Cooperation Centre for Scientific Research Relative to Tobacco

Smoke Analytes Sub-Group

CORESTA Recommended Method No. 95

DETERMINATION OF AROMATIC AMINES IN MAINSTREAM CIGARETTE SMOKE BY GAS CHROMATOGRAPHY MASS SPECTROMETRY WITH NEGATIVE CHEMICAL IONISATION (GC/MS(NCI))

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0. INTRODUCTION

From 2014 to 2019, the CORESTA Smoke Analytes Sub-Group, SMA, evaluated through a series of studies analytical methods for measurement of 7 aromatic amines in mainstream cigarette smoke. A method utilising derivatisation with heptafluorobutyric anhydride (HFBA) and analysis by Gas Chromatography Mass Spectrometry operated in Negative Chemical Ionisation (NCI) mode (GC/MS(NCI)) was selected for a full Collaborative Study (CS).

The CS included 11 laboratories, 2 smoking regimes (ISO 3308 and Health Canada Intense T-115/ISO 20778) and 7 samples, 4 commercial cigarette products and 3 reference products. Statistical analysis reported relatively large variation of the results, repeatability (r) was in the range 15 % - 64 % and reproducibility (R) was from 32 % to 193 % [1]. Challenges associated with analytical determination of aromatic amines were similar to those noted in previously published CORESTA work [2]. SMA decided to continue with a further evaluation of key method steps such as optimisation of Solid Phase Extraction (SPE) clean-up and derivatisation to investigate an impact on r&R values. Further method investigations were carried out by a focus group (6 laboratories) and from the findings it was concluded that modifications did not result in r&R improvements that would warrant another full collaborative study [3].

The 2016 full CS results were accepted as the basis for this CORESTA Recommended Method (CRM). A number of useful learnings and experiences were shared in reviews and discussion of this project during SMA meetings and several areas were recommended for consideration when implementing the method [3].

1. FIELD OF APPLICATION

This method is applicable for determination of seven aromatic amines (o-Toluidine, 2,6-Dimethylaniline, o-Anisidine, 1-Aminonaphthalene, 2-Aminonaphthalene, 3-Aminobiphenyl and 4-Aminobiphenyl) in mainstream cigarette smoke by Gas Chromatography/Mass Spectrometry in Negative Chemical Ionization mode (GC/MS-NCI).

This method was shown to be appropriate for analysis of aromatic amines in mainstream cigarette smoke (both ISO and intense smoking regimes) up to 14 mg/cigarette ISO Nicotine Free Dry Particulate Matter (NFDPM).

2. NORMATIVE REFERENCES

ISO 3402, *Tobacco and tobacco products; atmosphere for conditioning and testing*.

ISO 3308, *Routine analytical cigarette smoking machine; definitions and standard conditions.*

ISO 4387, *Cigarettes – Determination of Total and Nicotine-Free Dry Particulate Matter Using a Routine Analytical Smoking Machine.*

ISO 8243, *Cigarettes – Sampling.*

Health Canada, Official Method T-115, *Determination of Tar, Water, Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke.*

3. TERMS AND DEFINITIONS

No specific terms and definitions are listed in this document.

4. PRINCIPLE

Cigarettes are smoked on a standard smoking machine. The mainstream smoke is collected on a glass-fibre filter pad (Cambridge Filter Pad, CFP). After addition of internal standards, the CFP is extracted with dichloromethane (DCM) using a laboratory shaker for 20 minutes. The extract is filtered, derivatised with heptafluorobutyric anhydride (HFBA), purified on a Florisil SPE and analysed by GC/MS-NCI.

5. APPARATUS AND EQUIPMENT

Normal laboratory apparatus and equipment and, in particular, the following items:

5.1 Equipment needed to perform conditioning of cigarettes, in accordance with ISO 3402

5.2 Equipment needed to perform marking for butt length of cigarettes

5.3 Equipment needed to perform smoking of cigarettes, complying with ISO 3308

5.4 Analytical balance (0,0001g accuracy)

5.5 Orbital Shaker or equivalent

5.6 Automated volumetric pipettes (250 µl, 500 µl, 1000 µl, 10-100 µl and 1-10 ml)

5.7 Volumetric flasks (10 ml, 50 ml, 100 ml, 250 ml, 500 ml)

5.8 Extraction vessel (125ml Erlenmeyer flask or equivalent)

5.9 SPE automated workstation or manual SPE manifold

5.10 Glass culture tubes (dimensions relevant to the SPE system used)

5.11 GC/MS equipped with an autosampler, Mass Selective Detector (MSD) with Chemical ionization mode (CI) and data acquisition system

5.12 Inlet liner, splitless, single taper, glass wool, deactivated

5.13 GC Column: low/mid-polarity (14 % cyanopropyl-phenyl)-methylpolysiloxane stationary phase (30 m × 0,32 mm ID × 1 µm film thickness) [1] or equivalent

6. REAGENTS AND SUPPLIES

All reagents are Analytical Grade or equivalent unless otherwise stated.

6.1 1-Aminonaphthalene (CAS 134-32-7) ≥ 98 %

6.2 2,6-Dimethylaniline (CAS 87-62-7) ≥ 98 %

6.3 2,6-Dimethylaniline-d11 (CAS 1092805-08-7) ≥ 98 %

6.4 2-Aminonaphthalene (CAS 91-59-8) ≥ 98 %

6.5 2-Aminonaphthalene-d7 (CAS 93951-94-1) ≥ 98 %

6.6 3-Aminobiphenyl (CAS 2243-47-2) ≥ 98 %

6.7 4-Aminobiphenyl (CAS 92-67-1) ≥ 98 %

6.8 4-Aminobiphenyl-d9 (CAS 344298-96-0) ≥ 98 %

6.9 o-Anisidine (CAS 90-04-0) ≥ 98 %

6.10 o-Toluidine (CAS 95-53-4) ≥ 98 %

6.11 o-Toluidine-d9 (CAS 194423-47-7) ≥ 98 %

6.12 Dichloromethane HPLC grade or higher

6.13 Ethyl acetate HPLC grade or higher

6.14 Florisil SPE cartridge 3g/12 ml [2] or equivalent

6.15 Heptafluorobutyric anhydride (HFBA) (CAS 336-59-4) for GC derivatisation, ≥ 99 %

6.16 n-Hexane HPLC grade or higher

6.17 Glass fiber filter (Cambridge Filter Pad, CFP) 44 mm or 92 mm diameter depending on requirements for smoking machine type

6.18 Autosampler amber glass vials – 2 ml with Teflon lined septa

6.19 Filter paper, qualitative (15 cm diameter, 80 g/m^2)

NOTE 1: It is a responsibility of each laboratory to ensure appropriate risk assessments are carried out and relevant health and safety measures are in place before implementing this method.

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[1] The following separation column was found to have acceptable performance: DB-1701, 30 m × 0,3 2 mm ID × 1 µm film thickness (part number J&W 123-0733). This information is given for the convenience of users of this document and does not represent an endorsement by CORESTA of this product.

[2] The following SPE cartridges were found to be suitable: Florisil Spe-ed cartridges 3g/12 ml, catalogue number 5113, Applied Separations. This information is given for the convenience of users of this document and does not represent an endorsement by CORESTA of this product.

7. PREPARATION OF GLASSWARE

Glassware should be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.

It is important that all possible sources of contamination which may interfere with the analytical process are removed from the work area.

8. PREPARATION OF STANDARDS AND SOLUTIONS

All solutions and standards must be clearly and permanently labeled, including expiry date, and stored in a freezer set at ≤ 0 °C. The standard solutions listed below are stable for up to one year.

NOTE 2: Due to the inherent risks to the manipulation of the standards in their fundamental state, it is recommended that the weighing is done in the fewest possible steps. The mass of analytical standards and dilution volumes may differ from the example below depending on the frequency of method use; it is recommended to maintain the concentration levels, where possible.

8.1 Ethyl acetate in n-hexane (10 %, v/v)

Transfer 100 ml of ethyl acetate into a 1 litre volumetric flask and fill to the volume with n-hexane.

8.2 Stock Solution

Accurately weigh each aromatic amine standard into individual volumetric flasks (Table 1). Dilute each flask to volume with ethyl acetate/n-hexane solution (8.1) and mix well by gently inverting the flasks. The nominal concentration of each solution is given, as an example, in Table 1.

Table 1 - Individual Stock Solutions Preparation

Analyte	Weight (mg)	Volume (ml)	Concentration Stock solution [µg/ml]
o-Toluidine	20	100	200
2,6-Dimethylaniline	10	250	40
o-Anisidine	20	250	80
1-Aminonaphthalene	20	100	200
2-Aminonaphthalene	20	100	200
3-Aminobiphenyl	15	250	60
4-Aminobiphenyl	15	250	60
o-Toluidine-d9	20	100	200
2,6-Dimethylaniline-d11	20	100	200
2-Aminonaphthalene-d7	20	100	200
4-Aminobiphenyl-d9	10	100	100

8.3 Intermediate Stock Solution

Transfer the specified volumes of each aromatic amine stock solution into individual volumetric flasks (Table 2). Dilute each flask to volume with n-hexane and mix well by gently inverting the flasks. The nominal concentration of each solution is given in Table 2.

Table 2 - Intermediate Stock Solutions Preparation

Analyte	Stock Solution Volume (ml)	Final Volume (ml)	Concentration Stock solution [ng/ml]
o-Toluidine	0,250	250	200
2,6-Dimethylaniline	0,250	500	20
o-Anisidine	0,125	500	20
1-Aminonaphthalene	0,250	250	200
2-Aminonaphthalene	0,250	250	200
3-Aminobiphenyl	0,125	250	36
4-Aminobiphenyl	0,125	250	36

8.4 Internal Standard (IS) Intermediate Solution

Transfer the specified volumes (Table 3) of each deuterated aromatic amine stock solution (section 8.2) into a 500 ml volumetric flask, dilute to the mark with n-hexane and mix well. The nominal concentration of each standard is given in Table 3.

Table 3 - IS Intermediate Solution Preparation

Stock Solution	Stock Solution Volume (ml)	Final Volume (ml)	Concentration Intermediate solution [ng/ml]
o-Toluidine-d9	1,20	500	480
2,6-Dimethylaniline-d11	0,25		100
2-Aminonaphthalene-d7	0,25		100
4-Aminobiphenyl-d9	0,13		25

8.5 Calibration Standards Solutions

Transfer the specified volumes (Table 4) of each aromatic amine intermediate solution and 500 µl of internal standard (section 8.4) into a 50 ml volumetric flask, dilute to the mark with n-hexane and mix well. The nominal concentration of each standard is given in Table 4.

Table 4 - Calibration Standards Preparation

Level	Aliquot Intermediate Solution (ml)						
	o-Toluidine	2,6-Dimethylaniline	o-Anisidine	1-Aminonaphthalene	2-Aminonaphthalene	3-Aminobiphenyl	4-Aminobiphenyl
1	0,25	0,25	0,25	0,10	0,10	0,10	0,10
2	1,00	1,75	0,75	0,25	0,25	0,25	0,25
3	1,75	3,25	1,25	0,50	0,50	0,50	0,50
4	2,50	4,75	1,75	0,75	0,75	0,75	0,75
5	3,25	6,25	2,25	1,00	1,00	1,00	1,00
6	4,00	7,75	2,75	1,25	1,25	1,25	1,25
Level	Concentration Calibration Standards [ng/ml]						
	o-Toluidine	2,6-Dimethylaniline	o-Anisidine	1-Aminonaphthalene	2-Aminonaphthalene	3-Aminobiphenyl	4-Aminobiphenyl
1	1,00	0,10	0,10	0,40	0,40	0,07	0,07
2	4,00	0,70	0,30	1,00	1,00	0,18	0,18
3	7,00	1,30	0,50	2,00	2,00	0,36	0,36
4	10,00	1,90	0,70	3,00	3,00	0,54	0,54
5	13,00	2,50	0,90	4,00	4,00	0,72	0,72
6	16,00	3,10	1,10	5,00	5,00	0,90	0,90

Before GC/MS analysis, the calibration standards need to be derivatised following the sections 12.2 and 12.3.

9. SAMPLING

Sampling is performed in accordance with ISO 8243.

10. TOBACCO PRODUCTS PREPARATION

Cigarettes are conditioned in accordance with ISO 3402.

11. SAMPLE GENERATION – SMOKING

The method has been evaluated for two smoking regimes, ISO 3308 and Health Canada Intense (HCI) [3]. Table 5 summarises smoking parameters.

A routine analytical cigarette-smoking machine complying with the requirements of ISO 3308:2012, Health Canada Intense (HCI) regulations 1999 and ISO 20778:2018 [3] is required.

[3] *Health Canada Intense smoking regime was referred to in the study protocol. Since the last quarter of 2018 an ISO smoking machine standard ISO 20778 was adopted and is functionally equivalent to Health Canada Intense conditions.*

Table 5 - Smoking parameters applicable for the method

Smoking regime	Puff volume (ml)	Puff frequency (seconds)	Puff duration (seconds)	Ventilation blocking (%)
ISO 3308:2012	35	60	2	0
Health Canada Intense (HCI) [4]	55	30	2	100

The number of cigarettes per CFP and CFP size are selected to maximise analyte detection whilst avoiding breakthrough of 'tar' (Table 6). A CFP of 44 mm diameter can retain up to 150 mg of Total Particulate Matter (TPM) and CFP of 92 mm diameter up to 600 mg TPM. If this mass is exceeded, the number of cigarettes shall be reduced.

Table 6 - Recommended number of cigarettes for each smoking machine type and smoking regime:

Smoking regime	Machine type	CFP size (mm)	Cigarettes/CFP
ISO 3308:2012	Rotary	92	10
	Linear*	44	5
Health Canada Intense (HCI)[4]	Rotary	92	5
	Linear*	44 - 55	2 - 3

**The analytical method was validated on a rotary smoking machine (ISO, HCI, 92mm CFP) and used on linear smoking machines (HCI, 55 mm CFP). From the review of the information and the results in the previous joint experiments, participating laboratories who used linear smoking machines typically smoked 5 cigarettes with ISO smoking regime and 2-3 cigarettes with HCI smoking regime, but the CFP size was not required to be reported. The number of the cigarettes smoked on linear smoking machines may therefore require adjustment to avoid the breakthrough depending on the CFP size used.*

NOTE 3: To avoid losses by volatility the extraction should be done immediately after smoking is completed.

NOTE 4: The method was shown to be appropriate for the determination of 7 aromatic amines in products with up to 14 mg/cigarette of ISO tar yield. For testing of products with expected ISO tar yield above 14mg/cigarette, it is recommended to verify for breakthrough of all analytes. The analytical method may also require a dilution step prior to derivatisation.

[4] *Health Canada Intense smoking regime was referred to in the study protocol. Since the last quarter of 2018 an ISO smoking machine standard ISO 20778 was adopted and is functionally equivalent to Health Canada Intense conditions.*

12. SAMPLE ANALYSIS

12.1 Sample Extraction

After smoking, fold the CFP into quarters and place it into a 125 ml Erlenmeyer flask immediately. Add 1,0 ml of Internal Standard Intermediate Solution (see 8.4) and 50 ml of dichloromethane (DCM) and cap with a glass or Teflon stopper. Shake the extract for 20 minutes on an orbital shaker set at 200 rpm. Filter the extract with qualitative filter paper.

12.2 Sample derivatisation

Take a 5 ml aliquot and derivatise with HFBA, 25µl for ISO regime and 50 µl for intense regime. Mix well the extract and wait a minimum of 40 minutes to complete the reaction.

NOTE 5: The derivatisation agent HFBA (heptafluorobutyric anhydride) must be stored in a desiccator to prevent losses of reactivity. During handling it must be kept open for the shortest time possible to minimise moisture absorption. This is critical to assure that the reagent remains active. It is important to observe that when HFBA is added to the condensed smoke extract (extracted with DCM) almost instantly the extract turns from yellow to brown. If the colour change does not occur, use another aliquot of the extract and use a different vial.

12.3 SPE sample Clean-Up

Perform sample clean-up using Florisil SPE cartridges 3g/12 ml. In the SPE manifold, load Florisil SPE cartridges with 12,0 ml DCM and discard. Transfer the whole derivatised extract into SPE cartridge under vacuum and collect the eluent. Elute the cartridge with 8,5 ml DCM and collect the eluent. Combine both eluents, mix well and transfer an aliquot to a GC autosampler vial for GC/MS-NCI analysis.

NOTE 6: During clean-up process the elution must be slow, i.e. the flow should be below 2 ml/min (1 drop per second).

12.4 GC/MS Operating Conditions

The following conditions have been found to be acceptable but may need adjustment based on the system used:

Carrier gas:	Helium
Injector temperature:	250 °C
Injection mode:	splitless; constant flow 1,5 ml/min
Injection volume:	3,0 µl
Oven temperature programme:	40 °C (0.5 min)
	15 °C/min to 240 °C (5 min)
	50 °C/min to 270 °C (10 min)
Transfer line temperature:	240 °C
MS Source temperature:	150 °C
MS Quadrupole temperature:	106 °C
MS Mode:	NCI
Data acquisition mode:	Selected Ion Monitoring (SIM), see Table 7 for target ions
Reagent gas:	Methane at 40 % flow

NOTE 7: The MS mode (NCI) must be used to assure proper method selectivity and sensitivity. Example chromatograms are shown in Annex A, Figure 1 (standard solution, level 6) and Figure 2 (3R4F ISO mainstream smoke).

Table 7 - Target ions

Analytes	Target Ion (m/z)
o-Toluidine	283
2,6-Dimethylaniline	297
o-Anisidine	299
1-Aminonaphthalene	319
2-Aminonaphthalene	319
3-Aminobiphenyl	345
4-Aminobiphenyl	345
o-Toluidine-d9	290
2,6-Dimethylaniline-d11	306
2-Aminonaphthalene-d7	326
4-Aminobiphenyl-d9	354

12.5 Sample quantification

Aromatic amines are quantified using internally standardised calibration curve. Generate a linear calibration curve (ratio of each analyte's response to the ISTD response versus the amount of the analyte in ng/ml) at the beginning of analysis from the 6 working standards.

The amount of each analyte is reported in ng/cigarette and is calculated as follows:

$$C_{analyte(ng/cig)} = \frac{\left(\frac{A_i}{A_{pi}} - k\right) \times Q_{pi}}{S \times n}$$

Where:

A_i = analyte area in the sample

A_{pi} = internal standard area in the sample

k = linear coefficient of the calibration curve, y-intercept

Q_{pi} = amount of internal standard added to each sample (ng)

S = angular coefficient of the calibration curve, slope

n = number of cigarettes

13. REPEATABILITY AND REPRODUCIBILITY

An international Collaborative Study (CS) was conducted in 2016, involving 11 laboratories. The study included 7 cigarette products covering a wide range of blends and cigarette design constructions, University of Kentucky reference cigarettes (3R4F and 1R6F) and CORESTA Monitor Test Piece CM8. All samples were generated under two smoking regimes, ISO 3308 and Health Canada intense.

Table 8 – Products used in the study

Sample	Characteristics	ISO NFDPM (mg/cigarette, pack data)	Cigarette Length (mm)	Tipping Length (mm)	Filter Length (mm)	Butt Length (mm)
Sample 1	Dark air-cured product	8-10	83	25	21	29
Sample 2	American blended product	4-6	83	32	27	35
Sample 3	Virginia blended product	4-7	83	32	27	35
Sample 4	Charcoal filtered / blended product	1-2	84	32	27	35
3R4F	Kentucky Reference 3R4F	8	84	32	27	35
1R6F	Kentucky Reference 1R6F	8	84	32	27	35
CM8	CM8 Test Piece	12-14	83	29	21	33

Repeatability (r) and reproducibility (R) were calculated after the removal of outliers following an approach analogous to the ISO 5725-2 statistical procedures, but using an outlier detection approach based on MAD [5] rather than Grubbs' Test. Both r & R figures were calculated for each product for both ISO and HCI smoking regimes and for the seven analytes.

Repeatability and reproducibility were reported in absolute values and as a percentage of the mean. Repeatability ranged between 15-64 % of the mean and reproducibility between 32-193 % of the mean. The results for each product and smoking regimes are summarised in Tables 9 to 15.

[5] *Rousseeuw, Peter & Croux, Christopher. Alternatives to Median Absolute Deviation. Journal of the American Statistical Association. 88. 1273 – 1283, 1993. DOI 10.1080/01621459.1993.10476408.*

Table 9 - ISO and HCI mainstream smoke yields for 1-aminonaphthalene – r and R data

Sample	Regime	N	Mean (ng/cigarette)	Repeatability		Reproducibility	
				r (ng/cigarette)	r (%)	R (ng/cigarette)	R (%)
1R6F	HCI	8	21,9	5,0	22,7	11,8	53,9
	ISO	8	12,4	2,8	22,6	4,1	32,9
3R4F	HCI	9	22,6	6,3	27,9	15,8	70,1
	ISO	9	11,0	2,2	20,0	6,6	59,4
CM8	HCI	10	32,5	12,5	38,5	28,1	86,5
	ISO	10	15,9	3,4	21,3	15,3	95,8
Sample 1	HCI	9	33,9	7,1	21,1	28,3	83,5
	ISO	10	15,1	3,0	19,8	16,0	106,1
Sample 2	HCI	9	15,7	6,0	38,1	11,6	73,8
	ISO	8	7,4	2,1	28,5	5,1	69,5
Sample 3	HCI	8	16,8	5,5	32,5	12,4	73,9
	ISO	9	7,1	2,3	33,2	5,3	75,6
Sample 4	HCI	8	9,6	3,5	36,0	7,4	77,2
	ISO	4	1,6	0,5	29,4	1,3	81,2

Abbreviations: N -number of data sets, r -repeatability, R – reproducibility

Table 10 - ISO and HCI mainstream smoke yields for 2-aminonaphthalene – r and R data

Sample	Regime	N	Mean (ng/cigarette)	Repeatability		Reproducibility	
				r (ng/cigarette)	r (%)	R (ng/cigarette)	R (%)
1R6F	HCI	10	12,0	2,9	23,8	10,5	87,7
	ISO	9	6,6	1,8	27,4	4,8	73,3
3R4F	HCI	11	13,2	3,4	25,6	10,5	78,9
	ISO	9	6,2	1,8	29,1	4,6	74,9
CM8	HCI	10	15,9	4,4	27,8	14,0	88,0
	ISO	10	8,1	2,1	25,8	6,0	74,0
Sample 1	HCI	11	18,8	4,8	25,6	15,0	79,9
	ISO	9	9,5	2,5	26,4	5,0	53,2
Sample 2	HCI	9	8,8	2,6	29,3	7,6	86,8
	ISO	9	5,0	1,5	30,5	5,4	108,4
Sample 3	HCI	9	8,8	3,7	42,6	8,1	92,4
	ISO	8	3,9	0,9	21,7	2,7	69,6
Sample 4	HCI	9	5,0	1,9	38,0	5,8	115,9
	ISO	5	0,9	0,5	52,6	1,4	161,3

Abbreviations: N -number of data sets, r -repeatability, R – reproducibility

Table 11 - ISO and HCI mainstream cigarette smoke yields for 2,6-dimethylaniline – r and R data

Sample	Regime	N	Mean (ng/cigarette)	Repeatability		Reproducibility	
				r (ng/cigarette)	r (%)	R (ng/cigarette)	R (%)
1R6F	HCI	8	8,3	4,0	48,1	11,0	131,5
	ISO	6	3,8	1,4	35,7	1,7	44,8
3R4F	HCI	8	9,1	4,6	50,9	14,5	159,7
	ISO	6	2,8	1,2	44,1	1,8	62,9
CM8	HCI	8	13,0	4,7	36,3	12,0	92,2
	ISO	7	6,9	1,7	24,5	4,9	71,5
Sample 1	HCI	8	20,9	8,3	39,5	33,3	159,2
	ISO	8	9,9	3,5	35,0	14,0	141,7
Sample 2	HCI	8	6,9	3,1	45,0	10,2	147,4
	ISO	7	6,1	2,0	33,2	11,8	193,7
Sample 3	HCI	7	7,6	2,6	33,9	6,7	87,8
	ISO	6	3,2	1,1	34,6	3,0	95,6
Sample 4	HCI	6	3,5	1,3	38,6	3,8	108,2
	ISO	4	1,3	0,6	41,4	1,5	111,9

Abbreviations: N -number of data sets, r -repeatability, R – reproducibility

Table 12 - ISO and HCI mainstream cigarette smoke yields for o-anisidine – r and R data

Sample	Regime	N	Mean (ng/cigarette)	Repeatability		Reproducibility	
				r (ng/cigarette)	r (%)	R (ng/cigarette)	R (%)
1R6F	HCI	9	2,9	1,3	45,4	3,5	121,4
	ISO	8	1,6	0,6	36,3	1,8	116,5
3R4F	HCI	9	3,1	0,9	30,1	3,0	97,8
	ISO	8	1,5	0,4	26,2	1,6	101,3
CM8	HCI	9	5,3	2,1	39,3	5,7	106,2
	ISO	9	2,9	0,7	24,7	3,4	117,6
Sample 1	HCI	8	5,7	1,7	30,1	6,2	109,5
	ISO	8	2,6	0,7	28,5	2,9	112,9
Sample 2	HCI	9	2,9	1,1	37,3	4,0	138,6
	ISO	8	1,3	0,5	34,4	1,6	121,7
Sample 3	HCI	8	2,4	0,8	34,4	2,6	108,5
	ISO	6	1,1	0,4	39,2	1,4	124,9
Sample 4	HCI	7	1,3	0,5	41,9	1,3	97,2
	ISO	3	0,2	0,1	27,2	0,3	152,9

Abbreviations: N -number of data sets, r -repeatability, R – reproducibility

Table 13 - ISO and HCI mainstream cigarette smoke yields for o-toluidine – r and R data

Sample	Regime	N	Mean (ng/cigarette)	Repeatability		Reproducibility	
				r (ng/cigarette)	r (%)	R (ng/cigarette)	R (%)
1R6F	HCI	9	67,7	17,4	25,7	53,4	78,9
	ISO	7	36,7	7,8	21,3	12,2	33,2
3R4F	HCI	8	79,5	15,7	19,8	42,1	52,9
	ISO	9	34,8	7,4	21,2	24,4	70,0
CM8	HCI	9	94,3	30,6	32,5	75,2	79,8
	ISO	8	54,1	11,4	21,1	29,6	54,7
Sample 1	HCI	9	139,3	20,6	14,8	131,9	94,7
	ISO	9	62,0	14,1	22,7	58,9	95,0
Sample 2	HCI	8	49,6	14,1	28,4	37,6	75,7
	ISO	8	23,3	5,1	22,1	15,8	68,0
Sample 3	HCI	8	53,9	17,2	32,0	44,5	82,6
	ISO	8	23,0	4,2	18,1	16,7	72,6
Sample 4	HCI	7	29,6	9,4	31,7	26,5	89,7
	ISO	6	3,2	1,1	33,7	3,5	111,4

Abbreviations: N -number of data sets, r -repeatability, R – reproducibility

Table 14 - ISO and HCI mainstream cigarette smoke yields for 3- aminobiphenyl – r and R data

Sample	Regime	N	Mean (ng/cigarette)	Repeatability		Reproducibility	
				r (ng/cigarette)	r (%)	R (ng/cigarette)	R (%)
1R6F	HCI	11	3,5	1,1	30,0	3,9	109,7
	ISO	10	1,6	0,4	25,2	1,8	114,9
3R4F	HCI	11	4,0	1,2	29,6	4,5	112,2
	ISO	10	1,7	0,5	26,8	1,5	91,0
CM8	HCI	10	4,0	1,0	23,8	4,7	117,3
	ISO	11	1,9	0,5	26,1	2,3	121,2
Sample 1	HCI	11	6,3	1,6	24,8	7,2	113,7
	ISO	11	2,6	0,8	31,8	3,1	115,8
Sample 2	HCI	10	2,7	0,8	30,4	3,1	117,4
	ISO	10	1,3	0,5	37,3	2,3	175,3
Sample 3	HCI	9	2,6	0,7	26,6	3,4	131,4
	ISO	9	0,9	0,2	26,5	1,2	131,9
Sample 4	HCI	8	1,5	0,6	41,4	2,3	154,7
	ISO	4	0,2	0,1	37,3	0,3	185,8

Abbreviations: N -number of data sets, r -repeatability, R – reproducibility

Table 15 - ISO and HCI mainstream cigarette smoke yields for 4- aminobiphenyl – r and R data

Sample	Regime	N	Mean (ng/cigarette)	Repeatability		Reproducibility	
				r (ng/cigarette)	r (%)	R (ng/cigarette)	R (%)
1R6F	HCI	10	2,4	0,6	26,2	2,8	116,4
	ISO	10	1,0	0,3	30,5	1,2	111,9
3R4F	HCI	11	2,8	0,7	25,9	3,0	106,4
	ISO	10	1,1	0,7	64,4	1,4	130,4
CM8	HCI	11	2,5	0,8	30,3	2,8	112,6
	ISO	11	1,2	0,3	26,2	1,3	111,8
Sample 1	HCI	11	4,6	1,0	22,7	6,0	131,8
	ISO	11	1,8	0,4	24,6	2,3	127,5
Sample 2	HCI	9	2,1	0,4	21,2	1,7	82,1
	ISO	9	0,7	0,2	23,0	0,8	110,0
Sample 3	HCI	10	1,9	0,5	25,1	2,2	118,3
	ISO	7	0,7	0,2	25,3	0,7	93,7
Sample 4	HCI	7	1,2	0,4	37,8	1,3	108,4
	ISO	2	0,2	0,05	24,8	0,2	101,4

Abbreviations: N -number of data sets, r -repeatability, R - reproducibility

14. TEST REPORT

The expression of the laboratory data depends on the purpose for which the data are required, and the level of laboratory precision. Confidence limits shall be calculated and expressed on the basis of the laboratory data before any rounding has taken place.

Aromatic amines mainstream smoke yields are reported in ng/cig.

15. RECOMMENDATIONS

Before implementing the method, please refer to SMA report "2019 Small Group Collaborative Study on Aromatic Amines in Mainstream Cigarette Smoke" (SMA-048-1-CTR [6]) that contains a summary of learnings and recommendations for consideration.

[6] *Available at https://www.coresta.org/2019-small-group-collaborative-study-aromatic-amines-mainstream-cigarette-smoke-33656.html*

16. ANNEXES

Annex A – Examples of chromatograms:

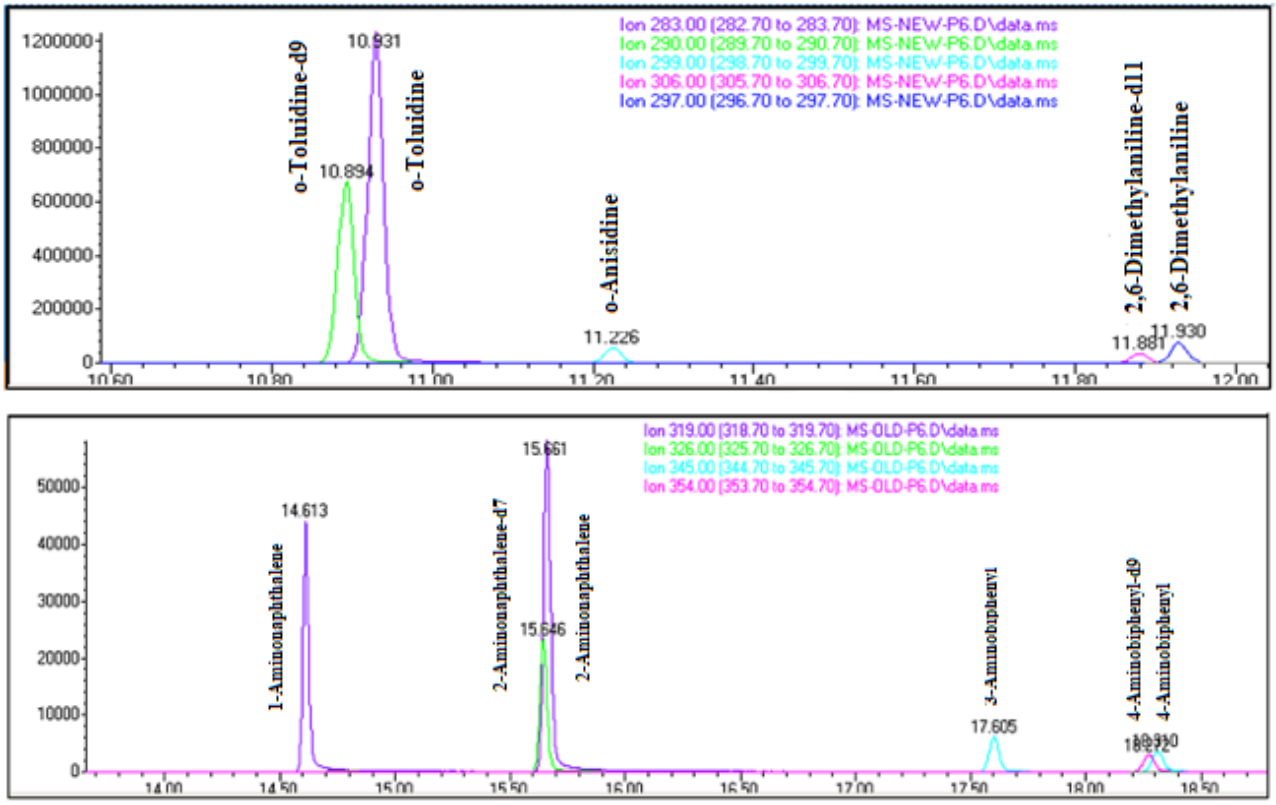

Figure 1 - Standard Solution (Level 6) extraction ions chromatogram

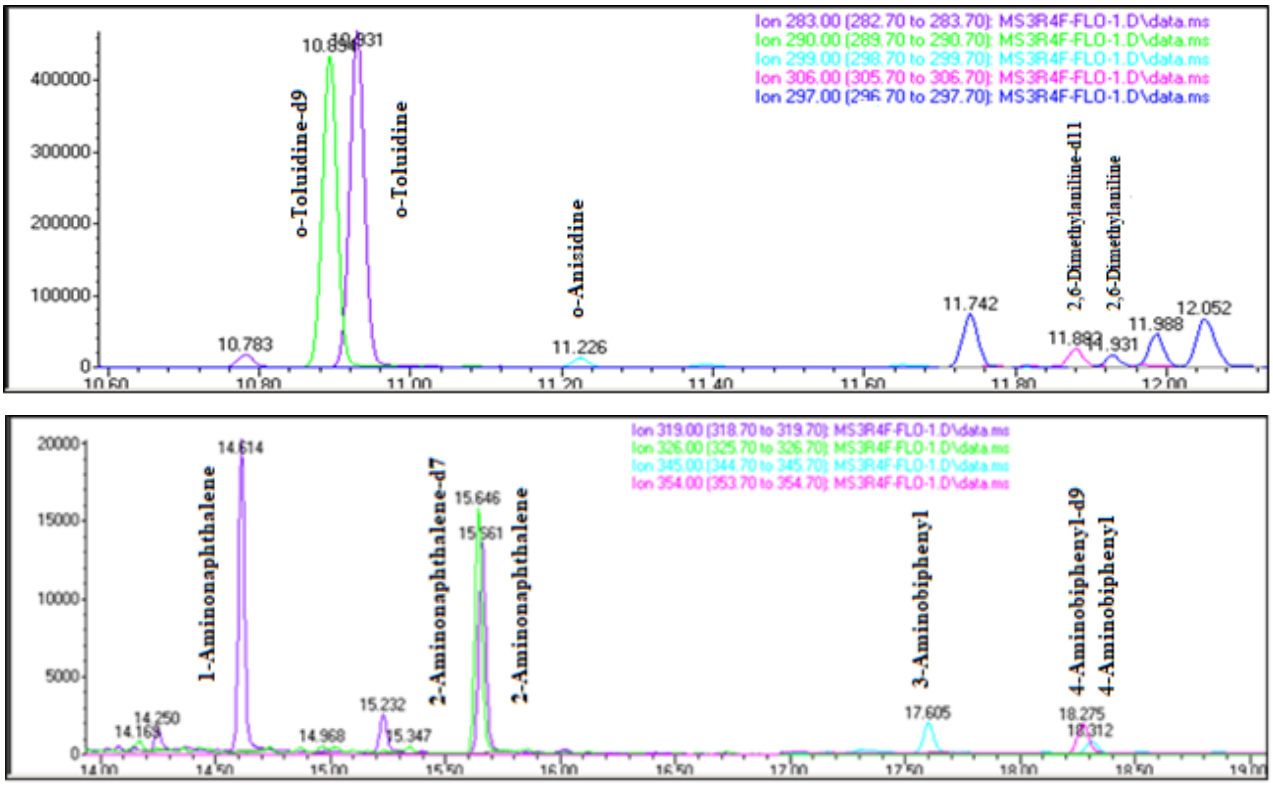

Figure 2 - Example of 3R4F (ISO smoking regime) extracted ions chromatogram

17. BIBLIOGRAPHY

[1] 2016 Collaborative Study on Aromatic Amines in Mainstream Cigarette Smoke, CORESTA SMA Technical Report SMA-048-2-CTR, Published March 2019. Available at www.coresta.org

[2] 2010 Intorp M., Purkis S. Determination of Aromatic Amines in Cigarette Mainstream Smoke - The CORESTA 2007 Joint Experiment, Beiträge zur Tabakforschung International, Contributions to Tobacco Research, Vol. 24, Issue 2 (78-92). Available at https://doi.org/10.2478/cttr-2013-0884

[3] 2019 Small Group Collaborative Study on Aromatic Amines in Mainstream Cigarette Smoke CORESTA SMA Technical Report SMA-048-1-CTR, Available at www.coresta.org

[4] ISO 5725-1, Accuracy (trueness and precision) of measurement methods and results – Part 1: General principles and definitions.

[5] ISO 5725-2, Accuracy (trueness and precision) of measurement methods and results – Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method.