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Cigarettes — Determination of selected phenolic compounds in cigarette mainstream smoke with an intense smoking regime using HPLC-FLD

Cigarettes — Dosage de composés phénoliques sélectionnés dans le courant principal de la fumée de cigarette avec un régime de fumage intense par CLHP-FLD

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Foreword

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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).

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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[2)] 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 interlaboratory study conducted in 2013 involving 18 laboratories using cigarettes manufactured from a range of blend styles. Cigarettes were smoked with the intense smoking regime specified in Health Canada Official Method T-115 (equivalent to ISO 20778).

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.

2) Until 2017, this was referred to as CORESTA Special Analytes Sub-group.

Cigarettes — Determination of selected phenolic compounds in cigarette mainstream smoke with an intense smoking regime 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 20778 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 20 mg/cigarette and 45 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 3402, *Tobacco and tobacco products — Atmosphere for conditioning and testing*

ISO 8243, *Cigarettes — Sampling*

ISO 20778, *Cigarettes — Routine analytical cigarette smoking machine — Definitions and standard conditions with an intense smoking regime*

ISO 20779, *Cigarettes — Generation and collection of total particulate matter using a routine analytical smoking machine with an intense smoking regime*

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 20779 (e.g. Cambridge filter pad, CFP), with intense smoking regime as specified in ISO 20778.

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 20778.

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 20778.

10.2 Smoking machine setup

An analytical cigarette-smoking machine complying with the requirements of ISO 20778 is required.

Check and adjust the puff volume drawn by the smoking machine at all channels, as described in ISO 20779.

Use a leak tester to determine whether a leak has occurred in the analytical smoking machine setup. 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 20779.

11 Sample analysis

11.1 Preparation of sample

After all samples have been smoked following ISO 20779, remove the CFP from the smoking machine, fold into quarters and place into a 125 ml extraction flask for 44 mm CFP (or in a 250 ml extraction flask for 92 mm CFP). Add 40 ml of 1 % acetic acid solution (see 7.2) for 44 mm CFP (80 ml for 92 mm CFP). Cover the flask with ground glass stopper, shake the flask until the CFP 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 minimise 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

Autosampler 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/min

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).