Characterization of Mainstream Tobacco Smoke

Analytical Test Report

Labstat®
Since 1976

Prepared for *Philip Morris Products SA*

Project Code: NS487-H

Date: July 26, 2019

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2 USE OF LABSTAT'S[1] ANALYTICAL REPORTS

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3 ADMINISTRATIVE INFORMATION

3.1 QUOTATION IDENTIFICATION

Quotation Number: 1964 (2)

Date of Quotation: July 17, 2019

Recipient's Name: (b) (6)

3.2 CLIENT IDENTIFICATION

Philip Morris Products SA

Quai Jeanrenaud 5

2000 Neuchatel

Switzerland

4 SAMPLE HANDLING

4.1 CHAIN OF CUSTODY

The samples to be tested for project NS487-H were received on June 18, 2019 via DHL.

4.2 SAMPLE CHARACTERIZATION AND CODING

4.2.1 SAMPLE CHARACTERISTICS

The shipment received on June 18, 2019 consisted of five cartons for each of two products. There was no physical damage to cartons. Individual test items were normal in appearance.

4.2.2 SAMPLE IDENTIFICATION

The following sample codes have been used to identify the products associated with the results in each of the tables that are part of this report.

Labstat Product ID	SMP Description	Sample Description	Batch #	Qty/ Type Received	Type of Product	Type of Analysis	Devices
1002	P1M2	N/A	N/A	N/A	N/A	N/A	IQOS 2.4 Plus White
1747695	SMP123290	P1M2 tested with IQOS 3 - new device	N/A	5/ cartons	Sticks	Emissions	IQOS 3.0
1747696	SMP123289	P1M2 tested with client supplied 2.4 Ref device - US configuration	N/A	5/ cartons	Sticks	Emissions	IQOS 2.4 US configuration

4.2.3 PHYSICAL MEASUREMENTS

Physical measurements were made on all tobacco products and no unusual findings were noted.

5 PROJECT-SPECIFIC INSTRUCTIONS

No special instructions, with respect to the selection of the test sample and/or compositing, were received. A shipment received June 26, 2019 contained IQOS 2.4 US configuration devices and IQOS 3.0 devices to be used for this project.

- iQOS devices activated 30s prior to first puff (device warm-up).

5.1 PLATFORM I TESTING

Test sticks were used in conjunction with a "tobacco heating system" by inserting the consumable (test stick) into a cigarette holder (CH). The CH was used in conjunction with a lighter bar to smoke the test sticks. Therefore, the following requirements of the ISO standard (see section 6.2.3) were either not applicable or could not be fulfilled for technical reasons:

- Air velocity control since the consumable is inserted into the CH.
- Butt length requirement since the heating of the consumable has no effect on the consumable length which remains unchanged after smoking.
- There was no ignition of the cigarette by an external lighter.

The use of the CH, in conjunction with the lighter bar for the test samples, required the reference cigarettes to be smoked on an independent run. All reference cigarettes were smoked to the ISO standard requirements for air velocity, butt length and ignition.

5.2 PLATFORM I END POINTS

Test sticks are puffed to a fixed puff number: 12 puffs per stick under Health Canada Intense (HCI) smoking regime[2].

[2] Health Canada Intense: (55.0±0.5)ml puff volume, (30±0.5)s puff frequency, (2.00±0.02)s puff duration, bell-shaped puff profile, no vent blocking; Canadian Tobacco Reporting Regulations: 21 June, 2000, Part 3(6)(b)(iii) - *Canada Gazette Part II, Vol. 134, No. 15*

6 EXPERIMENTAL DESIGN AND METHODS

The following is a summary of the instructions that have been received from the client in regard to the smoking and analysis of the tobacco products in this project.

6.1 SAMPLE GENERATION

All tobacco products were conditioned and smoked under the smoking regimes outlined in the following sub-sections.

6.1.1 CIGARETTE BUTT MARKING

Prior to testing, all reference cigarettes were marked with the standard butt length as specified in ISO 4387 (2000) *"Cigarettes -- Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine"*.

6.1.2 CIGARETTE CONDITIONING AND SMOKING ENVIRONMENTS

Cigarettes were conditioned and smoked under the environmental conditions specified in ISO 3402 (1999) *"Tobacco and tobacco products – Atmosphere for conditioning and testing"*. With respect to conditioning, this document states "*The conditioning atmosphere shall be as follows: temperature 22 ± 1 ℃; relative humidity 60 ± 3%*". Smoking requires an environment in which the temperature is 22 ± 2°C and the relative humidity 60 ± 5%.

6.1.3 MACHINE SMOKING CONDITIONS

Smoking of test and reference cigarettes were carried out on either a rotary smoking machine or a linear smoking machine. The smoking parameters and smoking machine specifications which were used are set out in the International Organization for Standardization standard ISO 3308:12, ***Routine analytical cigarette-smoking machine - Definitions and standard conditions*** with modifications as noted in the table below. A single port linear smoking machine was employed in the analysis of NO/NO_x contents of mainstream smoke.

The following table is a summary of the smoking parameters that were employed in this project.

Variable	"Canadian Modified"
Puff Volume (ml)	55
Interval (sec)	30
Duration (sec)	2
Vents	"open"

Mainstream yields (MS) were obtained under "Canadian Modified" conditions (as defined above). Yields obtained using "Canadian Modified" smoking parameters are referred to as "non-standard" (n). Data files ending in (n) denote results obtained under the conditions as noted in the previous sentence and in the above table.

6.2 ANALYTICAL METHODS[3]

6.2.1 SMOKE ANALYSIS

Test methods for the analysis of mainstream tobacco smoke are referenced in the table(s) below and were practiced as written unless otherwise indicated (see "Method Modifications").

METHODS FOR THE COLLECTION OF EMISSION DATA ON MAINSTREAM SMOKE[4]

Item	Emission	Labstat Method	Method Description
1.	Ammonia	TMS-00101 Appendix E	*Determination of Ammonia in Mainstream Tobacco Smoke*
2.	*(a)* Formaldehyde *(b)* Acetaldehyde *(c)* Acetone *(d)* Acrolein *(e)* Propionaldehyde *(f)* Crotonaldehyde *(g)* MEK (methyl ethyl ketone) *(h)* Butyraldehyde	TMS-00104 Appendix G	*Determination of Selected Carbonyls in Mainstream Tobacco Smoke*
3.	Hydrogen cyanide	TMS-00107 Appendix C	*Determination of Hydrogen Cyanide in Mainstream Tobacco Smoke*
4.	Mercury	TMS-00108 Appendix F	*Determination of Mercury in Mainstream Tobacco Smoke*
5.	*(a)* Lead *(b)* Cadmium *(c)* Chromium *(d)* Nickel *(e)* Arsenic *(f)* Selenium	TMS-00109 Appendix H	*Determination of Ni, Pb, Cd, Cr, As and Se in Mainstream Tobacco Smoke*
6.	*(a)* NO *(b)* NOx	TMS-00110 Appendix H	*Determination of Oxides of Nitrogen in Mainstream Tobacco Smoke*

[3] The most current version available at the time of testing was used for all test methods listed.

[4] Canadian Tobacco Reporting Regulations: 2000-07-19 Canada Gazette Part II, Vol. 134, No. 15 Part 3: Emissions from Designated Tobacco Products. Test method numbers refer to Health Canada methodologies which have been posted by Health Canada on the internet at site http://www.hc-sc.gc.ca/hl-vs/tobac-tabac/legislation/reg/indust/index_e.html

Item	Emission	Labstat Method	Method Description
7.	*(a)* Pyridine *(b)* Quinoline *(c)* Styrene *(d)* Nitrobenzene *(e)* Acetamide *(f)* Acrylamide	TMS-00112 Appendix G	*Determination of Pyridine, Quinoline and Styrene in Mainstream Tobacco Smoke*
8.	*(a)* NFDPM *(b)* Nicotine *(c)* Carbon Monoxide *(d)* Glycerol *(e)* Water	TMS-00115a Appendix E	*Determination of "NFDPM", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke*
9.	*(a)* Pyrene *(b)* Benzo(a)anthracene *(c)* Benzo(a)pyrene *(d)* Dibenz(a,h)anthracene	TMS-00120 Appendix H	*Determination of Selected Polynuclear Aromatic Hydrocarbons in Mainstream Tobacco Smoke*
10.	*(a)* 1,3-Butadiene *(b)* Isoprene *(c)* Acrylonitrile *(d)* Benzene *(e)* Toluene *(f)* Ethylene Oxide *(g)* Vinyl Chloride *(h)* Propylene Oxide	TMS-00124 Appendix G	*Determination of Vinyl Chloride, 1,3-Butadiene, Isoprene, Acrylonitrile, Benzene, Toluene, Styrene, and Acetamide in Mainstream Tobacco Smoke (expanded list)*
11.	*(a)* 1- aminonaphthalene *(b)* 2- aminonaphthalene *(c)* 3- aminobiphenyl *(d)* 4- aminobiphenyl *(e)* o- toluidine	TMS-00128 Appendix G	*Determination of Aromatic Amines in Mainstream Tobacco Smoke (expanded list)*
12.	*(a)* *N*-nitrosonornicotine *(b)* 4-(*N*-nitrosomethylamino)-1-(3-pyridyl)-1-butanone *(c)* *N*-nitrosoanatabine *(d)* *N*-nitrosoanabasine	TMS-00135 Appendix I	*Determination of Tobacco Specific Nitrosamines in Mainstream Tobacco Smoke by High-Performance Liquid Chromatography-Tandem Mass Spectrometry*
13.	*(a)* Hydroquinone *(b)* Resorcinol *(c)* Catechol *(d)* Phenol *(e)* *m*- Cresol *(f)* *p*-Cresol *(g)* *o*-Cresol	TMS-00139 Appendix F	*Determination of Phenolic Compounds In Mainstream Tobacco Smoke by a Modified High Performance Liquid Chromatography Method*

6.2.2 METHOD REFERENCES AND SYNOPSES

6.2.2.1 AMMONIA (T-101/TMS-00101)

6.2.2.1.1 METHOD SYNOPSIS

Five conditioned sticks (cigarettes) were smoked on a standard rotary smoking machine. The mainstream smoke was passed through a conditioned, pre-weighed 92mm glass fiber filter disk (pad) and then into two impingers placed in series after the pad, each containing 15mL of 0.1N sulfuric acid. The pad, after being weighed to determine the mainstream total particulate matter (TPM), was extracted with the contents of the two impingers. The mixture was then filtered through a 0.45 µm syringe filter into an auto-sampler vial and analyzed by cation exchange chromatography using an external standard calibration.

For this project, “heat-not-burn” products were “smoked” on a standard 20 port linear smoking machine using two impingers per port as smoked on a rotary machine.

6.2.2.2 CARBONYLS (T-104/TMS-00104)

6.2.2.2.1 METHOD SYNOPSIS

Two conditioned sticks (cigarettes) were smoked on a standard 20 port linear smoking machine that was fitted with Drechsel-type bottles or traps with fritted impingers. The unfiltered mainstream tobacco smoke was scrubbed of volatile carbonyls by passing each puff through an impinger into a trap containing 80mL of an acidified solution of 2,4-dinitrophenylhydrazine in acetonitrile. An aliquot of the reacted DNPH-smoke extract was then syringe-filtered and diluted with 1% trizma base in aqueous acetonitrile. The samples were subjected to reverse phase high performance liquid chromatography (HPLC) and quantified via ultra violet detection.

For the analysis of “heat-not-burn” products, no additional changes to the sample generation, collection or sample preparation of the base method for cigarettes were required.

6.2.2.3 HYDROGEN CYANIDE (T-107/TMS-00107)

6.2.2.3.1 METHOD SYNOPSIS

Five conditioned sticks (cigarettes) were smoked per port on a standard 20 port linear smoking machine, onto a conditioned, pre-weighed 44mm glass fiber filter disc (pad), with a trap containing 0.1N NaOH located directly behind the pad. The pad was extracted with 40 mL of 0.1N NaOH on a wrist action shaker for 30 minutes. Both the pad extract and impinger trapping solutions were analyzed by an automated continuous flow colorimetric analyzer where each sample undergoes on-line dilution. Hydrogen cyanide in the sample was converted to cyanogen chloride by an aqueous solution of chloramine-T. The cyanogen chloride then reacted with pyridine to give glutaconic aldehyde, which, upon reaction with a pyrazolone reagent, formed a coloured complex. A single channel monitored the complex, which was quantified by comparison to an external standard calibration.

For the analysis of “heat-not-burn” products, no additional changes to the sample generation, collection or sample preparation of the base method for cigarettes were required.

6.2.2.4 MERCURY (T-108/TMS-00108)

6.2.2.4.1 METHOD SYNOPSIS

Twenty conditioned sticks (cigarettes) were smoked on a standard rotary smoking machine. The mainstream smoke was passed directly through two impingers placed in series, each containing 30mL of an acidified potassium permanganate solution. The impinger solutions were then subjected to microwave digestion. Excess potassium permanganate was reduced with hydroxylamine hydrochloride and made to a final volume of 100mL. The digestate was then analyzed via cold vapour atomic absorption spectroscopy at 253.7 nm using a continuous flow vapour generator to reduce the divalent mercury to its atomic state with stannous chloride.

For this project, “heat-not-burn” products were “smoked” on a standard 20 port linear smoking machine instead of a rotary smoking machine. The mainstream smoke was passed through two midget impingers, each containing 10mL of the acidified potassium permanganate solution. The final volume of samples was 50mL instead of 100mL.

6.2.2.5 TRACE METALS (T-109/TMS-00109)

6.2.2.5.1 METHOD SYNOPSIS

Twenty conditioned sticks (cigarettes) were smoked on a standard rotary smoking machine equipped with an electrostatic precipitation generator to electrostatically precipitate the particulate matter onto a glass electrostatic precipitate (EP) tube. The total particulate matter (TPM) was extracted into 25 mL methanol. The methanol extract was then evaporated using gentle heating while under a constant stream of filtered ultra high purity (UHP) nitrogen. The sample was then subjected to microwave digestion using a mixture of hydrochloric acid, nitric acid and hydrogen peroxide. The gaseous phase metals were trapped by placing an impinger of a 10% v/v nitric acid solution between the EP tube and the puff drawing mechanism. The impinger solution was added to the same digestion vessel as the EP tube product and subjected to microwave digestion. The digestates were then analyzed by Inductively Coupled Plasma - Mass Spectrometer (ICP-MS) or Inductively Coupled Argon Plasma - Atomic Emission Spectrometer (ICP-AES).

For the analysis of “heat-not-burn” products, due to the nature of the sample, collection of the total particulate matter (TPM) required the use of two EP tubes. Each EP tube was extracted using half of the volume of methanol (12.5mL) that would have been used if only one EP tube was employed. The EP tubes and impinger samples were then collected in a single digestion vessel and subjected to the digestion process. For sample generation, sample preparation, and analysis high purity chemical reagents (Ultrapure Fisher Optima chemical grade) were used.

6.2.2.6 OXIDES OF NITROGEN (T-110/TMS-00110)

6.2.2.6.1 METHOD SYNOPSIS

One conditioned stick (cigarette) was smoked on a standard single port smoking machine, onto a conditioned, pre-weighed 44mm glass fiber filter disc (pad). The mainstream smoke was exhausted puff by puff into an evacuated smoke mixing chamber (SMC) located directly behind the pad. The gas was mixed and, at precise and consistent intervals, an aliquot of each puff was routed by vacuum through a filter to a dual channel real time chemiluminescence nitrogen oxides analyzer where the gas stream was split immediately into two channels. In channel A, the sample stream was reacted with ozone and the resultant chemiluminescent emission was directly proportional to the NO concentration in the sample. In channel B, the sample stream was chemically reduced first by a catalytic converter and then mixed with ozone in the reaction cell where the resultant chemiluminescent emission was due to NO_x or NO + NO_2. Selective photomultiplier detection monitored the reaction cell gas stream and the NO and NO_x found in the vapour phase of mainstream tobacco smoke were quantified by external standard calibration.

For the analysis of “heat-not-burn” products, the mainstream smoke from a single stick was exhausted puff by puff into an evacuated 500mL smoke mixing chamber. Calibration was done with three levels of concentration including 9, 30, and 50 ppm (NO/NOx).

6.2.2.7 SEMI VOLATILES (T-112/TMS-00112)

6.2.2.7.1 METHOD SYNOPSIS

Twenty conditioned sticks (cigarettes) were smoked on a standard rotary smoking machine. The mainstream smoke was passed through a conditioned, pre-weighed 92mm glass fiber filter disc (pad) and then into two cryogenic traps placed in series after the pad, each containing 20mL of methanol. The pad was then cut into quarters, spiked with the internal standard solution (containing d5-pyridine, d8-styrene and d7-quinoline) and extracted with the 40 mL of methanol from the two cryogenic traps. An aliquot of the extract was syringe filtered into an auto-sampler vial and analyzed using gas chromatography – mass spectrometry (GC/MS) with ion trap under full scan mode.

For this project, “heat-not-burn” products were “smoked” on a standard 20 port linear smoking machine using a 44mm pad instead of a rotary smoking machine. The pad was replaced after five sticks were smoked per port for HCI smoking conditions (two pads per replicate for HCI). d5-acetamide and d3-acrylamide were added as internal standards and analysis was done by gas chromatography – mass spectrometry (GC/MS) using a single quadrupole mass detector operating under selective ion monitoring (SIM) mode.

6.2.2.8 NFDPM, NICOTINE AND CARBON MONOXIDE (T-115/TMS-00115A)

6.2.2.8.1 METHOD SYNOPSIS

Five conditioned[5] sticks (cigarettes) were smoked using a standard 20 port linear smoking machine equipped with a CO analyzer, onto a conditioned, pre-weighed 44mm glass fiber filter disc (pad). The gas phase was collected into a Vapour Phase (VP) collection bag and then introduced into a Non-Dispersive Infra-Red (NDIR) analyzer and the % CO determined. The pad was re-weighed with the difference calculated as the Total Particulate Matter (TPM). The pad was extracted with isopropanol (IPA) containing the internal standards (trans-anethole for nicotine and methanol for water), and the extract analyzed for nicotine and water by gas chromatography (flame ionization detector (FID) for nicotine and thermal conductivity detector (TCD) for water). The nicotine-free dry particulate matter (NFDPM) value was determined by subtracting the water and nicotine from the TPM result.

For the analysis of "heat-not-burn" products, no additional changes to the sample generation, collection or sample preparation of the base method for cigarettes were required.

6.2.2.9 POLYNUCLEAR AROMATIC HYDROCARBONS (LABSTAT METHOD TMS-00120)

6.2.2.9.1 METHOD SYNOPSIS

Ten conditioned sticks (cigarettes) were smoked using a standard rotary smoking machine, onto a conditioned, pre-weighed 92mm glass fiber filter disc (pad). The pad was spiked with internal standards (d12-benzo[a]pyrene, d14-dibenz[a,h]anthracene, d10-anthracene and d10-pyrene) and extracted with 50 mL of methanol. The methanol extracts were filtered through a filter paper. A portion of filtered extract was cleaned up by Solid Phase Extraction (SPE) using a RapidTrace SPE Workstation and analyzed by gas chromatography-mass spectrometry for quantification. The mass detector was operated under Selected Ion Monitoring (SIM) mode. The ions of interest (i.e. molecular ions and in some cases specific fragment ions) were mass-selected and used for quantification.

For this project, "heat-not-burn" products were "smoked" on a standard 20 port linear smoking machine using a 44mm pad instead of a rotary smoking machine. After adding the internal standards solution to the pad, the pad was extracted with 20mL of methanol. The sample was then analyzed by GC/MS as described above.

[5] Conditioning parameters are defined by the client

6.2.2.10 VOLATILE ORGANICS (LABSTAT METHOD TMS-00124)

6.2.2.10.1 METHOD SYNOPSIS

Ten conditioned sticks (cigarettes) were smoked on a standard rotary smoking machine. The mainstream smoke was passed through a conditioned, pre-weighed 92mm glass fiber filter disc (pad) and then into two cryogenic traps placed in series after the pad, each containing 10mL of methanol. An aliquot of the solution from the two traps was spiked with the internal standards (d_6-benzene, d_6-vinyl acetate and d_4-furan) and analyzed by gas chromatography – mass spectrometry (GC/MS) with ion trap under full scan mode.

A second aliquot of the solution from the two traps was derivatized with 48% hydrobromic acid, then spiked with internal standards (d_4-2-bromorthanol) and analyzed for ethylene oxide via gas chromatography – mass spectrometry (GC/MS) operating under selective ion monitoring (SIM) mode.

For this project, “heat-not-burn” products were “smoked” on a standard 20 port linear smoking machine using a 44mm pad instead of a rotary smoking machine. The pad was replaced after five sticks were smoked per port for HCI smoking conditions (two pads per replicate for HCI). Lower calibrations standards were prepared and analysis of the trapping solution was done by gas chromatography – mass spectrometry (GC/MS) using a single quadrupole mass detector operating under selective ion monitoring (SIM) mode.

For the ethylene oxide analysis, a second aliquot of the trapping solution was dried on anhydrous sodium sulphate, derivatized with 48% hydrobromic acid, neutralized with sodium carbonate and spiked with the internal standards (d_4-2-bromorthanol). After centrifugation, the supernatant was analyzed with a capillary column by GC/MS operating under selective ion monitoring (SIM) mode.

6.2.2.11 AROMATIC AMINES (LABSTAT METHOD TMS-00128)

6.2.2.11.1 METHOD SYNOPSIS

Ten conditioned sticks (cigarettes) were smoked on a standard rotary smoking machine. The mainstream total particulate matter (TPM) was collected on a conditioned, pre-weighed 92mm glass fiber filter disc (pad). The pad was quartered and extracted with 100mL of 5% hydrochloric acid solution. The extraction flask was shaken for 30 minutes on a wrist-action shaker and the contents filtered into a 500mL separatory funnel. The internal standards (d_7-1-aminonaphthalene, d_7-2-aminonaphthalene, d_9-4-aminonabiphenyl, d_9-toluidine, d_8-benzidine, d_7-o-anisidine, d_5-o-aniline and d_6-2,6-dimethylaniline) were spiked into the solution. The filtrate was washed with dichloromethane, made basic with sodium hydroxide solution and extracted with hexane. The hexane extracts were dried with sodium sulphate, derivatized with pentafluoropropionic acid anhydride (PFPA) and trimethylamine, concentrated by rotary evaporation, passed through a florisil column, and quantified using gas chromatography – mass spectrometry (GC/MS) with ion trap under full scan mode.

For this project, “heat-not-burn” products were “smoked” on a standard 20 port linear smoking machine and a 44mm pad was used instead of a rotary smoking machine. The 44mm pad was replaced after five sticks were smoked per port for HCI smoking conditions (two pads per replicate for HCI). Quantification was achieved using negative chemical ionization (NCI) gas chromatography – mass spectrometry (GC/MS) operating in selective ion monitoring (SIM) mode.

6.2.2.12 TOBACCO SPECIFIC NITROSAMINES (LABSTAT METHOD TMS-00135)

6.2.2.12.1 METHOD SYNOPSIS

Five conditioned sticks (cigarettes) were smoked per port on a standard 20 port linear smoking machine. The mainstream total particulate matter (TPM) was collected on a conditioned, pre-weighed 44mm glass fiber filter disc (pad). The pad was spiked with a deuterium labeled internal standard solution (containing NNN-d4, NAT-d4, NAB-d4, and NNK-d4) and then extracted with a 100mM ammonium acetate solution. The extract was then filtered and subject to LC-MS/MS analysis with positive electrospray ionization (ESI). Two mass transition pairs for each analyte can be used to assist analyte confirmation and quantification. The most intense pairs are used for quantification while the less intense transition pairs are used as qualifiers for further compound confirmation.

For the analysis of “heat-not-burn” products, no additional changes to the sample generation, collection or sample preparation of the base method for cigarettes were required.

6.2.2.13 PHENOLIC COMPOUNDS (LABSTAT METHOD TMS-00139)

6.2.2.13.1 METHOD SYNOPSIS

Five conditioned sticks (cigarettes) were smoked per port on a standard 20 port linear smoking machine. The mainstream total particulate matter (TPM) was collected on a conditioned, pre-weighed 44mm glass fiber filter disc (pad). The pad was then extracted with 20 mL of 1% acetic acid (HOAc). An aliquot of the TPM extract was syringe filtered, diluted and subjected to reversed-phase gradient liquid chromatography. Phenols were monitored using selective fluorescence detection and quantified by comparison to an external standard calibration.

For the analysis of “heat-not-burn” products, ten sticks were smoked per port on a standard 20 port linear smoking machine. An impinger containing 20mL of 1% acetic acid (HOAc) was added in the smoke train after the pad. The pad was replaced after five sticks were smoked per port for HCI smoking conditions (two pads per replicate for HCI). The collection pad for ISO smoking conditions was extracted with the impinger solution (20mL). An additional 20mL of 1% acetic acid was added to the impinger solution to extract the two pads per replicate for HCI smoking conditions (total of 40mL per replicate).

6.3 METHOD MODIFICATIONS

6.3.1.1 METHOD MODIFICATIONS FOR THE DETERMINATION OF GLYCEROL AND MENTHOL (TMS-00115A APPENDIX E)

A second independent aliquot of the IPA extract was used to determine propylene glycol and menthol by gas chromatography (GC) with flame ionization detector (FID) using a megabore DB-Wax fused silica column that has a polyethylene glycol (PEG) stationary phase, as per Health Canada method T-304. Quantification was achieved using the internal standard calibration (internal standard: trans-anethole).

7 ACCEPTANCE OF DATA

7.1 EVALUATION OF RESULTS FROM CONTROL MATERIALS

Data obtained using control materials are deemed acceptable if the data are in keeping with Labstat's database for the control material and the specific method of analysis[6]. This is not a simple problem since there is no "yes" or "no" answer but rather one which is phrased in terms of probabilities. In the approach taken by Labstat, the measure of random variation in the procedure is taken to be the sample standard deviation (S.D. or "s").

To evaluate control data accuracy, a "*Z score*" statistic is determined as follows:

$$Z\text{ - }score = \frac{\text{Sample Average - Historical Average}}{\text{Historical Standard Deviation}}$$

To evaluate control data precision, a "*Chi-square*" statistic is determined as follows:

$$Chi\text{ - }square = (\text{Sample Size - 1}) \times \frac{(\text{Sample Standard Deviation})^2}{(\text{Historical Standard Deviation})^2}$$

P values are generated and the cut-off point (α) chosen in such a way as to minimize the chance of rejecting data which are legitimate members of the set (i.e. type 1 error). Thus, in most cases where the number of observed control samples is greater than or equal to 5, z-score p-values are generated from the Standard Normal distribution.

The standard deviation rather than the standard error for the mean has been chosen when determining the 'Z score'. This allows for both project-to-project variation, which is inherent in the historical data, and the 'normal' run-to-run variability, which is present in the data set. The cut-off point for P values is a matter of judgment and has been set at 0.005 assuming the probability of falsely rejecting a data point is 0.5% (i.e. α=0.01) or less for a two tailed test.

[6] A minimum of 30 results is normally required for the purpose of this comparison.

In instances where expected values are not known, a decision to accept the data is made based on observed levels of precision in comparison with that determined for similar analyses. Also, there are circumstances where the expected value may be “Below Detection Limits”. In this case the decision to accept or reject the data is made upon the ability of the method to recover the analyte of interest either in the form of a laboratory fortified blank (LFB) or laboratory fortified matrix (LFM). Acceptable recoveries are close to 100%, but vary depending on the analyte.

7.2 IDENTIFICATION OF OUTLIERS[7]

7.2.1 OUTLIER DEFINITION

An outlying observation, or "outlier," is one that appears to deviate markedly from other members of the sample in which it occurs. In this case, there are two alternatives:

1. An outlying observation may be merely an extreme manifestation of the random variability inherent in the data. If this is true, the value is retained and processed in the same manner as the other observations in the sample.
2. The observation may be the result of gross deviation from prescribed experimental procedure or an error in calculating or recording the numerical value. In such cases, an investigation must be carried out. When the experimenter is clearly aware that a gross deviation from prescribed experimental procedure has taken place, the resultant observation is discarded (assignable cause) without recourse to a statistical test. A statistical test may always be used to support a judgment that a physical reason does actually exist for an outlier, or the statistical criterion may be used routinely as a basis to initiate action to find a physical cause.

7.2.2 STATISTICAL CRITERIA

There are a number of criteria for testing outliers. In all of these, the doubtful observation is included in the calculation of the numerical value of a sample criterion (or statistic) that is then compared with a critical value. The critical value is that which would be exceeded by chance with some specified (small) probability on the assumption that all the observations did indeed constitute a random sample from a single parent population, distribution or universe. The specified small probability is called the "significance level" and can be thought of as the risk of erroneously rejecting a good observation. A level of significance of 0.02 has been chosen in conjunction with the statistical test and tables described in ASTM E178-08[8].

Significant departures from the expected results (i.e. “outliers”) are viewed seriously, requiring an investigation for an assignable cause. This is a documented procedure that, at a minimum, consists of the following steps:

1. Review of all associated calculations to ensure that arithmetic errors have not been made
2. Review of linearity range for any standards

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[7] The term "outlier" has been defined in International Standard ISO 3534-1:2006 entitled "*Statistics - Vocabulary and symbols - Part 1: General statistical terms and terms used in probability*"

[8] ASTM Designation: E178-08. Standard Practice for Dealing with Outlying Observations

3. Assessment of instrument status
4. Review of reagents, columns, standards etc. to ensure that contamination or decomposition has not occurred
5. Review of sample preparation and handling procedures as they relate to the result in question

If the outlier is present in the analyte data and an assignable cause is found, the test result is removed from the data set but recorded in the quality control section of the laboratory's record of test results for that project. The analysis must then be repeated. If the outlier is present in the ancillary[9] data and an assignable cause is found, the test result is not removed, but rather the outlying observation is replaced by the designation "AC" (Assignable Cause). If this investigation fails to determine an assignable cause, the test result is assumed to be a legitimate member of the data set and is included in all subsequent calculations.

8 RESULTS

8.1 QUALITY CONTROL

The control results for the variables of interest were acceptable as defined in section 7.1. Consequently, it is reasonable to assume that the values determined for the test samples are reflective of the characteristics of the products as received and tested as described in the Analytical Methods section.

8.2 ANALYTICAL DATA

Individual results and the corresponding sample statistics (consisting of means, standard deviations, and coefficients of variation or 95% confidence limits) may be found in the data files, labeled *NS487-H_ms_dataCF*.xls and *NS487-H_ms_controlsCF*.xls, which accompany this report.

8.2.1 SAMPLE STATISTIC CALCULATIONS

In cases where a sample result is below the limit of detection (LOD), the average of the value zero (0) and the LOD is used in the sample statistic calculation. In cases where a sample result is between the LOD and the limit of quantification (LOQ), the average of the LOD and the LOQ is used in the sample statistic calculation.

[9] Data, which are related, but not normally required as part of the reporting process (e.g. puff counts, TPM, cigarette weights etc.). Outliers in the analyte data that have an assignable cause are always repeated.

9 ACCREDITATION

Labstat International ULC has been accredited by the Standards Council of Canada to International Standard ISO/IEC 17025:2005 "*General requirements for the competence of testing and calibration laboratories*" with a scope[10] that includes all of the mandated tobacco-related Health Canada methods (see Tobacco Reporting Regulations dated 26 June 2000, Canada Gazette Part II, Vol. 134, No. 15 Schedules 1, 2 and 3 pages 1780 – 1785). The testing included in this report is within the scope of this accreditation, unless otherwise noted.

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10 AUTHORIZATION

This report has been reviewed by me and is certified, to the best of my knowledge, to be a true and accurate description of the procedures, protocols and test methods used to arrive at the data and/or findings that accompany this report.

Dated: July 26, 2019

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Mingliang Bao, Ph.D.

Principal Scientist

Labstat International ULC

11 APPENDIX A: "RAW" DATA AND SUMMARY STATISTICS

See Accompanying Data Files or Enclosed CD

[10] Labstat's accreditation scope is available on Standards Council of Canada website at: http://palcan.scc.ca/specs/pdf/180_e.pdf