



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

Smoke Analysis Sub-Group

**CORESTA Recommended Method
No. 97**

**DETERMINATION OF MENTHOL IN
MAINSTREAM CIGARETTE SMOKE
WITH AN
INTENSE SMOKING REGIME
BY GAS CHROMATOGRAPHY**

April 2021



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CORESTA RECOMMENDED METHOD N° 97

DETERMINATION OF MENTHOL IN MAINSTREAM CIGARETTE SMOKE WITH AN INTENSE SMOKING REGIME BY GAS CHROMATOGRAPHY

(April 2021)

0. INTRODUCTION

In 2020 the CORESTA Routine Analytical Chemistry Sub-Group (RAC) initiated a collaborative study for the determination of menthol in mainstream cigarette smoke condensates generated under ISO 3308 (non-intense) and ISO 20778 (intense) smoking conditions [1]. Menthol was determined following ISO 13110:2012 “Cigarettes - Determination of menthol in smoke condensates — Gas-chromatographic method” [2]. ISO 13110 was developed for smoke condensates generated under non-intense conditions but had not been evaluated in an interlaboratory study for smoke condensates generated under intense conditions. The results of the study demonstrate that ISO 13110 is suitable for the analysis of menthol in smoke condensates generated under both non-intense and intense conditions. These results are the basis for this CORESTA Recommended Method (CRM).

1. SCOPE

This Recommended Method is used to quantitatively determine the concentration of menthol in the total particulate matter (TPM) of mainstream cigarette smoke generated under ISO 20778 (intense) smoking conditions using gas chromatography (GC) with flame ionization detection (FID).

2. NORMATIVE REFERENCES

- 2.1 CORESTA Guide No. 24 — *Sample Handling of Mentholated Cigarettes and Cut Filler*
- 2.2 ISO 8243, *Cigarettes — Sampling*
- 2.3 ISO 20778, *Cigarettes — Routine analytical cigarette smoking machine — Definitions and standard conditions with an intense smoking regime*
- 2.4 ISO 20779, *Cigarettes — Generation and collection of total particulate matter using a routine analytical smoking machine with an intense smoking regime*

3. PRINCIPLE

Cigarettes are smoked on a routine analytical smoking machine under ISO 20778 conditions and the TPM of mainstream smoke is trapped on a glass fibre filter pad. The glass fibre filter pad is extracted with an organic solvent with internal standard. An aliquot of the extract is analyzed by GC-FID to determine the menthol content. Results are reported as milligrams of menthol per cigarette.

4. APPARATUS

- 4.1 Gas chromatograph equipped with a split/splitless multimode injector, flame ionization detector, autosampler, and computerized controlled data acquisition.
- 4.2 Column: DB-WAX^[1] (preferably 30 m, 0,53 mm internal diameter, 1 µm film thickness) or any other type of column showing equivalent separation capability. Example chromatograms are given in Figure A1 and A2 (Annex A).
- 4.3 Analytical balance: 0,1 mg accuracy
- 4.4 Extraction vessel: different vessel types may be utilized, including but not limited to: 100 ml Pyrex bottles with crimp seals and septa, 100 ml – 250 ml Erlenmeyer flasks with stoppers, and 25 mm × 200 mm culture tubes with PTFE lined caps.
- 4.5 Shaker: orbital or wrist-action acceptable.
- 4.6 Amber autosampler vials with PTFE lined septa
- 4.7 General laboratory equipment necessary for the preparation of samples, standards, and reagents.

5. REAGENTS

All reagents must be of recognized analytical grade or better.

5.1 Menthol [CAS: 2216-51-5] (≥ 99 % purity)

NOTE: It is recommended to store menthol in an air tight container not exposed to a heat source and light. Storage at a temperature lower than 4 °C is recommended.

5.2 Internal standard: n-heptadecane [CAS: 629-78-7] (≥ 99 % purity). Other internal standards such as anethol [CAS: 104-46-1] and carvone [CAS: 6485-40-1] may also be suitable if there is not an interference with another smoke component.

5.3 Propan-2-ol [CAS: 67-63-0] (≥ 99 % purity). Methanol [CAS: 67-56-1] or ethanol [CAS: 64-17-5] may also be used.

5.4 Carrier gas: helium [CAS: 7440-59-7] or nitrogen [CAS: 7727-37-9] or hydrogen [CAS: 1333-74-0] of high purity (at least 99,999 %).

5.5 Auxiliary gases: hydrogen [CAS: 1333-74-0] of high purity (at least 99,999 %) and air for the flame ionization detector.

6. PREPARATION OF EXTRACTION SOLVENT

Dissolve the internal standard in propan-2-ol to produce the extraction solvent whose mass concentration is in the range of 0,2 mg/ml to 0,5 mg/ml. Other solvents such as methanol and ethanol may also be used; however, extraction efficiency must be assessed.

^[1] DB-WAX is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement of this product.

7. STANDARDS

Dissolve the menthol in the extraction solvent to produce a series of at least four calibration solutions whose mass concentrations cover the range expected to be found in the test portion. Solvent and solutions stored at low temperatures shall be allowed to equilibrate to room temperature before use.

NOTE: Room temperature should be indicative of temperatures around 22 °C. If the room temperature is substantially different from 22 °C then all solvents, internal standards, and calibration standards need to be tested to prove their viability for use in the method under the temperature conditions of the laboratory.

The following is an example for the calibration standards. Different amounts and volumes can be used, if necessary, to prepare the standards, provided the concentrations of the prepared calibration standards cover the anticipated concentration range of the samples.

Table 1. Menthol Calibration Standards

| Calibration Level | Nominal menthol Concentration ¹ (mg/ml) |
|-------------------|--|
| 1 | 0,02 |
| 2 | 0,05 |
| 3 | 0,10 |
| 4 | 0,20 |
| 5 | 0,50 |
| 6 | 1,00 |

¹ The actual concentrations will vary depending upon the amount weighed and the purity of menthol.

The stock solutions have been shown to be stable for three months when stored in the refrigerator in tightly sealed bottles. The working calibration standards have been shown to be stable for one month when stored in the refrigerator in tightly sealed bottles. Laboratories should determine stability of all solutions under their conditions of use.

8. SAMPLING

Sampling is performed in accordance with ISO 8243.

9. SAMPLE GENERATION – SMOKING OF CIGARETTES

Samples shall be handled in accordance with CORESTA Guide N° 24. Sample Handling of Mentholated Cigarettes and Cut Filler.

9.1 General: Cigarettes are smoked in accordance with ISO 20778 and ISO 20779.

9.2 Linear Smoking: Typically, 3 cigarettes are smoked per 44 mm diameter glass fibre filter pad constituting one replicate.

9.3 Rotary Smoking: Typically, 10 cigarettes are smoked per 92 mm diameter glass fibre filter pad constituting one replicate.

NOTE: Glass fibre filter pads of 44 mm diameter are capable of retaining up to 150 mg of TPM while glass fibre filter pads of 92 mm diameter are capable of retaining 600 mg of TPM. If this mass is exceeded, the number of cigarettes should be reduced.

10. SAMPLE EXTRACTION

10.1 Preparation of sample:

Remove the glass fibre filter pad from the pad holder and wipe the inside of the holder with back side of the filter pad with no visible particulate matter. Add the filter pad to the extraction container.

Add 20 ml of extraction solution to the extraction vessel for 44 mm filter pads and add 50 ml of extraction solution to the extraction vessel for 92 ml filter pads and then cap the extraction vessel. Ensure the filter pad is fully submerged. The volume may be adjusted to give a concentration within the range of the calibration standards.

Shake the sample on an orbital shaker for at least 20 minutes at a speed sufficient to ensure adequate extraction of the smoke condensate.

11. SAMPLE ANALYSIS

11.1 Gas Chromatograph:

The instrument conditions specified below are recommendations and may be adjusted to obtain suitable chromatography.

Set up the apparatus and operate the gas chromatograph in accordance with the manufacturer's instructions. Ensure that the peaks for solvent, internal standard, menthol and other tobacco component peaks are well resolved. It is recommended to equip the gas chromatograph with an autosampler for sample injection.

Instrument operating conditions that have been found to be suitable for the specified column are as follows:

- carrier gas: helium at a flow rate at approximately 12 ml/min;
- make up gas: helium at a flow rate at approximately 5 ml/min;
- injection temperature: 200 °C;
- split ratio (approximately): 10/1;
- injection volume: 1 µl;
- oven temperature 1: 100 °C;
- time period 1 (initial): 2 min;
- temperature program 1: 10 °C/min;
- oven temperature 2: 150 °C;
- time period 2 (intermediate): 0,2 min;
- temperature program 2: 20 °C/min;
- oven temperature 3: 200 °C;
- time period 3 (final): 3 min;
- detector temperature: 250 °C.

Using the above conditions and the specified column, the analysis time is approximately 6 min to 8 min per sample.

11.2 Calibration of the gas chromatograph:

Inject a 1 µl aliquot of each of the calibration solutions into the gas chromatograph.

Calculate the ratio of the menthol peak to the internal standard peak from the peak area data for each of the calibration solutions. Generate a calibration curve by calculating a linear regression equation of the peak area ratios as a function of the concentration of menthol. The intercept of the regression line should be close to zero; if the intercept is not close to zero an investigation shall be performed, and the calibration should be repeated if necessary.

Perform this full calibration procedure daily before use.

In addition, verify the calibration by injecting an aliquot of an intermediate concentration standard, which should be prepared from a separate stock, after calibration and after approximately every 20 test portions. If the calculated concentration for this solution differs by more than 3 % from the original value, repeat the full calibration procedure.

11.3 Determination of the concentration of menthol in the test samples:

Inject 1 µl aliquots of each test portion into the gas chromatograph. Calculate the ratio of the menthol peak/internal standard peak from the peak area data and obtain the concentration of menthol in the solution by input of this ratio in the calibration curve.

11.4 Expression of Results:

From the concentration of menthol in the test portion, determine the amount of menthol per cigarette to the nearest 0,01 mg.

The menthol concentration (in mg/ml) in the test portion is determined by the internal standard calibration method using the regression equation derived from the calibration curve. Results are then converted and reported on a per cigarette basis, typically mg/cig.

$$C_M = \frac{C \times V}{q}$$

Where

M is the menthol concentration

C is the concentration of menthol obtained from the calibration curve, in mg/ml

V is the volume of extraction solution, in ml (normally 20 ml or 50 ml)

q is the number of test cigarettes extracted in the extraction vessel (normally 3 cigarettes or 10 cigarettes)

12. REPEATABILITY AND REPRODUCIBILITY

In 2020, an international collaborative study involving 18 laboratories was conducted for the determination of menthol in cigarettes. The study included four different mentholated cigarettes of differing blends, yields, and menthol content [1]. The cigarettes were smoked under ISO 20778 (intense) smoking conditions. The samples are described in Table 2. The values for repeatability limit, *r*, and reproducibility limit, *R*, obtained from this study are provided in Table 3. The statistical evaluation was performed according to ISO 5725-2 and assumed a test result to consist of the smoking of 20 cigarettes.

Table 2. Sample Description

| Sample Identification | Blend Type | ISO 20778 Mean TPM Yields (mg/cig) ¹ |
|-----------------------|------------|---|
| Sample A | American | 38,7 |
| Sample B | Virginia | 41,5 |
| Sample C | American | 38,3 |
| Sample D | American | 54,0 |

¹ Mean TPM (total particulate matter) yields were determined in this study

Table 3. Repeatability (r) and Reproducibility (R) Limits for Menthol Under ISO 20778 Conditions

| Product | No. of Labs * | Mean Menthol (mg/cig) | Repeatability | | Reproducibility | |
|----------|---------------|-----------------------|---------------|---------------|-----------------|---------------|
| | | | R (mg/cig) | r (% of mean) | R (mg/cig) | R (% of mean) |
| Sample A | 18 | 4,39 | 0,347 | 7,9 | 1,003 | 22,8 |
| Sample B | 18 | 3,59 | 0,235 | 6,5 | 0,765 | 21,3 |
| Sample C | 18 | 1,31 | 0,090 | 6,8 | 0,299 | 22,8 |
| Sample D | 18 | 1,18 | 0,087 | 7,4 | 0,206 | 17,5 |

* The number of laboratory data sets after removal of outliers.

13. TEST REPORT

The test report shall state the amount of menthol per cigarette, the method used, and shall include all conditions not specified in this document or regarded as optional. It shall also give all details necessary for the identification of the cigarettes smoked.

14. REFERENCES

- [1] CORESTA Routine Analytical Chemistry Sub-Group Technical Report: *2020 Collaborative Study for the Determination of Menthol in Cigarette Smoke Condensates*, April 2020.
- [2] ISO 13110:2012, *Cigarettes — Determination of menthol in smoke condensates — Gas-chromatographic method*

ANNEX A
(Informative)

Figures A1 and A2 illustrate typical chromatograms for a calibration standard and mainstream cigarette smoke sample.

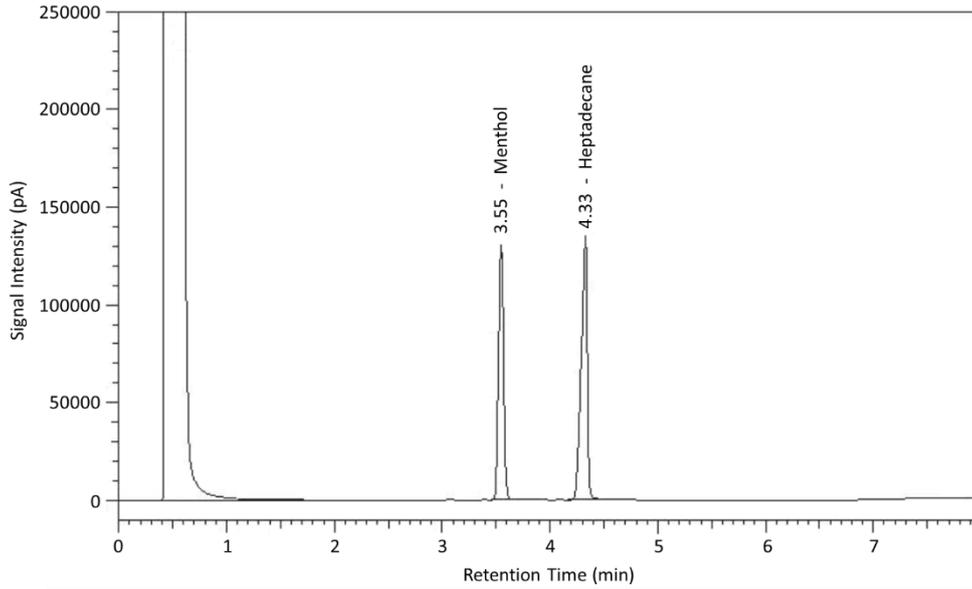


Figure A1 – Example chromatogram for a calibration standard (0.190 mg/ml)

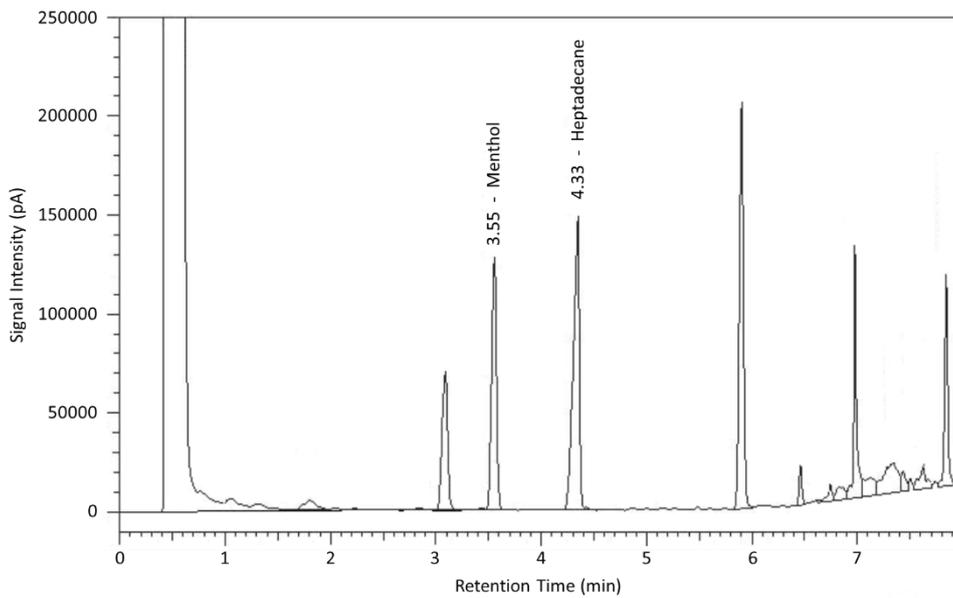


Figure A2 – Example chromatogram for menthol in mainstream cigarette smoke