INTERNATIONAL STANDARD

ISO 23905

First edition
2020-06

Cigarettes — Determination of selected phenolic compounds in cigarette mainstream smoke using HPLC-FLD

Cigarettes — Dosage de composés phénoliques sélectionnés dans le courant principal de la fumée de cigarette par CLHP-FLD

Reference number
ISO 23905:2020(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 126, *Tobacco and tobacco products*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

The CORESTA[1)] Smoke Analytes Sub-Group selected one method using reversed phase high performance liquid chromatography with fluorescence detection (HPLC-FLD) for the determination of selected phenolic compounds in cigarette mainstream smoke. Smoke was collected on a glass fibre filter pad and extracted with a 1 % acetic acid solution.

A CORESTA recommended method (CRM) was written[1] on the basis of the results obtained in a collaborative study conducted in 2013 involving 18 laboratories using cigarettes manufactured from a range of blend styles.

This document is based upon the CRM 78 and includes statistical evaluations carried out according to ISO 5725-1[2] and ISO 5725-2[3].

No machine smoking regime can represent all human smoking behaviour.

— It is recommended that cigarettes also be tested under conditions of a different intensity of machine smoking than those specified in this document.

— Machine smoking testing is useful to characterize cigarette emissions for design and regulatory purposes, but communication of machine measurements to smokers can result in misunderstandings about exposure and risk across brands.

— Smoke emission data from machine measurements may be used as inputs for product hazard assessment, but they are not intended to be nor are they valid as measures of human exposure or risks. Communicating differences between products in machine measurements as differences in exposure or risk is a misuse of testing using ISO standards.

1) Available at: www.coresta.org.

Cigarettes — Determination of selected phenolic compounds in cigarette mainstream smoke using HPLC-FLD

WARNING — The use of this document involves hazardous materials, operations and equipment. This document does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this document to establish appropriate safety and health practices and determine the applicability of any other restrictions prior to use.

1 Scope

This document specifies a method for the quantification of selected phenolic compounds by high performance liquid chromatography with fluorescence detection (HPLC-FLD) using ISO 3308 smoking parameters. The selected phenolic compounds are: hydroquinone, resorcinol, catechol, phenol, p-Cresol, m-Cresol and o-Cresol.

This method is applicable to cigarettes with total particulate matter (TPM) yields between 1 mg/cigarette and 16 mg/cigarette.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3308, *Routine analytical cigarette-smoking machine — Definitions and standard conditions*

ISO 3402, *Tobacco and tobacco products — Atmosphere for conditioning and testing*

ISO 4387, *Cigarettes — Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine*

ISO 8243, *Cigarettes — Sampling*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

4 Principle

Selected phenolic compounds are collected by passing the mainstream smoke of cigarettes through a glass fibre filter pad as specified in ISO 3308 (e.g. Cambridge filer pad, CFP).

The CFP is extracted by a 1 % acetic acid solution. The obtained filtered solution is diluted and analysed by HPLC-FLD.

5 Apparatus

The usual laboratory apparatus for use in preparation of samples, solutions and standards and, in particular, the following.

5.1 Routine analytical cigarette-smoking machine, complying with the requirements of ISO 3308.

5.2 High performance liquid chromatography system, consisting of a binary gradient pump, an auto sampler with sampling loop and cooling unit, a fluorescence detector, a data collection system.

5.3 HPLC column, with pentafluorphenylpropyl (PFP) stationary phase (e.g. 3 µm, 150 mm × 4,6 mm or equivalent).

5.4 Disposable guard column, such as PFP cartridge (e.g. 4 mm × 3,00 mm or equivalent).

5.5 Wrist action shaker, or equivalent.

5.6 Analytical balance, suitable for measuring to the nearest 0,1 mg.

5.7 Glassware, actinic red Erlenmeyer flasks of appropriate volumes with ground glass stoppers, actinic red volumetric flasks (10 ml, 25 ml and 50 ml).

5.8 Mechanical pipettes with disposable plastic tips.

6 Reagents

All reagents shall be at least of analytical reagent grade.

6.1 Methanol, HPLC grade.

6.2 Acetic acid, HPLC grade.

6.3 Hydroquinone, > 99 %.

6.4 Resorcinol, > 99 %.

6.5 Catechol, > 99 %.

6.6 Phenol, > 99 %.

6.7 p-Cresol, > 99 %.

6.8 m-Cresol, > 99 %.

6.9 o-Cresol, > 99 %.

6.10 Helium, (UHP), if necessary for sparging of HPLC mobile phase or equivalent degassing system.

6.11 Deionised water, with a resistivity > 18 MΩ·cm at 25 °C.

7 Preparation

7.1 General

Glassware equipment shall be cleaned and dried in such a manner which ensures that contamination does not occur.

7.2 Preparation of solutions — Acetic acid, with a volume fraction of 1 % solution

Add approximately 3 500 ml of deionized water to a 4 l volumetric flask. Add 40 ml of acetic acid to the flask. Mix and dilute to the volume with deionized water.

7.3 Preparation of standards

7.3.1 Primary phenolic compounds stock solutions

Weigh approximately 25 mg of each of the phenolic compounds as described in the Table 1 into individual 25 ml or 50 ml volumetric flasks and dissolve in 1 % acetic acid solution (see 7.2).

Table 1 — Preparation of primary phenolic compounds stock solutions

Compound	Weight (mg)	Purity (%)	Final volume (ml)	Concentration (mg/ml)
Hydroquinone	25,0	99,9	25	1,000
Resorcinol	25,0	99,9	50	0,500
Catechol	25,0	99,9	25	1,000
Phenol	25,0	99,9	25	1,000
p-Cresol	25,0	99,1	50	0,496
m-Cresol	25,0	99,5	50	0,498
o-Cresol	25,0	99,9	50	0,500

The tables for stock and standards given in the Tables 1 to 3 are given as examples. Each laboratory may prepare stock and calibration standards at different concentrations based on their samples. The primary phenolic compounds stock solutions are stored in the refrigerator and are to be prepared fresh every two weeks. Each laboratory may perform stability studies to determine the shelf life of the solutions.

7.3.2 Secondary phenolic compounds stock solutions

Pipette predetermined volumes, according to Table 2, of each primary phenolic compounds stock solution (see 7.3.1) into a 50 ml volumetric flask and dilute to volume with 1 % acetic acid solution (see 7.2).

Table 2 — Preparation of secondary phenolic compounds stock solutions

Compound	Volume of primary standard (ml)	Concentration (µg/ml)
Hydroquinone	0,500	10,00
Resorcinol	0,300	3,00
Catechol	0,500	10,00
Phenol	0,500	10,00
p-Cresol	0,200	1,98
m-Cresol	0,200	1,99
o-Cresol	0,200	2,00

The solutions are stable for about 5 days if stored in a refrigerator. Each laboratory may perform stability studies to determine the shelf life of the solutions.

7.3.3 Phenolic compounds working standards

Pipette appropriate volumes of each of the secondary phenolic compounds stock solutions (see 7.3.2) according to Table 3 into a 10 ml volumetric flask. Dilute to volume with 1 % acetic acid solution (see 7.2).

Table 3 — Preparation of phenolic compounds working 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
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

The solutions are stable for about 5 days if stored in a refrigerator. Each laboratory may perform stability studies to determine the shelf life of the solutions.

8 Sampling

Carry out sampling in accordance with ISO 8243.

9 Tobacco product preparation

Condition the cigarettes in accordance with ISO 3402.

10 Sample generation — Smoking of cigarettes

10.1 General

The smoking parameters for which the method has been studied are defined in ISO 3308.

10.2 Smoking machine setup

An analytical cigarette-smoking machine according to ISO 3308 is required.

Check and adjust the puff volume drawn by the smoking machine at all channels, in accordance with ISO 4387.

To determine whether a leak has occurred in the analytical smoking machine setup, use a leak tester. If the fluid column does not maintain its position but drops, there is a leak in the system.

10.3 Smoking

The cigarettes are smoked according to ISO 3308.

11 Sample analysis

11.1 Preparation of sample

After all samples have been smoked following ISO 3308, remove the glass fibre filter pad from the smoking machine, fold into quarters and place into a 125 ml extraction flask for 44 mm glass fibre filter pad (or in a 250 ml extraction flask for 92 mm glass fibre filter pad). Add 40 ml of 1 % acetic acid solution (7.2) for 44 mm glass fibre filter pad (80 ml for 92 mm glass fibre filter pad). Cover the flask with ground glass stopper, shake the flask until the glass fibre filter pad has disintegrated and filter the extract through a 0,45 µm syringe filter.

NOTE There might be a need to dilute (with 1 % acetic solution) the obtained solution so that the concentration of phenolic compounds is within the calibration range.

Transfer an aliquot of the filtered extract to a vial and fill the vial to minimize the headspace.

11.2 Determination

11.2.1 HPLC-FLD operating conditions

Set up and operate the HPLC-FLD in accordance with the manufacturer's instruction.

The following parameters have been found to be suitable for operation.

Chromatographic parameters:

Column temperature: ambient

Auto sampler temperature: 4 °C (±2 °C)

Injection volume: 10 µl to 20 µl

Mobile phase

Solvent A: 1 % acetic acid in deionized water

Solvent B: 1 % acetic acid in methanol

Flow: 0,8 ml/minute

Gradient (see Table 4)

Table 4 — Example of gradient

Time (min)	% A	% B
0	78	22
8	78	22
8,5	55	45
21	55	45
22	0	100
28	0	100

Wavelength programmable fluorescence detector settings (see Table 5).

Table 5 — Example of FLD settings

Time (min)	Excitation wavelength (nm)	Emission wavelength (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 is given as an example. A typical chromatogram is provided in Annex A.

11.2.2 Calibration

Analyse each calibration standard (see 7.3.3). Record the area, generate a calibration curve using a linear function. Calculate a response factor for each individual phenolic compound. The response obtained for test samples shall fall within the working range of the calibration curve.

The concentration of each phenolic compound in cigarette mainstream smoke samples is quantified by the external standard method. The identification of each phenolic compound peak in samples is determined by its comparison with the respective retention times in standards.

11.2.3 Calculation

The yield of individual phenolic compounds in the mainstream smoke of cigarettes, m_i expressed in micrograms per cigarette is given by Formula (1):

$$mi = \frac{C_i \times V \times Df}{N_{\text{cig}}} \tag{1}$$

where

C_i is the concentration of the phenolic compound in the diluted sample solution expressed in µg /ml;

V is the volume of 1 % acetic acid solution used for CFP extraction;

Df is the dilution factor;

N_{cig} is the number of cigarettes smoked.

The expression of the laboratory data depends on the purpose for which the data are required, and the level of laboratory precision. Any further statistical analyses should be calculated and expressed on the basis of the laboratory data before any rounding has taken place.

The yield of individual phenolic compounds in the mainstream smoke of cigarettes is reported in micrograms per cigarette (µg/cigarette) to the nearest 0,01 µg.

12 Repeatability and reproducibility

12.1 General

An interlaboratory study was conducted in 2013, involving 18 laboratories and 10 cigarette samples[4]. This provided data on the measurement of selected phenolic compounds in replicate analyses of 10 samples (see Table 6) performed under ISO 3308 smoking regime. The values for repeatability, *r*, and

reproducibility, *R*, given in Table 7, were obtained using this method. The statistical evaluation was performed according to ISO 5725-2.

The difference between two single results found on matched cigarette samples by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability limit, *r,* on average not more than once in 20 cases in the normal and correct operation of this method.

Single results on matched cigarette samples reported by two laboratories will differ by more than the reproducibility limit, *R*, on average not more than once in 20 cases in the normal and correct operation of the method.

Table 6 — Cigarette test samples of 2013 collaborative study

Sample	Product characterization	TPM yield (mg/cigarette)
Sample 1	Dark-air cured product	14
Sample 2	American blended product	9
Sample 3	American blended product	10
Sample 4	Virginia blended product	4
Sample 5	Virginia blended product	2
Sample 6	Virginia blended product	11
Sample 7	Charcoal filtered/blended product	1
Ky 3R4F	Kentucky reference cigarette 3R4F/American blend	10
Ky 1R5F	Kentucky reference cigarette 1R5F/American blend	2
CM 7	CM 7 test piece/Virginia blend	16

12.2 Results of the 2013 collaborative study

Calculated statistical data for selected phenolic compounds are given in Tables 7 to 13.

Table 7 — Statistical data for hydroquinone

Sample	Mean (µg/cigarette)	*r* (µg/cigarette)	*R* (µg/cigarette)	N[a]
Sample 1	42,78	8,92	13,40	14
Sample 2	36,98	5,97	10,99	18
Sample 3	46,88	6,72	11,75	18
Sample 4	20,38	2,89	9,49	14
Sample 5	9,30	1,78	3,68	18
Sample 6	46,69	6,31	12,73	16
Sample 7	8,09	1,41	2,97	18
Ky 3R4F	32,84	4,27	8,67	18
Ky 1R5F	8,45	1,58	3,97	17
CM 7	91,82	12,14	23,58	18

[a] N is the number of data sets taken for the statistical analysis after removal of outliers.

Table 8 — Statistical data for resorcinol

Sample	Mean (µg/cigarette)	*r* (µg/cigarette)	*R* (µg/cigarette)	N[a]
Sample 1	0,66	0,37	0,67	12
Sample 2	0,80	0,29	0,51	16
Sample 3	0,97	0,29	0,58	16
Sample 4	0,51	0,18	0,30	13
Sample 5	0,35	0,14	0,17	15
Sample 6	1,19	0,22	0,63	15
Sample 7	0,12	0,10	0,13	12
Ky 3R4F	0,63	0,19	0,46	15
Ky 1R5F	0,13	0,09	0,14	13
CM 7	1,79	0,52	0,95	17

[a] N is the number of data sets taken for the statistical analysis after removal of outliers.

Table 9 — Statistical data for catechol

Sample	Mean (µg/cigarette)	*r* (µg/cigarette)	*R* (µg/cigarette)	N[a]
Sample 1	35,30	7,32	7,83	14
Sample 2	39,87	6,84	9,19	18
Sample 3	42,61	4,91	7,46	17
Sample 4	24,17	4,77	7,99	15
Sample 5	12,89	1,98	2,61	18
Sample 6	58,19	7,58	11,00	16
Sample 7	7,84	1,88	2,35	18
Ky 3R4F	36,77	5,02	6,60	18
Ky 1R5F	8,13	1,60	2,25	17
CM 7	96,39	13,39	16,65	18

[a] N is the number of data sets taken for the statistical analysis after removal of outliers.

Table 10 — Statistical data for phenol

Sample	Mean (µg/cigarette)	*r* (µg/cigarette)	*R* (µg/cigarette)	N[a]
Sample 1	19,97	4,12	5,57	14
Sample 2	9,64	2,43	4, 20	18
Sample 3	12,47	2,52	3,93	18
Sample 4	4,58	1,24	3,30	15
Sample 5	1,21	0,36	0,77	16
Sample 6	13,91	3,04	4,81	16
Sample 7	0,49	0,29	0,39	13
Ky 3R4F	7,06	1,59	2,64	18
Ky 1R5F	0,93	0,44	0,65	14
CM 7	27,60	4,17	9,05	16

[a] N is the number of data sets taken for the statistical analysis after removal of outliers.

Table 11 — Statistical data for o-Cresol

Sample	Mean (µg/cigarette)	*r* (µg/cigarette)	*R* (µg/cigarette)	N[a]
Sample 1	5,75	1,22	2,11	14
Sample 2	2,62	0,63	1,18	18
Sample 3	3,30	0,67	1,14	18
Sample 4	1,34	0,29	0,78	15
Sample 5	0,42	0,15	0,31	16
Sample 6	3,40	0,66	1,35	16
Sample 7	0,21	0,12	0,23	13
Ky 3R4F	2,33	0,54	0,90	18
Ky 1R5F	0,38	0,15	0,25	15
CM 7	5,63	0,96	2,08	18

[a] N is the number of data sets taken for the statistical analysis after removal of outliers.

Table 12 — Statistical data for m-Cresol

Sample	Mean (µg/cigarette)	*r* (µg/cigarette)	*R* (µg/cigarette)	N[a]
Sample 1	3,73	0,77	1,43	13
Sample 2	2,03	0,42	0,99	17
Sample 3	2,50	0,43	0,90	17
Sample 4	1,10	0,23	0,64	14
Sample 5	0,38	0,11	0,23	17
Sample 6	2,78	0,53	0,92	16
Sample 7	0,24	0,10	0,36	13
Ky 3R4F	1,83	0,37	0,61	17
Ky 1R5F	0,32	0,12	0,29	13
CM 7	4,57	0,69	1,98	17

[a] N is the number of data sets taken for the statistical analysis after removal of outliers.

Table 13 — Statistical data for p-Cresol

Sample	Mean (µg/cigarette)	*r* (µg/cigarette)	*R* (µg/cigarette)	N[a]
Sample 1	10,40	2,24	3,16	13
Sample 2	5,44	1,19	2,26	17
Sample 3	6,49	1,07	1,93	17
Sample 4	2,49	0,47	1,47	14
Sample 5	0,88	0,20	0,40	17
Sample 6	6,25	1,02	2,24	16
Sample 7	0,44	0,16	0,39	17
Ky 3R4F	4,56	0,75	1,58	17
Ky 1R5F	0,73	0,27	0,46	16
CM 7	12,45	1,63	3,76	17

[a] N is the number of data sets taken for the statistical analysis after removal of outliers.

13 Report

The test report shall state all tested product(s) each with unique identification, reference to the smoking regime used for sample generation, the yield of selected phenolic compounds in micrograms per cigarette smoked, and the method used. The test report shall state the method used, and include all conditions not specified in this document or regarded as optional. All information should be recorded in fully traceable manner.