cutter similar to that shown in Picture 1.

7.2.1 Normalisation of part-filter lengths

Part-filters from machine and human smoking are normalised with respect to their nominal length. Digital callipers, such as illustrated in Picture 2, measure part-filter lengths to within ±0.1 mm.

The mean part-filter length is calculated for each extraction sample of five part-filters (three in the case of smoke regimes C, D and F). The calculation of the normalisation factor is shown in Equation 3.



Picture 1: Filter cutter

Picture 2: Digital callipers

Equation 3

normalisation factor = x / Σ(part-filter lengths) / n

Where *n* is the number of part-filters and *x* is the nominal part-filter length.

The normalisation factor (to 3 decimal places) is applied to tip nicotine measurements as shown in Equation 4. The solanesol analysis is treated similarly.

Equation 4

Normalised nicotine (mg/tip) = concentration nicotine (mg/mL) * extraction volume (mL) * normalisation factor / number of tips extracted

7.2.2 Extraction of part-filters

Once the part-filters are extracted they are referred to as tips. Five part-filters (three in the case of smoke regimes C, D and F) are extracted in 20 mL of TES in a 150 mL round-bottom flask. The extraction is completed on an orbital shaker operating at 160rpm for a minimum of 30 minutes. The resultant solution is vialed and analysed within 8 days (stored at +4°C) for nicotine or solanesol.

Human smoked tips must be analysed alongside the smoked calibration tips to ensure consistency with calibration smoking. It is important that part-filters are not exposed to prolonged high temperature conditions during shipping which may result in loss of nicotine by evaporation. Part-filters (calibration and human tips) may be frozen and stored

indefinitely, however, both sets of part-filters need to be treated the same. Tips are usually stored and shipped in screw top aluminium tins, example shown in Picture 3 and a set of smoked tips is shown in Picture 4. Any human tips which are contaminated, for instance contain lipstick staining, must be removed from the analysis set. Part-filters should be allowed to reach ambient temperature prior to length measurement.

Picture 3: Aluminium tin

Picture 4: Set of smoked part-filters

7.2.3 Analysis

In both nicotine and solanesol analyses calibration standards should be analysed at the start of the run, followed by the samples with a calibration check standard placed after every ten samples. Regime 0 provides blank tips to be analysed for tip nicotine and tip solanesol. The quantities measured in these tips will be low, however, it is important to include them in the calibration graphs.

A quality control chart is constructed by plotting CFP nicotine against CFP NFDPM yields (12 data points – Regime 0 not included), example depicted in Figure 1. Smoking linearity is demonstrated if the square of the linear coefficient of determination, R^2, is greater than 0.95. Failure to achieve linearity is most often caused by smoking engine errors. If solanesol analysis is also undertaken, it is advisable to construct a tip quality control chart by plotting tip solanesol against tip nicotine (14 data points – Regime 0 included), example depicted in Figure 2. Linearity is demonstrated if the square of the linear correlation coefficient, R^2, is greater than 0.95. Failure to achieve linearity here is most often ascribed to errors in the analytical methods.

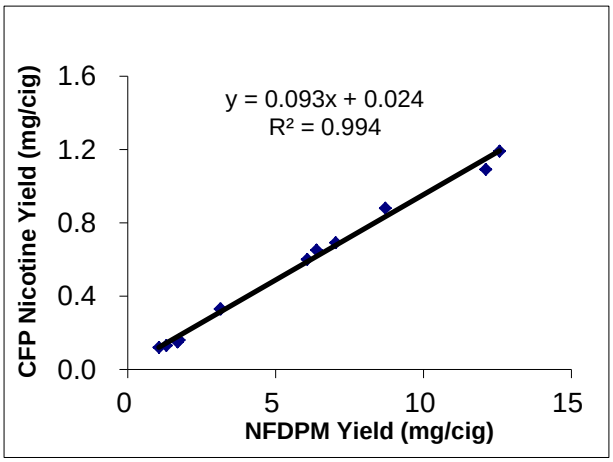


Figure 1: Smoke quality control chart

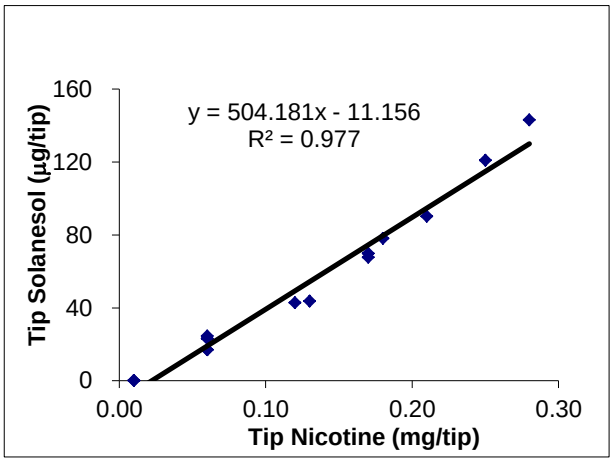


Figure 2: Tip quality control chart

The nicotine calibration graph is produced by plotting CFP nicotine yield against tip nicotine (14 data points – Regime 0 included), example depicted in Figure 3. The NFDPM calibration graph is produced by plotting NFDPM yield against tip solanesol (14 data points – Regime 0 included), example depicted in Figure 4. The square of the linear correlation coefficient, R^2, is expected to be greater or equal to 0.95 for both these graphs. From the graphs the parameters of gradient and y-axis intercept for the two the linear regression equations may be derived. Nicotine and NFDPM yields of human smokers may be estimated from tip nicotine and tip solanesol measurements by applying the relationships shown in Equations 5 and 6 respectively.

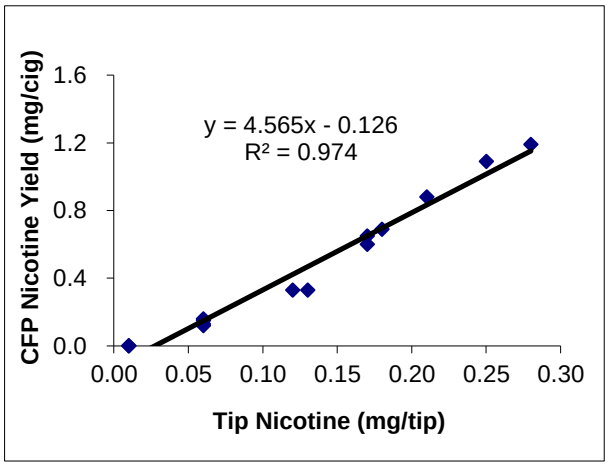


Figure 3: Nicotine calibration graph *G1*

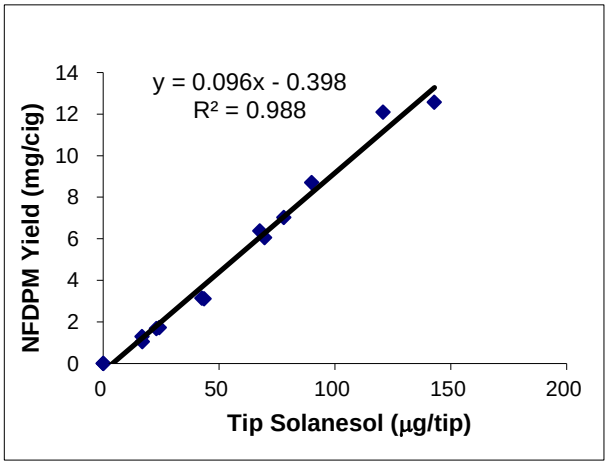


Figure 4: NDFPM calibration graph *G2*

Equation 5

Estimated nicotine yield = slope (*G1*) * tip nicotine + intercept (*G1*)

Equation 6

Estimated NFDPM yield = slope (*G2*) * tip solanesol + intercept (*G2*)

Occasionally human tip results will fall above the upper values of calibration smoking. The laboratory will need to decide if these results should be excluded; this may depend on number of subjects and how far beyond the upper smoking calibration point the results lie. Solutions can be diluted and re-analysed.

8. EXPRESSION OF RESULTS

Estimated nicotine and NFDPM yields (in mg/cig) are rounded to 3 decimal places.

9. REPEATABILITY AND REPRODUCIBILITY

An international collaborative study was conducted during 2013 using two cigarette products. Results from 8 laboratories (nicotine method) and 5 laboratories (solanesol method) were used in accordance with ISO 5725-6 procedures to calculate mean values, standard deviations and repeatability (r) and reproducibility (R) statistics (Tables 4 and 5).

Generally, a measurement system is deemed to be acceptable if the introduced error between participants remains under 10%. This was fulfilled for the estimation of nicotine yield by tip nicotine and very closely fulfilled for estimated NFDPM by tip solanesol.

Table 4: Results of International Collaborative Study – Estimated Nicotine yield

Estimated Nicotine Yield	Number of labs in statistical evaluation	Grand Mean (mg/cig)	Repeatability (r) (mg/cig)	Reproducibility (R) (mg/cig)	CV r (%)	CV R (%)
1 mg ISO tar low regime	8 of 8	0.29	0.050	0.104	6.0	12.5
1 mg ISO tar high regime	8 of 8	0.70	0.082	0.221	4.2	11.2
10 mg ISO tar low regime	8 of 8	1.01	0.161	0.289	5.6	10.1
10 mg ISO tar high regime	7 of 8	1.81	0.171	0.231	3.4	4.5

Table 5: Results of Inter-Laboratory Tests – Estimated NFDPM yield

Estimated NFDPM Yield	Number of labs in statistical evaluation	Grand Mean (mg/cig)	Repeatability (r) (mg/cig)	Reproducibility (R) (mg/cig)	CV r (%)	CV R (%)
1 mg ISO tar low regime	5 of 5	3.24	0.637	1.068	6.9	11.7
1 mg ISO tar high regime	4 of 5	7.30	0.499	1.773	2.4	8.6
10 mg ISO tar low regime	5 of 5	13.87	3.206	4.900	8.2	12.5
10 mg ISO tar high regime	5 of 5	25.15	3.066	6.168	4.3	8.7

ANNEX 1 and 2

It is recommended that nicotine extracted from tips is assayed by GC with flame ionisation detection. Two general methodologies are illustrated in Annex 1 and 2; however, any locally validated method may be used. Chromatograms from the two GC methods are illustrated showing standards and extracted tips examples.

Annex 1.

Gas chromatographic conditions for measurement of nicotine on CP Wax GC column

Gas Chromatograph: Agilent 6890 GC System, or equivalent

Column: CP Wax 52 CB (polyethylene glycol) 25 m x 0.53 mm x 2.0 µm or equivalent

Oven program:

oven temperature	160 °C
initial time	4 min
rate 1	80 °C/min
final temperature	230 °C
final time	2 min

Column conditions:

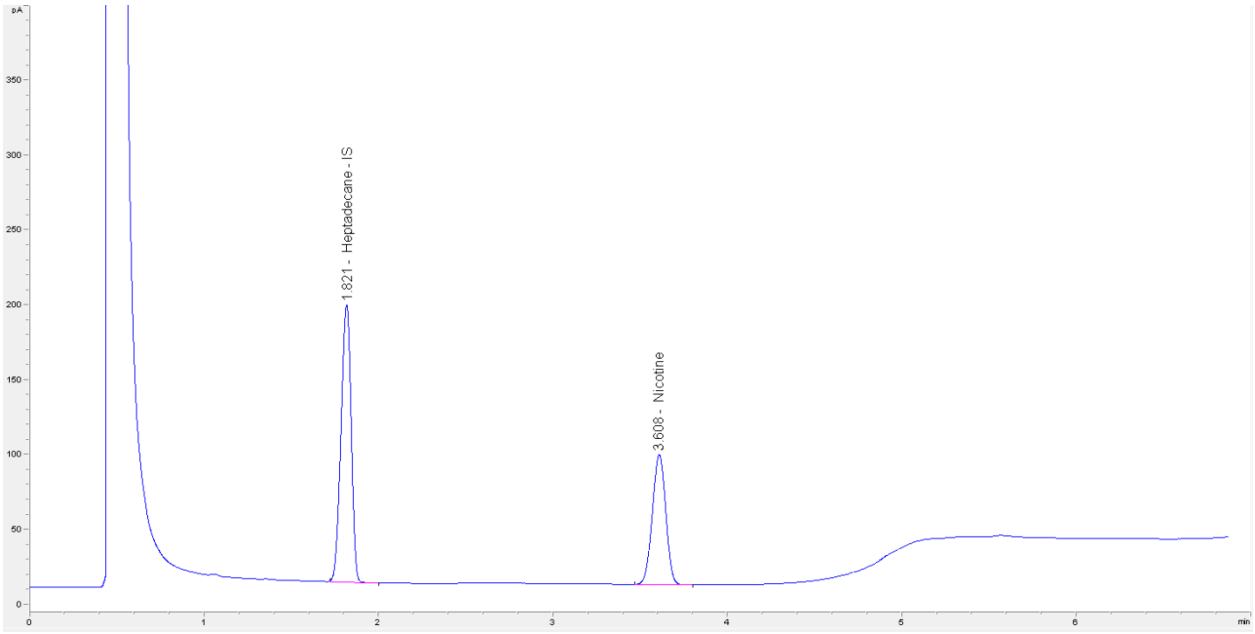
2	as above
column part number	Agilent Technologies CP7658
column type	polar phase
carrier gas	helium
inlet pressure	15 psi
column flow	~19 mL/min @ 160 °C
velocity	~137 cm/s
mode	constant pressure

Inlet:

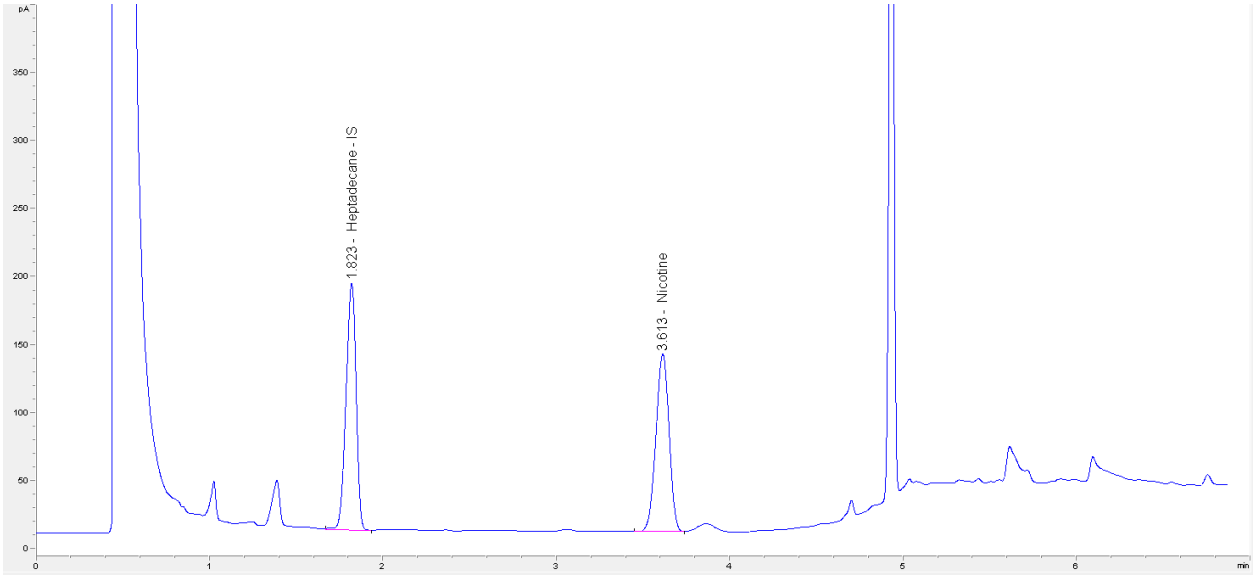
injection volume	1 µL
liner	focus
mode	splitless
temperature	250 °C
pressure	15 psi
purge time	0.1 min
purge flow	40.0 mL/min
gas saver	on
saver flow	20.0 mL/min
saver time	2.00 min

Detector: Flame Ionisation Detection

temperature	300 °C
H2 Flow	30 mL/min
air Flow	400 mL/min
make Up	constant (N2)
combined flow	30 mL
type	flame one
n-heptadecane elution time	~ 1.8 min
nicotine elution time	~ 3.6 min



Annex 1 Figure 1: Nicotine standard chromatogram using CP Wax 52 CB Column



Annex 1 Figure 2: Nicotine sample chromatogram using CP Wax 52 CB Column

Annex 2.

Gas chromatographic conditions for measurement of nicotine on HP-5 GC column

Gas Chromatograph: Agilent 6890 GC System or equivalent

Column: HP-5 (5% diphenyl, 95% dimethyl polysiloxane) 30 m x 0.32 mm x 0.25 μm or generic DB-5 equivalent

Oven program:

initial oven temperature	60 °C
initial time	2 min
ramp 1	6 °C/min
ramp temperature 1	180 °C
ramp 2	30 °C/min
ramp temperature 2	300 °C
hold time	2 min

Column conditions:

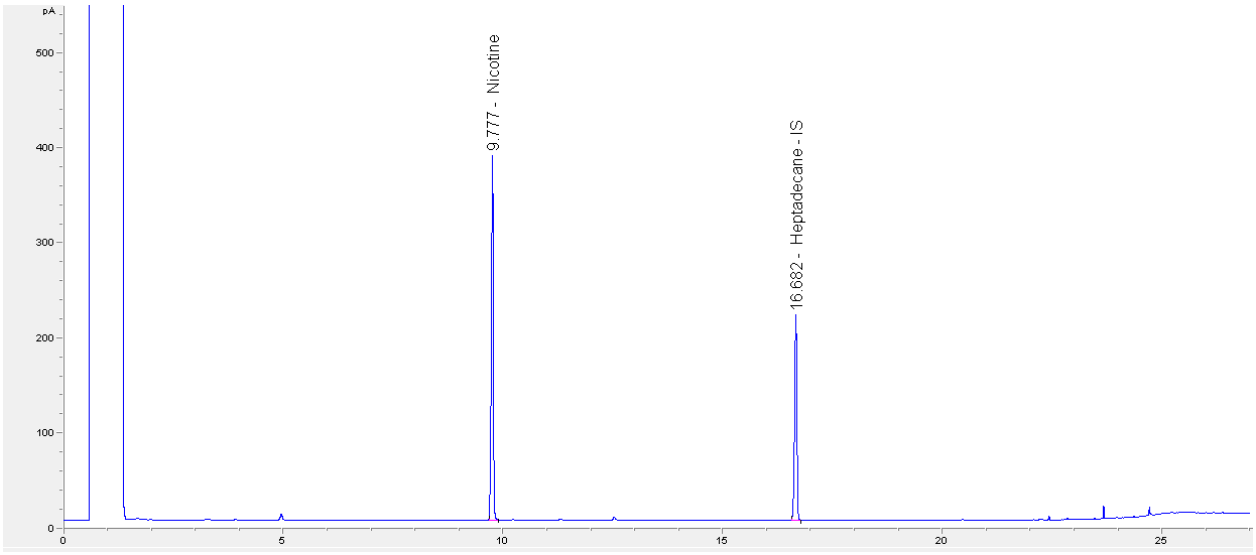
column	as above
column part number	Agilent Technologies 19091J-413
column type	non-polar phase
carrier gas	helium
inlet pressure	25 psi
velocity	82 cm/s
mode	constant flow

Inlet:

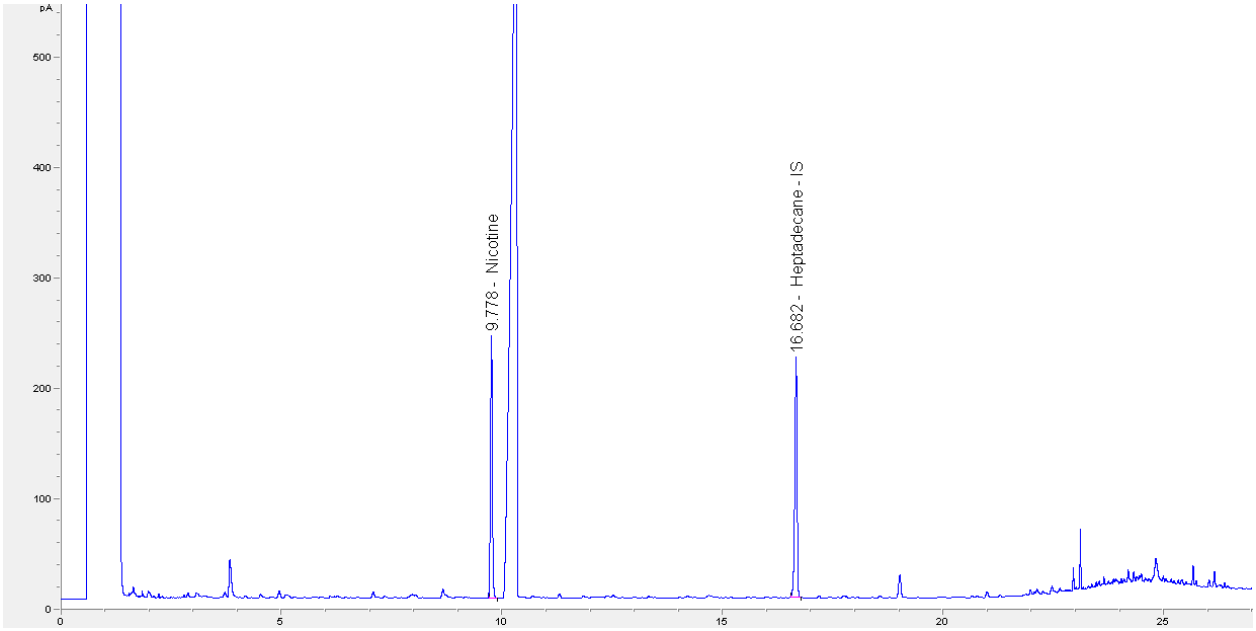
injection volume	1μL
mode	splitless
temperature	270 °C
pressure	25 psi
purge time	0.75 min
purge flow	60.0 mL/min
gas saver	on
saver flow	20.0 mL/min
saver time	2.00 min

Detector: Flame Ionisation Detection

temperature	300 °C
H2 Flow	30 mL/min
air flow	400 mL/min
make up	constant (N2)
nicotine elution time	~ 9.8 min
n-heptadecane elution time	~ 16.7 min



Annex 2 Figure 1: Nicotine standard chromatogram using HP-5 column



Annex 2 Figure 2: Nicotine sample chromatogram using HP-5 column

Annex 3.

Liquid chromatographic condition for measurement of solanesol

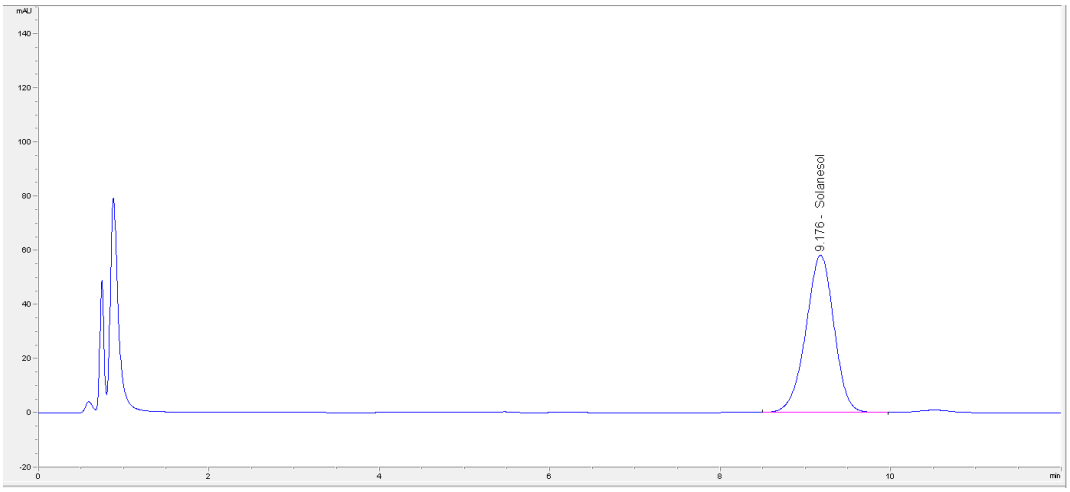
Liquid chromatograph Agilent 1100 series LC System or equivalent

column	Luna 3 µm C18(2) 100 Å (50 x 2 mm) (Phenomenex part number: 00B-4251-B0) or equivalent
guard cartridge	Security Guard C18 (4 x 3mm) (Phenomenex part number: AJO-4287) or equivalent
UV Detector	210 nm
injection volume	10 µL
column temperature	30 °C
auto sampler temperature	5 °C
mobile phase gradient	isocratic
mobile phase A	70 % HPLC grade methanol
mobile phase B	30 % HPLC grade acetonitrile
flow rate	0.3 mL/min
run time	12 min
solanesol elution time	~ 9.5 min

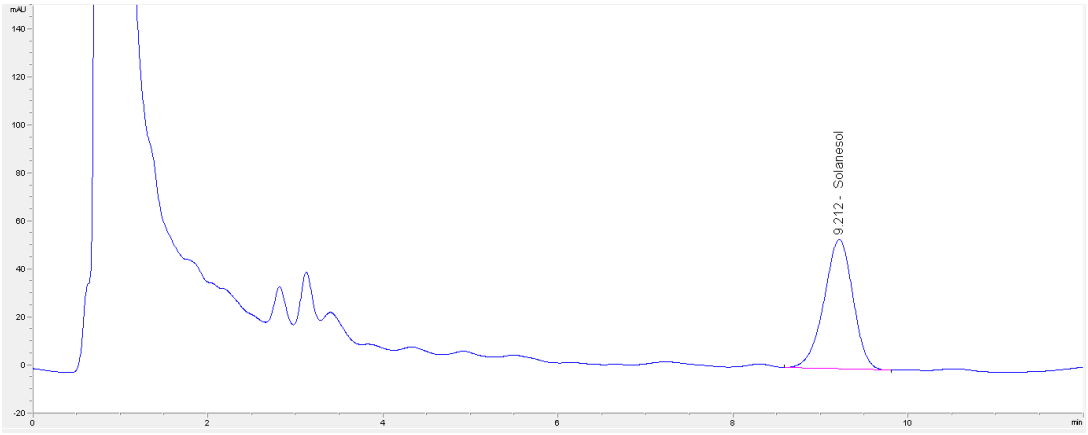
The system, with the UV lamp alight, is allowed to equilibrate with mobile phase for a minimum of 30 minutes before analysis.

Solanesol HPLC analysis

Reserve phase high performance liquid chromatography with UV detection is employed to quantify solanesol using a suitable column with isocratic elution. A suitable guard column may also be employed. The solanesol peak elutes at around 9.5 minutes. Example chromatograms from a standard and an extracted part-filter sample are shown in Annex 3 Figure 1 and Annex 3 Figure 2 respectively.



Annex 3 Figure 1: Solanesol standard chromatogram



Annex 3 Figure 2: Solanesol sample chromatogram