Routine Analytical Chemistry Sub-Group

Technical Report

Collaborative Study of Menthol in Cigarettes and Cut Filler

April 2019

Author and Study Project Leader:
Hiromoto Yamazaki, Japan Tobacco Inc., Japan

Co-Author and Statistical Analysis:
Michael Morton, Ph.D. Altria Client Services LLC, U.S.A.

Group Coordinator:
Hiromoto Yamazaki, Japan Tobacco Inc., Japan

Table of Contents

1. Introduction

1.1 Purpose of the Collaborative Study

The CORESTA Routine Analytical Chemistry Sub-Group (RAC) conducted this collaborative study of menthol in whole cigarettes and cut filler in the first quarter of 2018. The purpose of this study was to evaluate repeatability and reproducibility (r & R) values of the methodology and, if the results were favorable, draft a new CORESTA Recommended Method (CRM) for the determination of menthol in cigarettes and cut filler.

1.2 Background information

The CORESTA RAC Sub-Group conducted a proficiency study of menthol in cigarettes and cut filler in the first quarter of 2017. This study included four mentholated cigarettes with different levels of menthol and CORESTA Monitor Test Piece No.8 (CM8). Fifteen laboratories participated in this study. The purpose of the study was to compare the results coming from each participating laboratory's in-house analytical method for menthol. In the study each laboratory measured menthol in the tobacco filler, the whole cigarette, and the non-tobacco material of the cigarette. Most of the laboratories used GC/FID, though with a wide range of columns. Extraction times varied widely, though around one hour was the most common. The most common extraction solvent was methanol. One laboratory used ethanol as the extraction solvent and the laboratory performed similarly to the laboratories which used methanol. Three laboratories used isopropanol as the extraction solvent, and it appeared to be a less effective solvent in tobacco filler and in the whole cigarette. It is worth noting however, that the results for the three laboratories using isopropanol were comparable.

The results from the initial proficiency study were sued to develop a method for a follow-up collaborative study where all labs were required to use the prescribed method. Thirteen laboratories participated in this study. Preliminary results from 11 laboratories were presented to the RAC Sub-Group in April 2018 in Guildford, U.K.. Those results showed lab-to-lab differences that were larger than expected. Upon follow-up with the individual laboratories, it was found that two of the laboratories had calculation errors and another laboratory was found to be using isopropanol as the solvent instead of methanol. The two laboratories with calculation errors submitted corrected results, the data from the laboratory using the incorrect solvent were dropped, and three additional laboratories submitted data resulting in a total of 13 laboratory data sets for evaluation.

2. Organisation

2.1 Study participants

A list of the 15 participating laboratories is given in Appendix A. One of the 15 laboratories did not report results and, as noted above, one laboratory was excluded for not following the experimental protocol (isopropanol rather than methanol was used as the extraction solvent), leaving 13 laboratories for statistical evaluation. A code was assigned to each laboratory and the order of the assigned codes does not match the order of the list in Appendix A.

2.2 Protocol

Three commercial cigarettes and one prototype cigarette were tested. The parameters to be measured and subjected to statistical analysis were menthol in cigarettes and cut filler. Three independent sample preparations or replicates were required for each parameter. Each test

result was based on the extraction and analysis of two cigarettes or the tobacco filler from two cigarettes. The method of analysis is given in Appendix B.

The four products considered in this study are listed in following table.

Table 1: Studied products' characteristics

Product Code	Supplied by	Characteristic
Sample A	Japan Tobacco Inc.	Target menthol level: approx. 15 mg/cig
Sample B	Japan Tobacco Inc.	Target menthol level: approx. 9 mg/cig
Sample C	Japan Tobacco Inc.	Target menthol level: approx. 3 mg/cig
Sample D	Japan Tobacco Inc.	Target menthol level: approx. 2 mg/cig

Participating laboratories were requested to follow the protocol "RAC-116-1-CTR Collaborative Study of Determination Method of Menthol in Cigarettes and Cut Filler 171215" (provided in Appendix B) to analyse the Sample A to D and to report the 3 parameters listed in Table 2.

Note: The cut filler weights for each extraction shall be reported on a "as-is basis (wet-base; WB)" with no moisture correction.

Table 2: Parameters to be reported

Parameter	Number of Replicates	Unit	Code
Menthol in whole mentholated cigarettes	3	mg/cigarette	Menthol in CIG
Menthol in cut filler of mentholated cigarettes	3	mg/g	Menthol in CF
Cut filler weight for each extraction	3	mg	CF weight

2.3 Statistical analysis

The statistical analysis was conducted in basic conformance with ISO 5725-2:1994 and ISO/TR 22971:2005. A summary of the results from outlier detection and the calculated results for repeatability (r) and reproducibility (R) are given below in sections 3.1 and 3.2, respectively. Raw data are given in Appendix C and plots of the data are given in Appendix D.

3. Results

3.1 Outlier determination

An adaptation of Levene's Test[1] was used for eliminating laboratories with overly large repeatability standard deviations and Grubbs' Test was used to eliminate laboratories with outlying mean values. There were no outlying laboratories.

[1] The approach is discussed in detail by Michael Morton in "Within-Laboratory Variance Outlier Detection: An Alternative to Cochran's Test" in *Beiträge zur Tabakforschung International*, Vol 27 No. 7, pp135-144.

3.2 Repeatability and reproducibility estimation

The repeatability and reproducibility limits are shown in Table 3.

Table 3: r & R Limits

Product Code	Analyte	Nr. of Lab	Mean	Repeatability		Reproducibility	
				Limit (r)	r (% of mean)	Limit (R)	R (% of mean)
Sample A	Menthol in CIG (mg/cig)	13	14,71	0,688	4,7 %	1,76	12,0 %
	Menthol in CF (mg/g)	13	11,42	0,771	6,7 %	1,68	14,7 %
Sample B	Menthol in CIG (mg/cig)	13	8,97	0,443	4,9 %	1,31	14,6 %
	Menthol in CF (mg/g)	13	7,10	0,443	6,2 %	0,98	13,8 %
Sample C	Menthol in CIG (mg/cig)	13	3,47	0,235	6,8 %	0,61	17,5 %
	Menthol in CF (mg/g)	13	3,15	0,227	7,2 %	0,44	14,0 %
Sample D	Menthol in CIG (mg/cig)	13	2,03	0,161	7,9 %	0,40	19,8 %
	Menthol in CF (mg/g)	13	1,80	0,145	8,1 %	0,43	23,7 %

4. Recommendations

The RAC recommends that the results from this collaborative study be used to finalize the draft recommended method specified for use in this study and that the method be proposed as a new CRM.

5. Bibliography

[1] CORESTA Technical Report (2018), Proficiency Study of Menthol in Cigarettes and Cut Filler

[2] ISO 5725-2:1994, Accuracy (trueness and precision) of measurement method and results – Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method.

[3] ISO/TR 22971:2005, Accuracy (trueness and precision) of measurement methods and results - Practical guidance for the use of ISO 5725-2:1994 in designing, implementing and statistically analysing interlaboratory repeatability and reproducibility results.

[4] Within-Laboratory Variance Outlier Detection: An Alternative to Cochran's Test, Michael Morton. Beiträge zur Tabakforschung International, Vol. 27, No. 7, pp 135-144.

Appendix A – List of Participating Laboratories

Participating Laboratory	Country
Altria Client Services LLC	United States
ASL Analytic Service Laboratory GmbH	Germany
British American Tobacco Germany GmbH	Germany
C.I.T.MONTEPAZ S.A.	Uruguay
Enthalpy Analytical, LLC	United States
Imperial Tobacco Production Ukraine Test Lab Kiev	Ukraine
Japan Tobacco Inc.	Japan
JT International Germany GmbH	Germany
JTI (Ökolab)	Austria
KT&G	Korea
Labstat International ULC	Canada
Landewyck Tobacco s.a.	Luxembourg
R.J. Reynolds Tobacco Company	United States
Reemtsma / Imperial Tobacco Central Lab Hamburg	Germany
Tabacalera del Este S.A.	Paraguay

Appendix B – Experimental Protocol

Routine Analytical Chemistry Sub-Group
Collaborative Study
Determination of Menthol in Cigarettes and Cut Filler
Protocol

Hiromoto Yamazaki

Japan Tobacco Inc.

Japan

15 December 2017

Table of Contents - Protocol

Foreword

This CORESTA protocol describes the elements that the Standards Task Force has identified as important for describing elements of an experimental protocol for a collaborative study or a proficiency study as defined below (Horowitz 862):

- Collaborative Study
  "A collaborative study is an interlaboratory study in which each laboratory uses the defined method of analysis to analyse identical portions of homogeneous materials to assess the performance characteristics obtained for that method of analysis."

  CORESTA recognizes that collaborative studies require considerable effort and should be conducted only on methods that have received adequate prior testing.

- Proficiency Study
  "A proficiency study is an interlaboratory study consisting of one or more assays-conducted by a group of laboratories on one or more identical materials, by whatever method is in use in each laboratory, for the purpose of comparing the results of each laboratory with those of other laboratories, with the objective of evaluating or improving performance."

  Proficiency studies often serve as pre-work for selecting a method for a full collaborative study.

1. Introduction

The overall objective of this project is to develop a CORESTA Recommended Method (CRM) for the determination of menthol in mentholated cigarettes and in cut filler of mentholated cigarettes (most likely by gas chromatography). Other deliverables will be technical reports and a sample handling guide for mentholated cigarettes and cut filler of mentholated cigarettes.

The CORESTA Routine Analytical Chemistry Sub-Group (RAC) conducted a proficiency study of menthol in cigarettes and in cut filler in the first quarter of 2017. The study included four mentholated cigarettes with different levels of menthol and CORESTA Monitor Test Piece No.8 (CM8). This protocol was created based on the conclusions of the proficiency study and additional studies by a single lab.

2. Objective

The objective of this study is to estimate repeatability (r) and reproducibility (R) for the provided method.

Note: The use of any method other than that specified will not support the study objectives and the data cannot be included.

3. Study Coordinator

Hiromoto Yamazaki
Senior Scientist
Japan Tobacco Inc.
Product Quality Research Center
1-17-7 Yokokawa Sumida-ku, Tokyo
Japan
E-mail: [redacted] Tel: [redacted]

4. Study Time Table

Date	Activity
December, 2017 - January, 2018	Protocol & sample distribution by JT
January - February, 2018	Analysis at participant laboratories
End of February, 2018	Data submission by this date
March - April, 2018	Statistical analysis by Dr. Morton
Spring, 2018	Discuss results at RAC meeting

5. Samples

The test samples listed in Table 1 will be analysed.

Table 1: Cigarette test samples

Test Sample	Target Menthol Level	Sample Distribution
Sample A – commercial product	Approx. 15mg/cig	1 carton
Sample B – commercial product	Approx. 9 mg/cig	1 carton
Sample C – commercial product	Approx. 3 mg/cig	1 carton
Sample D – prototype product	Approx. 2 mg/cig	1 carton

Test samples A, B, C and D were produced in November, 2016. They were kept in a conditioned room (temperature: 22 °C, humidity: 60 %) until shipping.

6. Analysis

6.1 Parameters to be measured

- Menthol in whole mentholated cigarettes
- Menthol in cut filler of mentholated cigarettes
- Cut filler weight for each extraction

6.2 Method

Participating laboratories must use the supplied GC method for the determination of menthol in mentholated cigarettes and cut filler of mentholated cigarettes. Strict adherence to the protocol is required.

6.3 Replicates

Analyse three individual extractions of each test sample. Each extraction per test sample is from a separate pack.

6.4 Sample handling

All test samples (packed samples) should be kept in a conditioned room (temperature: 22 °C, humidity: 60 %) until analysis. Test cigarettes and cut filler should be extracted as soon as possible after opening a pack.

6.5 Test units

- Menthol in whole mentholated cigarettes: mg/cigarette
- Menthol in cut filler of mentholated cigarettes: mg/g
- Cut filler weight for each extraction: mg

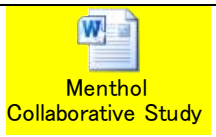
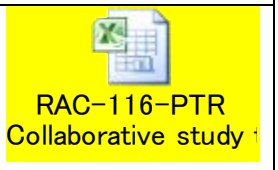
All results should be reported to two decimal places. The cut filler weights shall be reported on a "as-is basis (wet-base; WB)" with no moisture correction.

7. Data Submission

Participating laboratories should use the embedded Excel document for data reporting. Chromatograms (the extractions of cut filler and whole cigarette to Sample D and the lowest calibration standard) obtained with the supplied method should be reported in the data reporting sheet as well.

The completed data sheet should be sent to the following recipients by **end of February, 2018**:

Hiromoto Yamazaki: [redacted]
Linda Crumpler: [redacted]

Menthol Analysis Method	
Data Reporting Worksheet	

8. Explanation of Statistics to be Applied for a Collaborative Study

The data will be statistically evaluated in basic conformance with the recommendations of ISO 5725-2: 1994 and ISO/TR 22971:2005.

Select the appropriate description from the sections below and delete text that

For proficiency studies Z-scores are used to measure individual laboratory performance.

8.1 Z-Score Calculation

$$z = \frac{x - x_a}{\sigma_p}$$

where

x is the individual laboratory average

x_a is the assigned value

- It is ideal if the materials have an "assigned value" prior to the study. For example, certified values or a consensus value from a previous study. It is possible, however, to develop a consensus value from the proficiency study itself.

σ_p is the "standard deviation for proficiency assessment" and can be assigned/established several ways.

- It can be a "target" established, for example, by a desired or "fitness for purpose" relative error.
- Through use of something akin to the "Horowitz equation" which is a model fitted originally by William Horowitz relating concentration and method variability.
- Based on variability seen amongst labs in the current or previous studies.

The Horowitz Equation is used to estimate analytical method standard deviations as a function of the analyte concentration alone. It is defined separately in three different intervals.

$$\sigma_p = 0.01\sqrt{c},\ \ 13.8\ \% \leq c$$

$$\sigma_p = 0.02c^{0.8495},\ \ 120\frac{ng}{g} \leq c < 13.8\ \%$$

$$\sigma_p = 0.22c,\ \ c < 120\frac{ng}{g}$$

For collaborative studies repeatability and reproducibility estimation is performed

8.2 Repeatability and Reproducibility Estimation

The statistical evaluation of data for this collaborative study will follow the methods provided by ISO 5725-2. For outlier testing, the Grubbs and Cochran methods will be used.

ISO 5725-2 Tests - Consistency

Cochran's test - Within-laboratory variability: Suitable for detecting whether the highest value in a set of laboratory standard deviations is an outlier or not.

Grubbs' test - Between-laboratory variability: Suitable for detecting whether the highest (or lowest) laboratories averages are outliers or not.

The protocol "Harmonized statistical procedure" (defined by IUPAC 2006 IUPAC, Pure and Applied Chemistry 78, 145–196) is also applied. It consists of sequential applications of the Cochran's and Grubbs' tests until no further outliers are detected or until a drop of more than 22.2 % original number of laboratories would occur.

Appendix 1 – Protocol
International System of Units (SI) and their Symbols

SI base units

Base Quantity	Name	Symbol
Length	meter	m
Mass	kilogram	kg
Time	second	s
Electric current	ampere	A
Temperature	kelvin	K
Amount of substance	mole	mol
Luminous intensity	candela	cd

SI derived units

Derived Quantity	Name	Symbol	Equivalent SI Units
Frequency	hertz	Hz	s^{-1}
Force	newton	N	$m \cdot kg \cdot s^{-2}$
Pressure	pascal	Pa	N/m^2
Energy	joule	J	N·m
Power	watt	W	J/s
Electric charge	coulomb	C	s·A
Electric potential	volt	V	W/A
Electric resistance	ohm	Ω	V/A
Celsius temperature	Degrees Celsius	°C	K*

*Unit degree Celsius is equal in magnitude to unit kelvin

SI prefixes

Factor	Name	Symbol	Numerical Value
10^{12}	tera	T	1 000 000 000 000
10^{9}	giga	G	1 000 000 000
10^{6}	mega	M	1 000 000
10^{3}	kilo	k	1 000
10^{2}	hecto	h	100
10^{1}	deka	da	10
10^{-1}	deci	d	0.1
10^{-2}	centi	c	0.01
10^{-3}	milli	m	0.001
10^{-6}	micro	µ	0.000 001
10^{-9}	nano	n	0.000 000 001
10^{-12}	pico	p	0.000 000 000 001

Appendix 2 – Protocol – Method

DETERMINATION OF MENTHOL IN MENTHOLATED CIGARETTES AND CUT FILLER OF MENTHOLATED CIGARETTES – PROTOCOL FOR COLLABORATIVE STUDY

1. INTRODUCTION

This document has been prepared for use by CORESTA RAC members participating in the 2017-2018 collaborative study to quantitatively measure the amount of menthol in mentholated cigarettes and in cut filler of mentholated cigarettes.

2. SCOPE

This method is applicable to the gas-chromatographic determination of the menthol in mentholated cigarettes and in cut filler of mentholated cigarettes. It is not recommended for the analysis of the menthol content in mentholated cigarettes with filters containing any adsorbents (e.g. charcoal). The applicability of this method to filters containing them is unproven, possibly due to adsorption of the menthol or internal standard.

3. PRINCIPLE

Menthol is extracted from mentholated cigarettes or cut filler of mentholated cigarettes with methanol containing an internal standard. After shaking for 2 hours, the sample is analyzed by GC with flame ionization detector (FID).

4. REAGENTS

Use only reagents of recognized analytical grade.

4.1 **Carrier gas**: helium, nitrogen or hydrogen of high purity

4.2 **Auxiliary gases**: air and hydrogen of high purity for the flame ionization detector

4.3 **Methanol** [67-56-1] of purity at least 99 %

4.4 **Internal standard**: anethole [104-46-1], n-heptadecane [629-78-7], 1,3-butanediol [107-88-0] or decanol [112-30-1] of purity at least 99 %

4.5 **Extraction solvent**: methanol (4.3) containing an appropriate mass concentration of internal standard (4.4)

This is normally in the range of 0,2 mg/mL to 1,0 mg/mL.

4.6 **Reference substance**: menthol [2216-51-5] of purity at least 99 %

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.

4.7 **Calibration standards**

Dissolve the menthol (4.6) in the extraction solvent (4.5) 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 concentration of the calibration standards prepared covers the anticipated concentration range of the samples.

Table 1. Menthol calibration standards – Nominal Concentrations
(Actual concentrations will vary depending upon the amount weighed and the purity of menthol.)

Standard ID	**Nominal menthol Concentration** (mg/mL)
1	0,02
2	0,05
3	0,10
4	0,20
5	0,50
6	1,00

5. APPARATUS

5.1 **Extraction vessel**, several different styles of may be utilized, including but not limited to: 100 mL Pyrex bottles with crimp seals and septa, 100 – 250 mL Erlenmeyer flasks with stoppers, and 25 mm x 200 mm culture tubes with teflon lined caps.

5.2 **Shaker**, preferably horizontal, but wrist-action acceptable.

5.3 **Gas-chromatograph**, equipped with a split/splitless multimode injector, a flame ionization detector, and a computerized controlled data acquisition and processing system.

Note: A recorder and integrator are acceptable if proven to be operational for intended purpose.

5.4 **Column**, DB-WAX (preferably 15 m x 320 µm x 0,25 µm) or any other type of column showing equivalent separation capability.

6. PROCEDURE

6.1 Test portion

6.1.1 Whole mentholated cigarettes

Take out two cigarettes from one test sample. Slit them longitudinally and separate them into cut filler and non-tobacco materials (NTMs; cigarette paper, filter and tipping paper etc.). Put all materials (cut filler and NTMs) into the extraction vessel (5.1) and pipette 40,0 mL of extraction solvent (4.5) into the extraction vessel. Place the extraction vessel in the shaker (5.2) and shake for 2 hours.

6.1.2 Cut filler of mentholated cigarettes

Take out two cigarettes from one test sample. Slit them longitudinally and separate them into cut filler and non-tobacco materials (NTMs; cigarette paper, filter and tipping paper etc.). Weigh, to the nearest 0,01 mg, the cut filler of two cigarettes and record it. Put the cut filler into the extraction vessel (5.1) and pipette 40,0 mL of extraction solvent (4.5) into the extraction vessel. Place the extraction vessel in the shaker (5.2) and shake for 2 hour.

If the extract is not analysed on the same day, store in a refrigerator. After conditioning to room temperature, the extract should be analysed.

6.2 Setting up the apparatus

Set up the apparatus and operate the gas chromatograph (5.3) 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.

Suitable operating conditions may be as follows for the column described in 5.4:

- carrier gas: helium at a flow rate of about 2,5 mL/min;
- make up gas: nitrogen at a flow rate of about 25 mL/min;
- injection temperature: 220 °C;
- split ratio (approximately): 8 / 1;
- injection volume: 1 µL;
- oven temperature 1: 80 °C;
- time period 1 (initial): 0 min;
- temperature program 1: 10 °C/min;
- oven temperature 2: 170 °C;
- time period 2 (intermediate): 0 min;
- temperature program 2: 70 °C/min;
- oven temperature 3: 250 °C;
- time period 3 (final): 3,9 min;
- detector temperature: 250 °C.

Using the above conditions, the analysis time is about 14 min per sample.

6.3 Calibration of the gas chromatograph

Inject an aliquot (1 µL) of each of the calibration solutions (4.7) into the gas chromatograph. Record the peak areas of the menthol and internal standard (4.4).

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 should occur to explain the situation, and the calibration should be repeated if necessary.

Perform this full calibration procedure daily before use. In addition, inject an aliquot of an intermediate concentration standard, which should be prepared from a separate stock, after about 20 sample determinations. If the calculated concentration for this solution differs by more than 3 % from the original value, repeat the full calibration procedure.

6.4 Determination

Inject aliquots (1 µL) of the test portion (6.1) 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.

7. EXPRESSION OF RESULTS

From the concentration of menthol in the test portion, determine the amount of menthol in mentholated cigarettes or cut filler of mentholated cigarettes.

7.1 Whole mentholated cigarettes

The menthol concentration (in mg/mL) 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, typically mg/cig.

$$Menthol\ conc.\ (mg/cig.) = \frac{Menthol\ conc.\ (mg/mL) \times Solvent\ Volume (40mL)}{Number\ of\ Test\ Cigarette\ (2\ cig.)}$$

7.2 Cut filler of mentholated cigarettes

The menthol concentration (in mg/mL) 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 weight basis, typically mg/g.

$$Menthol\ conc.\ (mg/g) = \frac{Menthol\ conc.\ (mg/mL) \times Solvent\ Volume (40mL)}{Cut\ Filler\ Weight\ (mg) \times 1000}$$

CHROMATOGRAMS

Figures 1-2 illustrate typical chromatograms that can be expected to be obtained with this method.

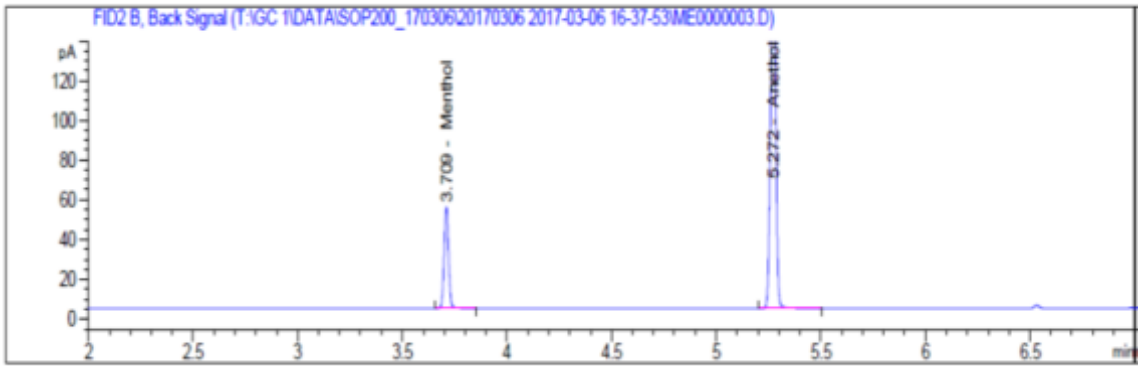


Figure 1. Chromatogram for menthol in a calibration standard

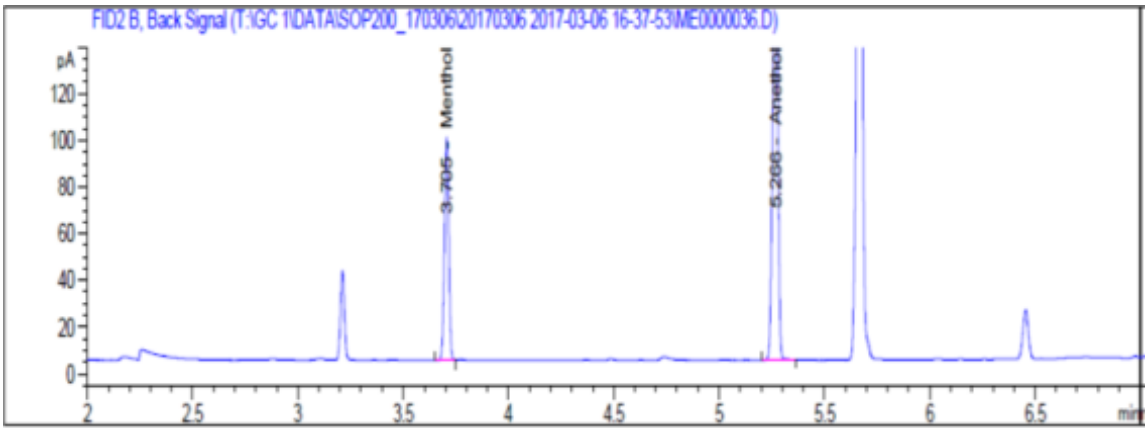


Figure 2. Chromatogram for menthol in whole mentholated cigarettes

Appendix C – Raw Data Set

Laboratory	Sample	Replicate	Cut filler Weight (mg)	Menthol in cut filler (mg/g)	Menthol in whole cigarette (mg/cig)
1	A	1	1122	11.64	15.88
1	A	2	1095	11.67	16.12
1	A	3	1127	12.30	15.51
2	A	1	1121	11.46	14.50
2	A	2	1117	11.61	14.64
2	A	3	1142	11.74	14.24
4	A	1	1132	10.73	14.04
4	A	2	1097	11.40	14.05
4	A	3	1115	10.98	13.87
5	A	1	1075	4.43	10.89
5	A	2	1076	4.38	10.54
5	A	3	1105	4.34	10.87
6	A	1	1091	11.47	14.65
6	A	2	1088	11.95	14.40
6	A	3	1125	11.68	14.82
7	A	1	1110	11.62	14.12
7	A	2	1120	11.66	14.52
7	A	3	1112	11.22	14.22
8	A	1	1062	10.83	13.92
8	A	2	1089	10.86	14.19
8	A	3	1083	10.74	13.42
9	A	1	1077	11.51	14.16
9	A	2	1094	11.29	14.37
9	A	3	1049	11.38	14.40
10	A	1	1113	10.89	15.16
10	A	2	1072	10.74	15.33
10	A	3	1067	10.98	14.91
11	A	1	1116	11.77	14.77
11	A	2	1110	11.55	14.82
11	A	3	1098	11.86	14.70
12	A	1	1068	9.99	14.71
12	A	2	1084	9.82	14.40
12	A	3	1109	11.06	14.57
13	A	1	1129	11.49	14.33

Laboratory	Sample	Replicate	Cut filler Weight (mg)	Menthol in cut filler (mg/g)	Menthol in whole cigarette (mg/cig)
13	A	2	1126	11.64	14.77
13	A	3	1134	11.78	15.04
14	A	1	5001	11.34	15.01
14	A	2	5004	11.61	15.04
14	A	3	5002	11.75	15.13
15	A	1	1081	12.43	15.53
15	A	2	1109	12.38	15.41
15	A	3	1083	12.66	16.19
1	B	1	1151	7.44	9.38
1	B	2	1083	7.31	9.24
1	B	3	1128	7.28	9.91
2	B	1	1126	7.14	8.94
2	B	2	1141	6.99	9.06
2	B	3	1115	7.06	9.20
4	B	1	1133	6.94	8.40
4	B	2	1117	6.67	8.45
4	B	3	1167	6.61	8.48
5	B	1	1149	2.51	6.17
5	B	2	1081	2.50	6.21
5	B	3	1163	2.53	6.09
6	B	1	1113	7.33	8.73
6	B	2	1150	7.23	9.17
6	B	3	1101	7.25	8.76
7	B	1	1102	7.04	9.02
7	B	2	1097	7.06	8.73
7	B	3	1078	7.22	8.87
8	B	1	1133	6.65	8.44
8	B	2	1148	6.56	8.45
8	B	3	1138	6.58	8.38
9	B	1	1124	7.25	8.70
9	B	2	1122	7.35	8.60
9	B	3	1135	6.94	8.67
10	B	1	1068	6.88	8.55
10	B	2	1093	6.59	8.53
10	B	3	1077	6.67	8.68
11	B	1	1125	7.13	8.84

Laboratory	Sample	Replicate	Cut filler Weight (mg)	Menthol in cut filler (mg/g)	Menthol in whole cigarette (mg/cig)
11	B	2	1096	7.38	8.98
11	B	3	1093	7.28	9.18
12	B	1	1119	6.67	8.86
12	B	2	1111	7.08	9.05
12	B	3	1116	6.76	8.94
13	B	1	1131	7.42	9.17
13	B	2	1114	7.43	8.96
13	B	3	1110	7.50	8.80
14	B	1	5001	7.02	9.10
14	B	2	5001	6.97	8.95
14	B	3	5001	7.01	9.19
15	B	1	1116	8.11	10.16
15	B	2	1102	7.38	10.10
15	B	3	1116	7.74	10.05
1	C	1	1161	3.27	3.76
1	C	2	1188	3.36	3.58
1	C	3	1162	3.35	3.76
2	C	1	1216	3.38	3.69
2	C	2	1144	3.30	3.40
2	C	3	1177	3.32	3.60
4	C	1	1138	3.13	3.24
4	C	2	1166	3.00	3.25
4	C	3	1157	3.16	3.27
5	C	1	1149	1.16	2.26
5	C	2	1023	1.22	2.18
5	C	3	1156	1.21	2.25
6	C	1	1138	3.33	3.50
6	C	2	1135	3.24	3.49
6	C	3	1160	3.28	3.54
7	C	1	1124	3.23	3.40
7	C	2	1115	3.15	3.26
7	C	3	1109	3.12	3.42
8	C	1	1142	3.05	3.11
8	C	2	1136	2.94	3.04
8	C	3	1141	2.99	3.15
9	C	1	1156	3.10	3.26

Laboratory	Sample	Replicate	Cut filler Weight (mg)	Menthol in cut filler (mg/g)	Menthol in whole cigarette (mg/cig)
9	C	2	1140	3.13	3.39
9	C	3	1129	2.99	3.37
10	C	1	1081	3.27	3.20
10	C	2	1147	2.90	3.21
10	C	3	1119	2.87	3.30
11	C	1	1173	3.32	3.66
11	C	2	1157	3.29	3.58
11	C	3	1187	3.27	3.66
12	C	1	1111	3.10	3.52
12	C	2	1116	3.15	3.55
12	C	3	1132	3.07	3.37
13	C	1	1166	3.27	3.50
13	C	2	1158	3.29	3.58
13	C	3	1166	3.18	3.46
14	C	1	5000	2.84	3.57
14	C	2	5004	2.89	3.55
14	C	3	5002	2.90	3.58
15	C	1	1157	3.12	3.89
15	C	2	1179	3.16	3.67
15	C	3	1172	3.04	3.97
1	D	1	1117	1.93	2.25
1	D	2	1136	2.01	2.32
1	D	3	1156	1.86	2.19
2	D	1	1133	1.76	2.00
2	D	2	1167	1.81	2.02
2	D	3	1168	1.84	1.97
4	D	1	1172	1.70	1.90
4	D	2	1157	1.74	1.88
4	D	3	1150	1.71	1.75
5	D	1	1174	0.58	1.27
5	D	2	1140	0.62	1.34
5	D	3	1146	0.58	1.29
6	D	1	1135	1.81	2.04
6	D	2	1157	1.84	2.08
6	D	3	1146	1.78	2.02
7	D	1	1116	1.91	1.94

Laboratory	Sample	Replicate	Cut filler Weight (mg)	Menthol in cut filler (mg/g)	Menthol in whole cigarette (mg/cig)
7	D	2	1136	1.94	1.93
7	D	3	1145	2.00	2.04
8	D	1	1187	1.72	1.92
8	D	2	1105	1.70	1.93
8	D	3	1133	1.68	1.90
9	D	1	1168	1.99	1.99
9	D	2	1121	2.02	2.10
9	D	3	1108	2.01	2.14
10	D	1	1150	1.51	1.86
10	D	2	1118	1.55	1.76
10	D	3	1125	1.46	1.72
11	D	1	1152	1.86	2.01
11	D	2	1207	1.86	2.11
11	D	3	1150	2.01	2.07
12	D	1	1127	1.65	1.91
12	D	2	1106	1.64	2.03
12	D	3	1159	1.72	2.07
13	D	1	1194	2.03	2.11
13	D	2	1177	1.90	2.07
13	D	3	1139	1.95	2.19
14	D	1	5001	1.79	2.11
14	D	2	5001	1.65	2.15
14	D	3	5002	1.63	2.09
15	D	1	1119	1.76	2.22
15	D	2	1152	1.74	2.18
15	D	3	1163	1.72	2.25

Appendix D – Data Graphs