



**Cooperation Centre for Scientific Research
Relative to Tobacco**

Smoke Analytes Sub-Group

**CORESTA Recommended Method
No. 78**

**DETERMINATION OF SELECTED
PHENOLIC COMPOUNDS
IN MAINSTREAM CIGARETTE
SMOKE BY HPLC-FLD**

December 2018



CORESTA RECOMMENDED METHOD N° 78

Title:

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DETERMINATION OF SELECTED PHENOLIC COMPOUNDS IN MAINSTREAM CIGARETTE SMOKE BY HPLC-FLD

(December 2018)

0. INTRODUCTION

The CORESTA Special Analytes Sub-Group (SPA)¹, selected the Health Canada Test Method T-114 as the basis of this CORESTA Recommended Method (CRM). Mainstream cigarette smoke was collected on a Cambridge Filter Pad (CFP), extracted using 1 % acetic acid solution and the extract was analysed by Reversed Phase High Performance Liquid Chromatography with Fluorescence Detection (RP HPLC-FLD). The separation of m-cresol and p-cresol isomers was achieved by employing a column with pentafluorophenylpropyl (PFP) stationary phase.

The CRM was produced through a full collaborative study involving 18 laboratories from 11 countries smoking 10 samples with a range of blend styles (Virginia flue-cured, American Blend and Dark air-cured) and ISO tar yields (1 mg – 13 mg) under both the ISO and Health Canada Intense (HCI) regimes. The method also includes notes about some of the main features that need to be controlled to provide data as robust and consistent as the repeatability and reproducibility data provided. Statistical evaluations were made according to ISO 5725 recommendations.

1. FIELD OF APPLICATION

This method is applicable to the determination of selected phenolic compounds (hydroquinone, resorcinol, catechol, phenol, p-cresol, m-cresol, and o-cresol) in the Total Particulate Matter (TPM) of mainstream cigarette smoke by RP HPLC-FLD.

The described method is specified using ISO 3308 and Health Canada T-115 smoking parameters. The use of these machine smoking parameters reflects their inclusion in the reporting requirements of various national regulations rather than an endorsement of their appropriateness by CORESTA.

2. NORMATIVE REFERENCES

- 2.1** *ISO 3308*
Routine analytical cigarette-smoking machine – Definitions and standard conditions.
- 2.2** *ISO 8243*
Cigarettes – Sampling.
- 2.3** *ISO 3402*
Tobacco and tobacco products – Atmosphere for conditioning and testing.

¹ In January 2017, the Sub-Group name was changed to "Smoke Analytes Sub-Group" (SMA)

2.4 *ISO 4387*

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

2.5 *Health Canada, Official Method T-115*

Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke.

2.6 *Health Canada, Official Method T-114*

Determination of Phenolic Compounds in Mainstream Tobacco Smoke.

3. METHOD SUMMARY

3.1 Cigarettes are smoked on a routine analytical smoking machine under ISO 3308 and/or Health Canada T-115 smoking regimes and mainstream smoke is trapped on a glass fibre filter pad (CFP).

3.2 The CFP is extracted with aqueous acetic acid.

3.3 An aliquot of the TPM extract is syringe filtered, diluted and analysed by RP HPLC-FLD.

4. APPARATUS AND EQUIPMENT

Laboratory apparatus and equipment, in particular the following items:

- Equipment for conditioning of tobacco products
- Equipment for marking for butt length
- Equipment for smoking of tobacco products complying with ISO 3308 and equipped for smoking according to ISO 4387 and/or Health Canada T-115

Laboratory equipment for the preparation of samples, standards, and reagents – for example:

- 125 ml or 150 ml actinic red Erlenmeyer flasks (or Erlenmeyer flasks wrapped with aluminium foil) with ground glass stoppers
- Analytical balance, capable of measuring to four decimal places
- Wrist action shaker or equivalent
- Actinic red volumetric flasks (10 ml, 25 ml and 50 ml)
- Syringe filter, 0,45 µm polyvinylidene difluoride (PVDF) or equivalent
- Auto-sampler vials, caps and polytetrafluoroethylene (PTFE) faced septa
- HPLC system equipped with a binary gradient pump or equivalent, a cooled auto-sampler with appropriate sampling loop and a fluorescence detector. The instrument must be also equipped with computerised control and data acquisition and processing system.
- HPLC column with pentafluorophenylpropyl (PFP) stationary phase; an example of the column dimensions are 3 µm, 150 mm × 4,6 mm or equivalent
- Disposable Guard Column such as PFP cartridge; an example of the cartridge dimensions are 4 mm × 3,0 mm or equivalent

5. REAGENTS AND SUPPLIES

All reagents shall be, at the least, recognized as analytical reagent grade.

- Methanol – HPLC Grade
- Acetic acid, glacial – HPLC Grade (~ 17.4 M)
- Deionised water
- Hydroquinone (min. 99 %)
- Resorcinol (min. 99 %)
- Catechol (min. 99 %)
- Phenol (min. 99 %)
- p-Cresol (min. 99 %)
- m-Cresol (min. 99 %)
- o-Cresol (min. 99 %)
- Helium (UHP) – if necessary for sparging of HPLC system mobile phase or equivalent degassing system
- Argon and nitrogen

Note: The solvents and chemicals used in this method are classified as toxic, highly toxic, harmful, carcinogenic, mutagenic, sensitising, teratogenic, irritant, corrosive, easily flammable and dangerous for the environment. The instructions specified in the individual material safety data sheets concerning safe handling, storage and waste disposal as well as protective equipment are to be followed.

6. PREPARATION OF GLASSWARE

For the preparation of samples, standards and reagents, all glassware shall be thoroughly cleaned before use to avoid any contamination.

7. PREPARATION OF SOLUTIONS

7.1 Preparation of 1 % (v/v) Acetic Acid Solution

- Add approximately 3500 ml of deionized water to a 4 l volumetric flask.
- Add 40 ml of acetic acid to the flask.
- Make up the solution to volume with deionised water and mix well.

8. PREPARATION OF STANDARDS

The calibration should cover the concentration range of interest.

8.1 Primary (1°) Phenolic Compound Standards

As an example, weigh approximately 25 mg of phenolic compounds as described in **Appendix 1A** into individual 25 ml or 50 ml volumetric flasks and dissolve in 1 % acetic acid solution (7.1). The concentrations of phenolic compounds are recorded in mg/ml.

Note: If the stability of the phenolic compound standards is of concern, then the headspace of each bottle can be purged with an inert gas (*e.g.* argon or nitrogen). Primary phenolic compound standards are stored in the refrigerator and are to be prepared fresh every ten working days.

8.2 Secondary (2°) Phenolic Compound Standard

Pipette predetermined volumes (see **Appendix 1**) of each primary phenolic compound standard into a 50 ml volumetric flask and dilute to the mark with 1 % acetic acid (7.1).

The concentrations of phenolic compounds are recorded in µg/ml.

Note: If stored in the refrigerator (at approximately 4 °C), the standards are stable for five working days.

8.3 Phenolic Compound Working Standards

Take appropriate volumes (0,2 ml to 8 ml) of the 2° phenolic compound standard (8.2) and dilute to 10 ml with 1 % acetic acid to prepare calibration standards with approximate phenolic compound concentrations in the ranges noted in **Appendix 1**.

Transfer to auto-sampler vials.

The concentrations of phenolic compounds are recorded in µg/ml.

Note: If stored in the refrigerator (at approximately 4 °C), the standards are stable for five working days.

Note: The stability of the phenol standards is known to be limited. The stability data presented is a guide only and stability should be evaluated by each laboratory.

9. SAMPLING

Sampling is performed in accordance with ISO 8243.

10. TOBACCO PRODUCT PREPARATION

Cigarettes are conditioned in accordance with ISO 3402.

11. SAMPLE GENERATION – SMOKING OF CIGARETTES

The smoking parameters for which the method has been studied are set out in ISO 3308 and in the Health Canada, Official Method T-115.

Table 1: Smoking parameters for ISO and HCI smoking regimes

Smoking regime	Puff volume (ml)	Puff frequency (seconds)	Puff duration (seconds)	Ventilation blocking (%)
ISO 3308	35	60	2	0
HCI T-115	55	30	2	100

The cigarettes are smoked according to ISO 4387. Typically, 5 cigarettes should be smoked onto a 44 mm CFP, and 10 cigarettes onto a 92 mm CFP. CFP of 44 mm diameter is capable of retaining up to 150 mg of Total Particulate Matter (TPM) and pads of 92 mm diameter up to 600 mg TPM. If this mass is exceeded, the number of cigarettes shall be reduced. For low tar products under the ISO 3308 smoking regime, a greater number of cigarettes may be smoked to achieve a minimum TPM of 10 mg for a 44 mm pad and 20 mg for a 92 mm pad.

12. SAMPLE ANALYSIS

- Remove the CFP from its holder, fold the CFP into quarters (with the condensate inside) and wipe the inside of the holder with the pad.
- Transfer the CFP into an actinic red Erlenmeyer flask or Erlenmeyer flask wrapped with aluminium foil (125 ml for a 44 mm pad; 250 ml for a 92 mm pad).

Note: The compounds are sensitive to light exposure.

- For a 44 mm CFP, add 40 ml of 1 % acetic acid solution into the flask. For a 92 mm pad, add 80 ml of 1 % acetic acid solution into the flask.
- Cover the flask with a ground glass stopper held in place using a clamp and where necessary wrap the flask with aluminium foil.
- Shake the flask vigorously until the CFP has disintegrated and filter the extract through a 0,45 µm syringe filter.
- The CFP extracts may need to be diluted so that the concentrations of target phenolic compounds fall within the desired calibration ranges.
 - Dilution example: Transfer 3 ml of filtered pad extract into a 10 ml volumetric flask and dilute to the mark with 1 % acetic acid solution.
- Transfer an aliquot of the extract to a vial and fill the vial to minimise the headspace. It is recommended for analysis to use an auto-sampler that is cooled to approximately 4 °C.

Note: Phenolic compounds, especially hydroquinone, will decompose with prolonged storage at room temperature. It is recommended to analyse all samples as soon as they are smoked and within 24 hours.

Note: Ensure the concentration levels of phenolic substances are within the concentration range of the method.

12.1 Determination

12.1.1 HPLC Operating Conditions

Set up and operate the HPLC-FLD system in accordance with the manufacturer's instructions. The following conditions are suitable for analysis:

- Column Temperature: Ambient
- Auto-sampler Tray Temperature: 4 °C
- Injection volume: 10 µl or 20 µl
- Mobile Phase:
 - Mobile phase A: Prepare 2 l of 1 % acetic acid in deionized water and degas
 - Mobile phase B: Prepare 2 l of 1 % acetic acid in methanol and degas
 - Flow rate: 0,8 ml/min
 - The following is an example of gradient conditions:

Time (min)	% A	% B
0	78	22
8	78	22
8,5	55	45

Time (min)	% A	% B
21	55	45
22	0	100
28	0	100

- Column conditioning: 6 minutes

- Wavelength Programmable Fluorescence Detector Settings (example):

Time (min)	Excitation (nm)	Emission (nm)
0,0	280	310
12,4	280	310
12,5	274	298
23,0	274	298
24,0	280	310
28,0	280	310

Note: The choice of chromatographic conditions may be different for different instrument configurations and columns applied for separation. Elution pattern should be similar to the example chromatogram shown in **Appendix 2A**.

12.2 Calculations

12.2.1 Calibration Curve

- A linear calibration curve for each individual phenolic compound is prepared by plotting the concentration of the standards versus their respective peak responses.
- Response factors are calculated for each individual phenolic compound from the calibration curves.

12.2.2. Sample Quantification

- The concentration of selected phenolic compounds in mainstream smoke samples is quantified by the external standard method. A typical chromatogram is shown in **Appendix 2B**. The identification of peaks is by comparison of their retention times with corresponding standards, and the spiking of smoke samples.
- Phenolic compound concentrations are reported in $\mu\text{g/ml}$ by the chromatography software.
- The yields of phenolic compounds in mainstream smoke in $\mu\text{g/cigarette}$ are calculated as follows:

$$M = \frac{C * V * DF}{N}$$

Where :

- M is the yield of the phenolic compound in cigarette smoke expressed in $\mu\text{g} / \text{cigarette}$,
- C is the concentration of the phenolic compound in the diluted sample solution expressed in $\mu\text{g/ml}$,
- V is the volume of 1 % acetic acid solution used for CFP extraction,
- DF is the dilution factor, *e.g.*, 10/3,
- N is the number of cigarettes smoked.

13. REPEATABILITY AND REPRODUCIBILITY

The Collaborative Study was conducted in 2013, involving 18 laboratories and five replicate analyses of 10 cigarette samples including the University of Kentucky reference cigarettes 3R4F and 1R5F and the CORESTA Monitor Test Piece CM 7 and covering a wide range of blends and cigarette design constructions. However, not all laboratories reported data for all samples and all analytes under both regimes. Some data were also removed as outliers.

Repeatability (r) and reproducibility (R) were calculated following the ISO 5725 Part 1 and 2 statistical procedures.

13.1 Explanation of Outliers and Data Analysis

ISO 5725-2 can be applied directly in determining estimates of r and R since the data were collected under repeatability and reproducibility conditions as defined by ISO 5725-1 (*i.e.* the participating laboratories all used the same method as opposed to their own internal method, thus satisfying the reproducibility condition).

13.2 Data Removal

Individual data points that were reported as non-numeric (*i.e.* below the LOQ) were removed prior to evaluation of numeric data for outliers.

13.3 Among-Laboratory Variability

For evaluation of agreement among laboratories, the Grubbs' test (ISO 5725-2, section 7.3.4) was used to detect single or multiple outlying laboratories. If the Grubbs statistic was significant at the 5 % level, the sample data from that laboratory was removed.

If the Grubbs statistic for a single high outlier was significant for certain analytes, samples or laboratories, the data were removed prior to the calculations of the mean, repeatability and reproducibility variance.

13.4 Determination of Inter-Laboratory Repeatability and Reproducibility Variance

For each of the j test samples ($j = 1, \dots, 8$), the mean (\hat{m}_j) was determined (ISO 5725-2, section 7.4.4) across the p participating laboratories whose data remained following the removal of outliers.

Repeatability variance (s_{rj}) and reproducibility variance (s_{Rj}) were determined (ISO 5725-2, section 7.4.5.1 and ISO 5725-2 Section 7.4.5.5, respectively).

Repeatability (r) and Reproducibility (R) figures calculated for 95 % confidence level were also indicated for the individual compounds and sample cigarettes.

13.5 Results

Results of mean yields, number of laboratories upon which the statistical analysis was based (N), calculated statistical data (s_r , s_R , r and R), the percentage of data reported and the percentage of data removed for the individual phenolic compounds are indicated in the following tables.

Table 2: Statistical data for hydroquinone ($\mu\text{g}/\text{cigarette}$) in mainstream cigarette smoke under the ISO 3308 smoking regime

Sample	Blend Type	ISO Tar Yield (mg/cig)	MEAN	sr	sR	r	R	N
CM7	NA	NA	91,82	4,34	8,42	12,14	23,58	18
KR 1R5F	NA	NA	8,45	0,56	1,42	1,58	3,97	17
KR 3R4F	NA	NA	32,84	1,53	3,09	4,27	8,67	18
Sample 1	Dark Air-Cured	10	42,78	3,19	4,79	8,92	13,4	14
Sample 2	American	7	36,98	2,13	3,93	5,97	10,99	18
Sample 3	American	8	46,88	2,4	4,2	6,72	11,75	18
Sample 4	Virginia	3	20,38	1,03	3,39	2,89	9,49	14
Sample 5	Virginia	1	9,3	0,64	1,32	1,78	3,68	18
Sample 6	Virginia	10	46,69	2,25	4,55	6,31	12,73	16
Sample 7	Charcoal Filtered	1	8,09	0,5	1,06	1,41	2,97	18
% Reported			99,41					
% Removed			0,58					

Table 3: Statistical data for resorcinol ($\mu\text{g}/\text{cigarette}$) in mainstream cigarette smoke under the ISO 3308 smoking regime

Sample	Blend Type	ISO Tar Yield (mg/cig)	MEAN	sr	sR	r	R	N
CM7	NA	NA	1,79	0,19	0,34	0,52	0,95	17
KR 1R5F	NA	NA	0,13	0,03	0,05	0,09	0,14	13
KR 3R4F	NA	NA	0,63	0,07	0,16	0,19	0,46	15
Sample 1	Dark Air-Cured	10	0,66	0,13	0,24	0,37	0,67	12
Sample 2	American	7	0,80	0,10	0,18	0,29	0,51	16
Sample 3	American	8	0,97	0,10	0,21	0,29	0,58	16
Sample 4	Virginia	3	0,51	0,06	0,11	0,18	0,30	13
Sample 5	Virginia	1	0,35	0,05	0,06	0,14	0,17	15
Sample 6	Virginia	10	1,19	0,08	0,22	0,22	0,63	15
Sample 7	Charcoal Filtered	1	0,12	0,04	0,05	0,10	0,13	12
% Reported			94,1					
% Removed			5,9					

Table 4: Statistical data for catechol ($\mu\text{g}/\text{cigarette}$) in mainstream cigarette smoke under the ISO 3308 smoking regime

Sample	Blend Type	ISO Tar Yield (mg/cig)	MEAN	sr	sR	r	R	N
CM7	NA	NA	96,39	4,78	5,94	13,39	16,65	18
KR 1R5F	NA	NA	8,13	0,57	0,80	1,60	2,25	17
KR 3R4F	NA	NA	36,77	1,79	2,36	5,02	6,60	18
Sample 1	Dark Air-Cured	10	35,30	2,61	2,80	7,32	7,83	14
Sample 2	American	7	39,87	2,44	3,28	6,84	9,19	18
Sample 3	American	8	42,61	1,75	2,67	4,91	7,46	17
Sample 4	Virginia	3	24,17	1,70	2,85	4,77	7,99	15
Sample 5	Virginia	1	12,89	0,71	0,93	1,98	2,61	18
Sample 6	Virginia	10	58,19	2,71	3,93	7,58	11,00	16
Sample 7	Charcoal Filtered	1	7,84	0,67	0,84	1,88	2,35	18
% Reported			99,4					
% Removed			0,6					

Table 5: Statistical data for phenol ($\mu\text{g}/\text{cigarette}$) in mainstream cigarette smoke under the ISO 3308 smoking regime

Sample	Blend Type	ISO Tar Yield (mg/cig)	MEAN	sr	sR	r	R	N
CM7	NA	NA	27,60	1,49	3,23	4,17	9,05	16
KR 1R5F	NA	NA	0,93	0,16	0,23	0,44	0,65	14
KR 3R4F	NA	NA	7,06	0,57	0,94	1,59	2,64	18
Sample 1	Dark Air-Cured	10	19,97	1,47	1,99	4,12	5,57	14
Sample 2	American	7	9,64	0,87	1,50	2,43	4,20	18
Sample 3	American	8	12,47	0,90	1,40	2,52	3,93	18
Sample 4	Virginia	3	4,58	0,44	1,18	1,24	3,30	15
Sample 5	Virginia	1	1,21	0,13	0,27	0,36	0,77	16
Sample 6	Virginia	10	13,91	1,09	1,72	3,04	4,81	16
Sample 7	Charcoal Filtered	1	0,49	0,10	0,14	0,29	0,39	13
% Reported			97,5					
% Removed			2,5					

Table 6: Statistical data for o-cresol ($\mu\text{g}/\text{cigarette}$) in mainstream cigarette smoke under the ISO 3308 smoking regime

Sample	Blend Type	ISO Tar Yield (mg/cig)	MEAN	sr	sR	r	R	N
CM7	NA	NA	5,63	0,34	0,74	0,96	2,08	18
KR 1R5F	NA	NA	0,38	0,05	0,09	0,15	0,25	15
KR 3R4F	NA	NA	2,33	0,19	0,32	0,54	0,90	18
Sample 1	Dark Air-Cured	10	5,75	0,44	0,75	1,22	2,11	14
Sample 2	American	7	2,62	0,22	0,42	0,63	1,18	18
Sample 3	American	8	3,30	0,24	0,41	0,67	1,14	18
Sample 4	Virginia	3	1,34	0,10	0,28	0,29	0,78	15
Sample 5	Virginia	1	0,42	0,05	0,11	0,15	0,31	16
Sample 6	Virginia	10	3,40	0,23	0,48	0,66	1,35	16
Sample 7	Charcoal Filtered	1	0,21	0,04	0,08	0,12	0,23	13
% Reported			99,4					
% Removed			0,6					

Table 7: Statistical data for m-cresol ($\mu\text{g}/\text{cigarette}$) in mainstream cigarette smoke under the ISO 3308 smoking regime

Sample	Blend Type	ISO Tar Yield (mg/cig)	MEAN	sr	sR	r	R	N
CM7	NA	NA	4,57	0,25	0,71	0,69	1,98	17
KR 1R5F	NA	NA	0,32	0,04	0,10	0,12	0,29	13
KR 3R4F	NA	NA	1,83	0,13	0,22	0,37	0,61	17
Sample 1	Dark Air-Cured	10	3,73	0,28	0,51	0,77	1,43	13
Sample 2	American	7	2,03	0,15	0,35	0,42	0,99	17
Sample 3	American	8	2,50	0,15	0,32	0,43	0,90	17
Sample 4	Virginia	3	1,10	0,08	0,23	0,23	0,64	14
Sample 5	Virginia	1	0,38	0,04	0,08	0,11	0,23	17
Sample 6	Virginia	10	2,78	0,19	0,33	0,53	0,92	16
Sample 7	Charcoal Filtered	1	0,24	0,04	0,13	0,10	0,36	13
% Reported			100,0					
% Removed			0,0					

Table 8: Statistical data for p-cresol ($\mu\text{g}/\text{cigarette}$) in mainstream cigarette smoke under the ISO 3308 smoking regime

Sample	Blend Type	ISO Tar Yield (mg/cig)	MEAN	sr	sR	r	R	N
CM7	NA	NA	12,45	0,58	1,34	1,63	3,76	17
KR 1R5F	NA	NA	0,73	0,09	0,16	0,27	0,46	16
KR 3R4F	NA	NA	4,56	0,27	0,56	0,75	1,58	17
Sample 1	Dark Air-Cured	10	10,40	0,80	1,13	2,24	3,16	13
Sample 2	American	7	5,44	0,43	0,81	1,19	2,26	17
Sample 3	American	8	6,49	0,38	0,69	1,07	1,93	17
Sample 4	Virginia	3	2,49	0,17	0,52	0,47	1,47	14
Sample 5	Virginia	1	0,88	0,07	0,14	0,20	0,40	17
Sample 6	Virginia	10	6,25	0,37	0,80	1,02	2,24	16
Sample 7	Charcoal Filtered	1	0,44	0,06	0,14	0,16	0,39	17
% Reported			100,0					
% Removed			0,0					

Table 9: Statistical data for hydroquinone ($\mu\text{g}/\text{cigarette}$) in mainstream cigarette smoke under the Health Canada T-115 smoking regime

Sample	Blend Type	MEAN	sr	sR	r	R	N
CM7	NA	191,17	9,79	17,51	27,40	49,02	18
KR 1R5F	NA	53,41	4,23	6,33	11,85	17,74	17
KR 3R4F	NA	89,62	4,17	8,37	11,67	23,43	18
Sample 1	Dark Air-Cured	91,44	6,50	9,88	18,19	27,67	14
Sample 2	American	101,85	5,62	9,96	15,75	27,88	18
Sample 3	American	114,04	6,52	8,59	18,27	24,05	18
Sample 4	Virginia	76,44	3,57	6,20	9,98	17,37	14
Sample 5	Virginia	43,56	3,34	5,24	9,36	14,67	18
Sample 6	Virginia	106,50	5,70	10,77	15,97	30,16	16
Sample 7	Charcoal Filtered	60,19	6,14	7,75	17,18	21,70	18
% Reported		99,4					
% Removed		0,6					

Table 10: Statistical data for resorcinol ($\mu\text{g}/\text{cigarette}$) in mainstream cigarette smoke under the Health Canada T-115 smoking regime

Sample	Blend Type	MEAN	sr	sR	r	R	N
CM7	NA	3,77	0,25	0,82	0,70	2,31	18
KR 1R5F	NA	1,07	0,13	0,19	0,38	0,55	14
KR 3R4F	NA	1,87	0,16	0,42	0,45	1,18	15
Sample 1	Dark Air-Cured	1,35	0,21	0,45	0,60	1,27	11
Sample 2	American	2,55	0,23	0,71	0,65	1,99	17
Sample 3	American	2,77	0,27	0,83	0,75	2,32	18
Sample 4	Virginia	2,21	0,29	0,54	0,80	1,52	15
Sample 5	Virginia	2,07	0,24	0,49	0,68	1,39	17
Sample 6	Virginia	2,70	0,26	0,45	0,71	1,26	16
Sample 7	Charcoal Filtered	1,46	0,20	0,45	0,57	1,27	17
% Reported		96,3					
% Removed		3,7					

Table 11: Statistical data for catechol ($\mu\text{g}/\text{cigarette}$) in mainstream cigarette smoke under the Health Canada T-115 smoking regime

Sample	Blend Type	MEAN	sr	sR	r	R	N
CM7	NA	198,81	9,77	17,45	27,35	48,87	18
KR 1R5F	NA	41,73	3,50	4,74	9,81	13,26	17
KR 3R4F	NA	90,24	5,21	8,17	14,58	22,87	18
Sample 1	Dark Air-Cured	76,29	5,01	7,91	14,03	22,16	14
Sample 2	American	100,18	5,16	9,17	14,46	25,68	18
Sample 3	American	96,21	7,21	11,15	20,19	31,23	18
Sample 4	Virginia	79,79	4,12	7,23	11,53	20,24	14
Sample 5	Virginia	54,38	4,58	6,13	12,81	17,16	18
Sample 6	Virginia	127,00	8,85	10,23	24,78	28,64	15
Sample 7	Charcoal Filtered	51,10	4,69	6,74	13,13	18,86	18
% Reported		98,8					
% Removed		1,2					

Table 12: Statistical data for phenol ($\mu\text{g}/\text{cigarette}$) in mainstream cigarette smoke under the Health Canada T-115 smoking regime

Sample	Blend Type	MEAN	sr	sR	r	R	N
CM7	NA	43,05	3,19	4,58	8,93	12,83	18
KR 1R5F	NA	8,20	0,67	1,09	1,88	3,05	17
KR 3R4F	NA	13,13	1,18	1,49	3,30	4,17	18
Sample 1	Dark Air-Cured	36,65	3,00	2,94	8,39	8,22	14
Sample 2	American	16,99	1,48	1,97	4,14	5,51	18
Sample 3	American	19,19	1,72	2,22	4,82	6,21	18
Sample 4	Virginia	12,00	1,06	1,63	2,96	4,56	15
Sample 5	Virginia	6,64	0,62	0,84	1,74	2,34	18
Sample 6	Virginia	19,33	1,64	1,99	4,59	5,58	15
Sample 7	Charcoal Filtered	6,93	1,12	1,15	3,13	3,23	18
% Reported		98,8					
% Removed		1,2					

Table 13: Statistical data for o-cresol ($\mu\text{g}/\text{cigarette}$) in mainstream cigarette smoke under the Health Canada T-115 smoking regime

Sample	Blend Type	MEAN	sr	sR	r	R	N
CM7	NA	8,674	0,674	1,293	1,89	3,62	18
KR 1R5F	NA	2,533	0,207	0,333	0,58	0,93	16
KR 3R4F	NA	4,150	0,344	0,584	0,96	1,64	18
Sample 1	Dark Air-Cured	10,884	0,900	1,280	2,52	3,58	14
Sample 2	American	4,663	0,375	0,688	1,05	1,93	18
Sample 3	American	5,219	0,437	0,717	1,22	2,01	18
Sample 4	Virginia	3,139	0,235	0,435	0,66	1,22	14
Sample 5	Virginia	1,900	0,187	0,278	0,52	0,78	17
Sample 6	Virginia	4,532	0,412	0,554	1,15	1,55	15
Sample 7	Charcoal Filtered	1,974	0,324	0,404	0,91	1,13	17
% Reported		98,8					
% Removed		1,2					

Table 14: Statistical data for m-cresol ($\mu\text{g}/\text{cigarette}$) in mainstream cigarette smoke under the Health Canada T-115 smoking regime

Sample	Blend Type	MEAN	sr	sR	r	R	N
CM7	NA	7,08	0,45	1,15	1,27	3,22	17
KR 1R5F	NA	1,78	0,17	0,27	0,48	0,76	16
KR 3R4F	NA	3,25	0,26	0,51	0,72	1,42	17
Sample 1	Dark Air-Cured	7,14	0,53	0,88	1,49	2,47	13
Sample 2	American	3,58	0,24	0,55	0,68	1,55	17
Sample 3	American	3,90	0,32	0,47	0,90	1,31	16
Sample 4	Virginia	2,65	0,23	0,48	0,64	1,34	14
Sample 5	Virginia	1,58	0,16	0,27	0,45	0,74	17
Sample 6	Virginia	3,91	0,30	0,46	0,84	1,28	16
Sample 7	Charcoal Filtered	1,67	0,24	0,41	0,69	1,16	17
% Reported		99,4					
% Removed		0,6					

Table 15: Statistical data for p-cresol ($\mu\text{g}/\text{cigarette}$) in mainstream cigarette smoke under the Health Canada T-115 smoking regime

Sample	Blend Type	MEAN	sr	sR	r	R	N
CM7	NA	20,16	1,28	2,49	3,58	6,97	16
KR 1R5F	NA	5,53	0,39	0,80	1,09	2,24	16
KR 3R4F	NA	8,54	0,64	0,92	1,78	2,56	16
Sample 1	Dark Air-Cured	21,25	1,53	2,70	4,29	7,55	13
Sample 2	American	9,99	0,71	1,27	1,98	3,56	16
Sample 3	American	10,77	0,78	1,27	2,19	3,55	16
Sample 4	Virginia	6,49	0,47	0,91	1,31	2,56	14
Sample 5	Virginia	4,14	0,33	0,46	0,91	1,30	17
Sample 6	Virginia	9,08	0,66	1,01	1,84	2,82	15
Sample 7	Charcoal Filtered	4,12	0,65	0,71	1,82	1,98	16
% Reported		96,3					
% Removed		3,7					

14. REPORT

14.1 Test results

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.

The amount of individual selected phenols in the mainstream smoke of cigarettes is reported in $\mu\text{g}/\text{cig}$ to the nearest 0,1 μg .

Appendix 1

Example of Preparation of Calibration Standards

A: Stock Standards

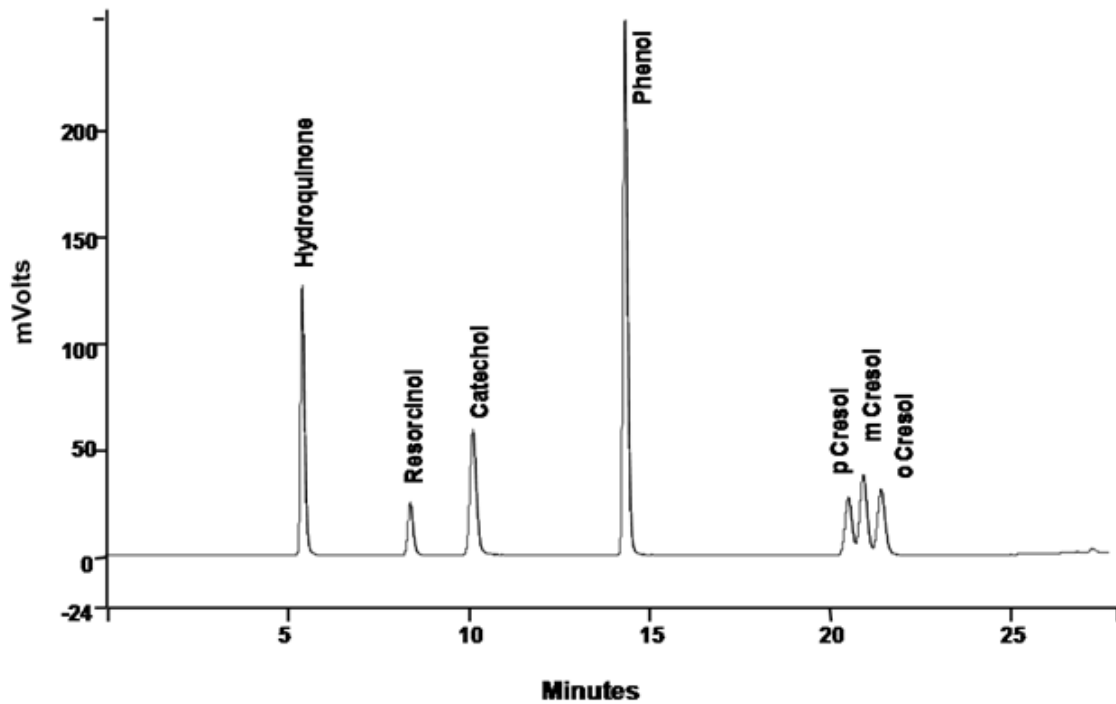
Phenolic Compound	Primary Standards			Secondary Standards			
	Weight [mg]	Purity [%]	Final volume with 1 % Acetic Acid [ml]	Concentration [mg/ml]	Volume of Primary Standard [ml]	Final Volume with 1 % Acetic Acid [ml]	Concentration [µg/ml]
Hydroquinone	25,0	99,9	25	1,000	0,500	50	10,00
Resorcinol	25,0	99,9	50	0,500	0,300		3,00
Catechol	25,0	99,9	25	1,000	0,500		10,00
Phenol	25,0	99,9	25	1,000	0,500		10,00
p-Cresol	25,0	99,1	50	0,496	0,200		1,98
m-Cresol	25,0	99,5	50	0,498	0,200		1,99
o-Cresol	25,0	99,9	50	0,500	0,200		2,00

B: Calibration Standards

Standard Level		1	2	3	4	5	6
Volume of Secondary Standard	[ml]	0,2	1,0	2,0	4,0	6,0	8,0
Final Volume with 1 % Acetic Acid	[ml]	10	10	10	10	10	10
Hydroquinone	[µg/ml]	0,200	1,000	2,000	4,000	5,99	7,99
Resorcinol	[µg/ml]	0,060	0,300	0,599	1,200	1,80	2,40
Catechol	[µg/ml]	0,200	1,000	2,000	4,000	5,99	7,99
Phenol	[µg/ml]	0,200	1,000	2,000	4,000	5,99	7,99
p-Cresol	[µg/ml]	0,040	0,199	0,398	0,796	1,19	1,59
m-Cresol	[µg/ml]	0,040	0,198	0,396	0,793	1,19	1,59
o-Cresol	[µg/ml]	0,040	0,200	0,400	0,799	1,20	1,60

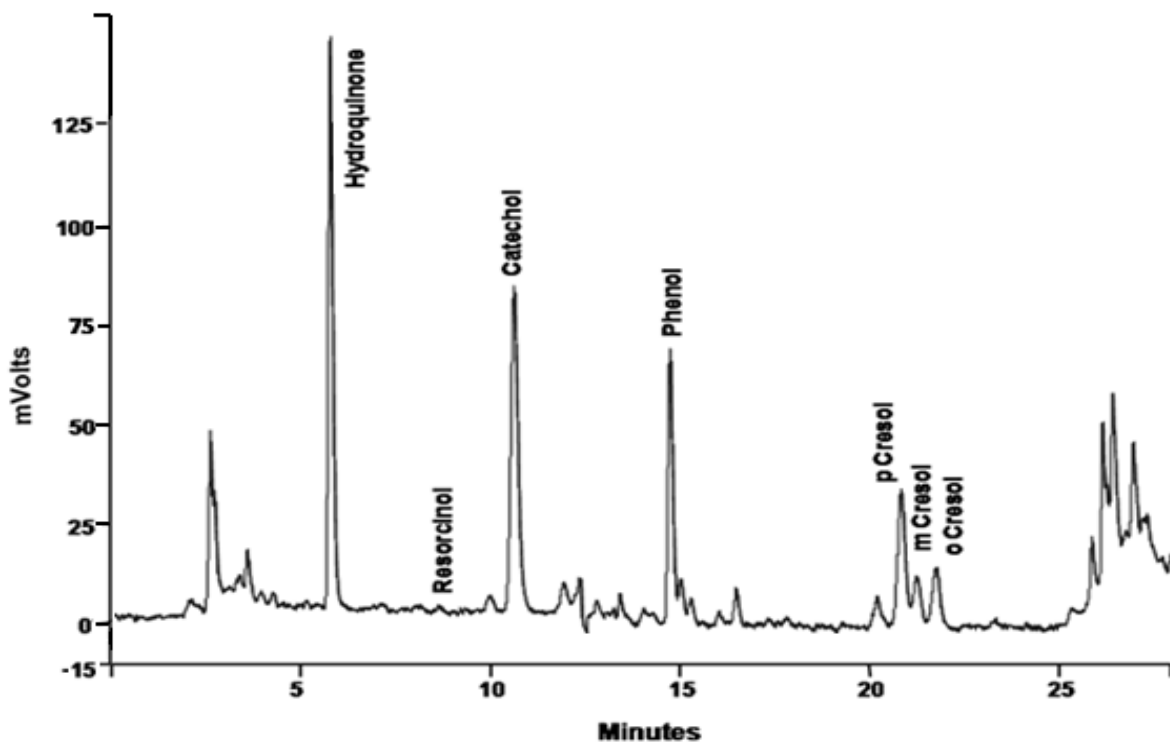
Appendix 2A

Example of a Chromatogram of a Phenolic Calibration Standard



Appendix 2B

Typical Chromatogram of a Mainstream Cigarette Smoke Sample of KR 3R4F



15. BIBLIOGRAPHY

- [1] ISO 5725-1: Accuracy (trueness and precision) of measurement methods and results – Part 1: General principles and definitions.
- [2] ISO 5725-2: Accuracy (trueness and precision) of measurement methods and results – Part 2: Basic method for the determination of repeatability (r) and reproducibility (R) of a standard measurement method.