Cooperation Centre for Scientific Research Relative to Tobacco

Smoke Analytes Sub-Group

CORESTA Recommended Method No. 74

DETERMINATION OF SELECTED CARBONYLS IN MAINSTREAM CIGARETTE SMOKE BY HPLC

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Title:

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0. INTRODUCTION

At the outset of this work, discussions in the CORESTA Special Analytes Sub-Group determined that most laboratories used a method involving derivatisation of carbonyls with 2,4-dinitrophenylhydrazine (DNPH) because they considered it the most suitable and so this was chosen as the basis of the Recommended Method. The method comprised smoke collection in impinger traps, derivatisation of carbonyls with DNPH followed by their determination using reversed phase High Performance Liquid Chromatography with Ultra Violet or Diode Array Detection (HPLC-UV or HPLC-DAD).

Initial Joint Experiments and on-going discussions addressed some methodological aspects that needed to be evaluated before drafting a Recommended Method. The CORESTA Recommended Method (CRM) was produced through a Collaborative Study undertaken in 2010 involving 15 laboratories from 11 countries using the ISO 3308 smoking regime (Intorp et al., 2012). Further data are provided for the same selected carbonyl compounds from 10 samples with different tar yields from a Collaborative Study in 2012 using both the ISO 3308 and Health Canada T-115 (HCI) smoking regimes, which involved 19 laboratories from 11 countries. This method includes recommendations about some of the critical steps that should be controlled to provide data as robust and consistent as the repeatability and reproducibility data provided in the CRM. Statistical evaluations were carried out according to ISO 5725 recommendations.

At that time, when the collaborative study was conducted, the study protocol stipulated the use of Health Canada Official Method (T-115) for Intense conditions as there was not an ISO standard that defined Intense smoking conditions. ISO 20778, Routine analytical cigarette-smoking machine — Definitions and standard conditions was published in 2018 and is equivalent to Health Canada Intense conditions and is referenced hereafter.

1. FIELD OF APPLICATION

This method is applicable to the determination of selected carbonyls (formaldehyde, acetaldehyde, acetone, acrolein, propionaldehyde, crotonaldehyde, 2-butanone and n-butyraldehyde) as their 2,4-dinitrophenylhydrazones in mainstream smoke from cigarettes with ISO NFDPM yields between 1 mg/cigarette and 15 mg/cigarette using reversed phase HPLC-UV/DAD.

The described method is specified using ISO 3308 and ISO 20778 (Intense) smoking parameters. The use of these machine smoking parameters reflects their inclusion in the reporting requirements of various national regulations rather than an endorsement of their appropriateness by CORESTA.

2. NORMATIVE REFERENCES

2.1 *ISO 3308:2012*
Routine analytical cigarette-smoking machine – Definitions and standard conditions

2.2 *ISO 3402:1999*
Tobacco and tobacco products – Atmosphere for conditioning and testing

2.3 *ISO 3696:1987*
Water for analytical laboratory use – specification and test methods

2.4 *ISO 4387:2008/Amd 1:2008*
Cigarettes – Determination of Total and Nicotine-free Dry Particulate Matter Using a Routine Analytical Smoking Machine

2.5 *ISO 5725-1:1994*
Accuracy (trueness and precision) of measurement methods and results – Part 1: General principles and definitions

2.6 *ISO 5725-2:1994*
Accuracy (trueness and precision) of measurement methods and results – Part 2: Basic method for the determination of repeatability (r) and reproducibility (R) of a standard measurement method

2.7 *ISO 8243:2013*
Cigarettes – Sampling

2.8 *Health Canada Official Method T-115: December 1999*
Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke

2.9 *ISO 20778:2018*
Cigarettes - Routine analytical cigarette-smoking machine — Definitions and standard conditions with an intense smoking regime

3. METHOD SUMMARY

3.1 Cigarettes are smoked on a smoking machine as specified in either ISO 3308 or in ISO 20778 that has been fitted with impingers, but without the specified filter pad holder containing the glass fiber filter (e.g. Cambridge Filter Pad, CFP, or similar product).

3.2 The carbonyls in mainstream tobacco smoke are trapped by passing each puff through an impinger device containing an acidified solution of 2,4-dinitrophenylhydrazine (DNPH) in acetonitrile.

3.3 An aliquot of the smoke extract is then syringe-filtered and diluted with 1 % Trizma™ base in aqueous acetonitrile.

3.4 The samples are subjected to analysis using reverse phase HPLC-UV or HPLC-DAD.

4. APPARATUS AND EQUIPMENT

Laboratory apparatus and equipment, in particular the following items:

- Equipment for conditioning of tobacco products
- Equipment for butt length marking
- Equipment for smoking of tobacco products complying with ISO 3308:2009
- Impingers for trapping mainstream smoke

- Erlenmeyer flasks (150 ml) with ground glass stoppers (or equivalent for combining impinger solutions)
- Polyvinylchloride tubing appropriate for connection of the trapping system, dimensions 1/4" ID × 3/8" OD

Laboratory equipment for the preparation of samples, standards, and reagents - examples:

- Analytical balance, capable of measuring to four decimal places
- Amber glass volumetric flasks 10 ml, 25 ml, 200 ml, 1 l, and 2 l
- Glass micropipettes – 50 µl, 100 µl, 150 µl, 300 µl, 400 µl, 500 µl, 800 µl, 1000 µl and 2000 µl
- Volumetric pipettes – 1 ml, 2 ml, 5 ml, 6 ml, 7 ml, 8 ml, and 20 ml
- Glass graduated measuring cylinders 25 ml, 50 ml and 100 ml
- Dispenser capable of delivering 35 ml
- Hot Plate/Stirrer
- Syringe filter - 0,45 µm PVDF or equivalent
- Disposable syringes - 5 ml
- Disposable glass Pasteur pipettes
- Rubber bulbs
- Autosampler vials, caps and Teflon faced septa

HPLC system consisting of:

- Tertiary gradient pump
- Auto-sampler with appropriate sampling loop
- UV and/or DAD detector
- Data Collection System
- LC column: 250 mm × 4 mm, Reversed Phase (RP) C18 (5 µm), or equivalent
- Disposable Guard Column: 4 mm × 4 mm, RP C18 (5 µm), or equivalent
- Vacuum filter
- Amber glass bottles 1 l and 4 l
- Desiccator

5. REAGENTS AND SUPPLIES

- Acetonitrile (MeCN) – HPLC Grade
- Isopropanol (IPA) – HPLC Grade
- Ethyl Acetate – HPLC Grade
- Tetrahydrofuran (THF) – HPLC Grade
- Ethanol – HPLC Grade
- Phosphoric acid (85 %)
- Deionised water (resistivity >18,0 MΩ.cm @ 25 °C)
- Formaldehyde-DNPH (min. 99 %)
- Acetaldehyde-DNPH (min. 99 %)
- Acetone-DNPH (min. 99 %)
- Acrolein-DNPH (min. 99 %)
- Propionaldehyde-DNPH (min. 98 %)
- Crotonaldehyde-DNPH (min. 99 %)
- 2-Butanone-DNPH (min. 98 %); methyl ethyl ketone-DNPH derivative
- n-Butyraldehyde-DNPH (min. 99 %)

- Tris-(hydroxymethyl)-aminomethane; (Trizma™ Base, ACS Reagent Grade).
  Note – for reader's convenience, Trizma™ Base will be used throughout the document.
- 2,4-dinitrophenylhydrazine (DNPH)
- Helium (UHP) – if necessary for sparging of HPLC system mobile phase or equivalent degassing system

Note: All reagents shall be at least analytical grade.

Warning notice: The solvents and chemicals used in this method are classified as toxic, highly toxic, harmful, carcinogenic, mutagenic, sensitising, teratogenic, irritant, corrosive, easily flammable and dangerous for the environment. The instructions specified in the individual material safety data sheets concerning safe handling, storage and waste disposal as well as protective equipment must be followed. For example, DNPH solutions must not be sonicated as this can be explosive.

6. PREPARATION OF GLASSWARE

Glassware shall be cleaned and dried in such a manner to ensure that contamination from glassware does not occur.

Note: All possible sources of contamination shall be removed from the work area (e.g. acetone solvent wash bottles).

7. PREPARATION OF SOLUTIONS

DNPH solution *(using Phosphoric Acid*)

- Add approximately 150 ml deionised water to a 200 ml volumetric flask, then carefully add 28 ml of 85 % phosphoric acid and mix the solution.
- Make up the solution to volume with deionised water.
- Weigh 6,8 g (0,024 mole) of DNPH (approximately 30 % water) into a 2 l amber volumetric flask and add 1 l of acetonitrile. Dissolve DNPH by alternately gently swirling and warming the flask. Make sure there are no crystals remaining.

  Warning notice: Do not sonicate as a precipitation of DNPH may occur.

  Note: If using re-crystallized DNPH, weigh 4,8 g to achieve the same molality (Appendix 1)
- After the DNPH is dissolved, add 58 ml of the diluted phosphoric acid solution whilst gently mixing. Dilute to volume with deionised water. The colour of the solution will become bright orange upon addition of the deionised water.

  Note: The addition of water will cool the solution and may initiate the precipitation of the DNPH. Add the water slowly. Gentle heating and stirring may be required to maintain the solution at room temperature and to prevent the precipitation of DNPH. If crystals appear do not sonicate.
- Store the solution in a 4 l amber bottle at room temperature in the dark to prevent or significantly reduce the chances of DNPH precipitation. This solution, if properly sealed, will remain stable for one week.

  Note: Perchloric acid was investigated in a joint experiment (Intorp et al., 2012) and no differences were observed in comparison to phosphoric acid.

Trizma™ Base Dilution Solution (80:20 v/v, MeCN:1 % aqueous Trizma™)

- Dissolve 2,00 g of Trizma™ Base in 200 ml of deionised water in a 1 l volumetric flask. Dilute to volume with acetonitrile.

Store in a 1 l amber bottle with Teflon-lined cap or equivalent at ambient temperature.

8. PREPARATION OF STANDARDS

8.1 HPLC Calibration Standards and Working Solutions

- The calibration should cover the concentration range of interest. The following calibration steps were found to be suitable for this purpose.

8.1.1 Primary (1°) Carbonyl Standards

- Weigh the hydrazones as described in Appendix 2 into individual 25 ml volumetric flasks and dissolve in acetonitrile. Record the concentrations of the free aldehyde equivalents in µg/ml.

Note: When properly stored (sealed and refrigerated at ~ 4 °C), solutions are stable for up to one year.

8.1.2 Secondary (2°) Carbonyl Standards

- Pipette predetermined volumes (Appendix 2) of each primary hydrazone standard into a 25 ml volumetric flask and dilute to the mark with acetonitrile.

Note: Store at approximately 4 °C. Stability and storage time should be checked by the laboratory.

8.2. Carbonyl Working Standards

- Take appropriate volumes (0,050 ml to 10 ml) of the 2° carbonyl standard (8.1.2) and dilute to 10 ml with acetonitrile to prepare calibration standards with approximate carbonyl concentrations (Appendix 2)**.**
- Transfer to auto-sampler vials.

Note: Store carbonyl working standards refrigerated and prepare a fresh set of working standards every 20 days.

9. SAMPLING

Sampling is performed in accordance with ISO 8243:2013.

10. TOBACCO PRODUCT PREPARATION

Conditioning of the cigarettes is done in accordance with ISO 3402:1999.

11. SAMPLE GENERATION – SMOKING OF CIGARETTES

The smoking parameters for which the method has been studied are defined in ISO 3308:2012 and in ISO 20778:2018 (Table 1).

Table 1. Smoking Parameters for ISO and Intense Smoking Regimes

Smoking regime	Puff volume (ml)	Puff frequency (seconds)	Puff duration (seconds)	Ventilation Blocking (%)
ISO 3308:2012	35	60	2	0
ISO 20778:2018	55	30	2	100

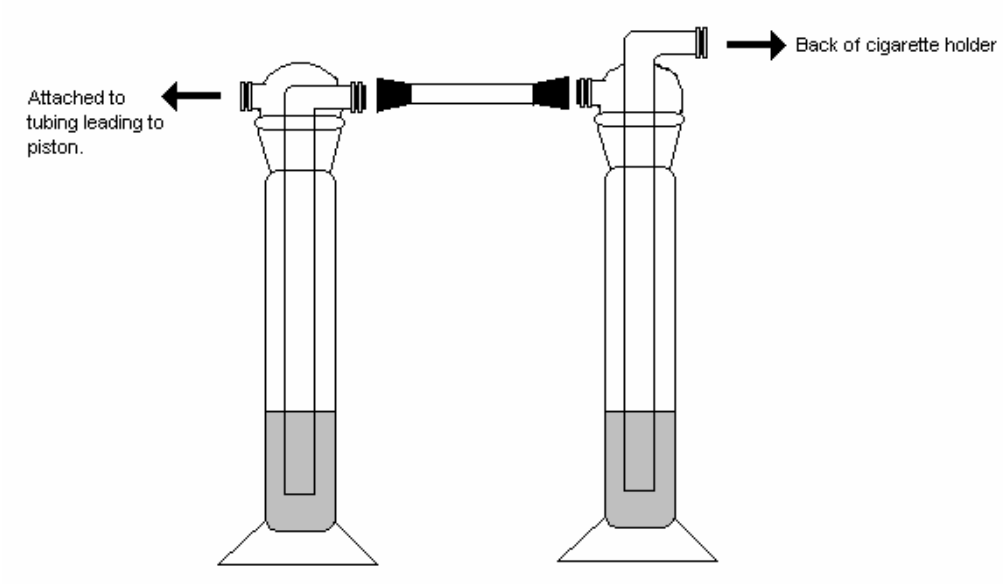
- A cigarette-smoking machine complying with the requirements of ISO 3308 or ISO 20778 is required with the following modifications as detailed below:

Note: No filter pad is required in the set up and therefore puff count information is the only means to monitor whether the smoking process is controlled.

- Assemble the carbonyl mainstream apparatus on the smoking machine without using the filter pads and filter holders.
- Check and adjust the puff volume drawn by the smoking machine at all channels at the cigarette end of the port as described in ISO 4387 with the impingers and DNPH in line.

Figure 1. Example of a suitable trapping system

This shows two impingers, each containing 35 ml of DNPH solution.



Note: It is recommended to check the trapping efficiency when validating this method under both the ISO and Intense smoking regimes. To check the trapping efficiency of the method, add an additional 3rd impinger and follow the method accordingly. Analyse each impinger individually for the compounds of interest. If no compounds are detected in the additional impinger then only the prescribed number of impingers is required to trap all the carbonyls effectively, otherwise an additional impinger is required.

When applying the Intense smoking regime with a higher smoke velocity (e.g. ISO = 17,5 ml/sec; Intense = 27,5 ml/sec), the trapping efficiency should be checked during laboratory validation in order to possibly adjust the volume of the trapping solution or introduce another liquid trap.

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

- The cigarettes are smoked according to ISO and/or Intense regimes with the following modifications:

Note: The number of cigarettes smoked may need to be adjusted depending on smoking regimes to prevent analyte breakthrough (see Note above).

Linear Smoking

- Two cigarettes are smoked per replicate for both ISO and Intense smoking regimes.

Rotary Smoking

- Five cigarettes are smoked per replicate for both ISO and Intense smoking regimes.

12. SAMPLE ANALYSIS

12.1 Preparation of Mainstream Smoke Extract Solution

- Rinse the tubing with the impinger solution by forcing the solution back up the impinger e.g. by using positive air pressure, then with negative air pressure until air is drawn back through the solution.
- Repeat this rinsing procedure at least three times for each impinger to dissolve any smoke condensate in the gas transfer lines.
- Allow the DNPH smoke extract solution to sit for five to thirty minutes (Intorp et al, 2012) before continuing with sample preparation.
- Pipette 6 ml of 1 % Trizma™ base solution into a 10 ml volumetric flask.
- Add 4 ml of syringe-filtered DNPH smoke extract to the volumetric flask.
- Mix the volumetric flask well. Transfer a portion of this solution to an auto-sampler vial.
- Cap the vials with Teflon faced septa and store at room temperature until analysed.
- Repeat above steps for each smoke extract sample.

12.2 Reversed Phase High Performance Liquid Chromatography

12.2.1 Chromatographic Conditions (Example)

- Column Temperature: 30 °C
- Auto-sampler Tray Temperature: ambient
- Injection volume: 20 µl
- UV or DAD detection at 365 nm

Note: These settings are detector-dependent and may require modification in order to achieve a linear response over the concentration range of analytes of interest.

12.2.2 Mobile Phase Reagents

- Solvent A: Prepare 2 l of 30 % Acetonitrile, 10 % THF, 1 % IPA in Type I water, filter and degas (UHP Helium sparged).
- Solvent B: Prepare 2 l of 65 % Acetonitrile, 1 % THF, 1 % IPA in Type I water, filter and degas (UHP Helium sparged).
- Solvent C: Acetonitrile (UHP Helium sparged).

Note: Adjustments to the mobile phase may be required depending upon column differences or resolution of the analytes.

- Sample Wash: Solvent A.

12.2.3 HPLC Separation Conditions

Standards and samples are analysed by HPLC operated at a flow rate of 1,5 ml/min (Table 2). The injection volume is 20 µl.

Table 2. HPLC Mobile Phase Gradient

Time (min)	Composition		
0,0	100 % A	0 % B	0 % C
8,0	70 % A	30 % B	0 % C
20,0	47 % A	53 % B	0 % C
27,0	0 % A	100 % B	0 % C
30,0	0 % A	0 % B	100 % C
32,0	0 % A	0 % B	100 % C
34,0	95 % A	5 % B	0 % C
Method End			
Equilibration 10,0	100 % A	0 % B	0 % C

- The chromatographic conditions may require adjustment for different instrument manufacturers, configurations and columns, however the elution pattern should be similar to the example chromatograms shown in Appendices 3 and 4**.**

12.3 Calculations

12.3.1 Calibration Curve

- Generate a calibration curve for each individual carbonyl by plotting standards peak areas against their respective concentrations.

12.3.2 Determination of Response Factor

- From the calibration curves calculate response factors for each individual carbonyl compound.

12.3.3 Sample Quantification

- The concentration of selected carbonyls in smoke samples is quantified by the external standard method. An example of a typical chromatogram is shown in **Appendix 4**. The identification of peaks is by comparison of retention times with standards, and the spiking of smoke samples.

- Carbonyl concentrations are reported in µg/ml by the chromatography software.
- Determination of Mainstream Carbonyl Yields is in µg/cigarette, e.g.

$$c = \frac{A}{RF} \times \frac{DF}{n}$$

where

c is the concentration of the respective carbonyl expressed in µg/cigarette

n is the number of cigarettes

A is a peak area of the respective carbonyl in the sample

RF is the response factor that is determined from calibration curve

DF is the dilution factor that is calculated as

$$DF = V_I \times \frac{V_F}{V_{Al}}$$

where

V_I is the impinger volume expressed in ml

V_F is the final volume expressed in ml

V_{Al} is the aliquot volume expressed in ml

Note: It was observed that under the conditions chosen for the derivatisation an isomerization of acetaldehyde hydrazone occurs. The resulting additional isomer can be separated by HPLC and elutes under the described separation conditions in front of the main isomer (**Appendix 4**). For the calculation of acetaldehyde yield the area of both isomers should be calculated to obtain correct results.

- If the concentration levels of carbonyls are above the highest calibration standard, the sample should be diluted to fit in the calibration curve and re-analysed.

13. REPEATABILITY AND REPRODUCIBILITY

Two Collaborative Studies were conducted assessing the methodology. In 2010, mean selected carbonyl yields, repeatability (r) and reproducibility (R) values were determined from a Collaborative Study involving 15 laboratories and five replicate analyses of two reference cigarettes (KR 3R4F and KR 1R5F), the CORESTA Monitor CM 6 and five commercial cigarettes covering a wide range of blends and designs[1]. This study included the smoking conditions described in ISO 3308. The samples are identified in Table 3 below.

[1] Intorp M., Purkis S.W., Wagstaff W., Determination of carbonyl compounds in cigarette mainstream smoke: the CORESTA 2010 Collaborative Study and Recommended Method. Beiträge zur Tabakforsch., 25(2), p. 361-374, June 2012.

Table 3. 2010 Collaborative Study Sample Identification

Sample ID	ISO Tar Yield (mg)	Product/ Blend Type
CM6	15	CORESTA Monitor, Virginia blend
1R5F	2	Kentucky Reference, American blend
3R4F	8	Kentucky Reference, American blend
Sample 1	10	Dark air-cured blend
Sample 2	6	American blend
Sample 3	8	Virginia blend
Sample 4	1	American blend/charcoal filter
Sample 5	10	American blend/charcoal filter

In 2012, mean yield, r and R data were obtained from another Collaborative Study involving 19 laboratories. This provided data on the measurement of the same selected carbonyls in five replicate analyses for 10 samples (seven commercial products and KR 3R4F, KR 1R5F, CORESTA Monitor CM6) performed under both the ISO 3308 and ISO 20778 (Intense) smoking regimes[2]. The samples are identified in Table 4 below. Carbonyls are collected using impingers only; therefore, carbonyl data does not include TPM values. For this reason, the TPM yields for Benzo[a]pyrene (B[a]P) from the same study are included in Table 3. TPM yields are included because they demonstrate the range of deliveries for the products used in this study.

Table 4. 2012 Collaborative Study Sample Identification

Sample ID	ISO TPM Yield† (mg/cig)	Intense TPM Yield† (mg/cig)	Product/ Blend Type
CM6	17.6	42.0	CORESTA Monitor 6 Test Piece
1R5F	2.1	26.7	Kentucky Reference 1R5F
3R4F	9.9	39.9	Kentucky Reference 3R4F
Sample 1	11.8	37.1	Dark air-cured
Sample 2	9.8	35.3	American blended
Sample 3	7.4	30.7	American blended
Sample 4	4.2	24.7	Virginia blended
Sample 5	2.1	17.0	Virginia blended
Sample 6	12.0	32.8	Virginia blended
Sample 7	1.5	21.3	Charcoal filtered

† *TPM (total particulate matter): the TPM values are the collaborative study means from the B[a]P analysis after removal of outliers[2]*

[2] "CORESTA Smoke Analytes Sub-Group Technical Report – 2012 Collaborative Study on B[a]P, VOCs, and Carbonyls in Mainstream Cigarette Smoke, August 2019.

The statistical evaluation of both Collaborative Studies was conducted according the ISO 5725 Parts 1 and 2. The results are summarised in Tables 5-12 for the 2009 Collaborative Study and Tables 13-20 for the 2012 Collaborative Study.

13.1. ISO 3308 Results from the 2010 Collaborative Study

Calculated statistical data of the individual selected carbonyl compound are summarised in the Tables 5 - 12.

Table 5. Formaldehyde (ISO 3308 Smoking)

Sample description	NFDPM yield (mg/cigarette)	N*	Mean	sr**	r	sR***	R
			(µg/cigarette)				
1	10	14	15,1	1,9	5,4	4,8	14
2	6	14	8,4	1,1	3,1	2,3	7
3	8	14	22,8	2,7	7,6	6,1	17
4	1	14	2,4	0,5	1,3	1, 1	3
5	10	13	27,7	2,9	8,2	7,9	22
CM 6	15	14	42,7	4,3	12,1	10,2	29
1R5F	2	15	3,0	0,4	1,2	1,3	4
3R4F	8	15	18,8	1,7	4,9	4,6	13
% Reported	98,3						
% Removed	1,7						

*Abbreviations: *N - number of data sets taken for statistical analysis after removing of outliers;*
***sr - repeatability standard deviation; ***sR - reproducibility standard deviation*

Table 6. Acetaldehyde (ISO 3308 Smoking)

Sample description	NFDPM yield (mg/cigarette)	N*	Mean	sr**	r	sR***	R
			(µg/cigarette)				
1	10	13	485	27	77	56	158
2	6	13	335	21	60	47	134
3	8	14	445	28	79	46	131
4	1	14	86,4	7,8	22,1	16,6	46,8
5	10	11	502	20	56	49	139
CM 6	15	13	651	27	77	55	156
1R5F	2	15	141	13	37	30	86
3R4F	8	15	538	28	79	63	177
% Reported	93,9						
% Removed	6,1						

*Abbreviations: *N - number of data sets taken for statistical analysis after removing of outliers;*
***sr - repeatability standard deviation; ***sR - reproducibility standard deviation*

Table 7. Acetone (ISO 3308 Smoking)

Sample description	NFDPM yield (mg/cigarette)	N*	Mean	sr**	r	sR***	R
			(µg/cigarette)				
1	10	14	199	11	31	35	99
2	6	13	141	9	26	28	78
3	8	14	171	12	33	30	85
4	1	14	36,5	4,0	11,4	14,1	40
5	10	13	180	12	33	31	88
CM 6	15	15	251	17	48	37	104
1R5F	2	15	62	5,8	16,3	18,4	52
3R4F	8	15	206	12	35	35	99
% Reported	98,3						
% Removed	1,7						

*Abbreviations: *N - number of data sets taken for statistical analysis after removing of outliers;*
***sr - repeatability standard deviation; ***sR - reproducibility standard deviation*

Table 8. Acrolein (ISO 3308 Smoking)

Sample description	NFDPM yield (mg/cigarette)	N*	Mean	sr**	r	sR***	R
			(µg/cigarette)				
1	10	14	42,0	2,6	7,3	8,5	24
2	6	14	29,9	2,5	7,0	6,4	18
3	8	14	39,6	3	8,4	7,1	20
4	1	14	6,2	0,8	2,2	1,6	5
5	10	13	44,4	2,8	7,8	9,0	26
CM 6	15	15	63,1	4,2	11,8	10,1	29
1R5F	2	14	9,2	1,0	2,7	2,3	7
3R4F	8	15	47,6	2,9	8,1	8,4	24
% Reported	98,3						
% Removed	1,7						

*Abbreviations: *N - number of data sets taken for statistical analysis after removing of outliers;*
***sr - repeatability standard deviation; ***sR - reproducibility standard deviation*

Table 9. Propionaldehyde (ISO 3308 Smoking)

Sample description	NFDPM yield (mg/cigarette)	N*	Mean	sr**	r	sR***	R
			(µg/cigarette)				
1	10	13	36,2	2,4	6,7	5,1	14
2	6	13	26,9	1,7	4,7	3,9	11
3	8	13	33,2	2,5	7,2	4,3	12
4	1	14	6,7	0,7	2,1	1,8	5
5	10	11	36,9	1,9	5,3	3,7	10
CM 6	15	14	48,5	3,3	9,3	5,3	15
1R5F	2	14	11,3	1,0	2,9	2,8	8
3R4F	8	15	39,8	2,2	6,3	5,5	16
% Reported	93						
% Removed	7						

*Abbreviations: *N - number of data sets taken for statistical analysis after removing of outliers;*
***sr - repeatability standard deviation; ***sR - reproducibility standard deviation*

Table 10. Crotonaldehyde (ISO 3308 Smoking)

Sample description	NFDPM yield (mg/cigarette)	N*	Mean	sr**	r	sR***	R
			(µg/cigarette)				
1	10	14	16,9	1,4	3,9	5,3	15
2	6	12	7,2	0,8	2,1	3,1	9
3	8	12	12,2	1,3	3,6	4	11
4	1	13	1,5	0,3	0, 9	0,7	2
5	10	13	11,1	1,1	3,2	4,8	14
CM 6	15	11	19,1	1,2	3,5	4,3	12
1R5F	2	14	2,2	0,4	1,0	1,1	3
3R4F	8	14	12,1	1,0	2,7	5,1	14
% Reported	91						
% Removed	9						

*Abbreviations: *N - number of data sets taken for statistical analysis after removing of outliers;*
***sr - repeatability standard deviation; ***sR - reproducibility standard deviation*

Table 11. 2-butanone (ISO 3308 Smoking)

Sample description	NFDPM yield (mg/cigarette)	N*	Mean	sr**	r	sR***	R
			(µg/cigarette)				
1	10	14	45,9	3,4	9,6	10,4	30
2	6	13	31,9	2,2	6,2	8,1	23
3	8	14	40,5	3,3	9,3	9,6	27
4	1	14	7,2	0,9	2,6	2,3	7
5	10	14	37,5	4,0	11,3	8,6	24
CM 6	15	15	58,3	6,0	16,9	12,7	36
1R5F	2	15	12,6	1,4	4,1	4	11
3R4F	8	15	48,0	3,4	9,5	10,6	30
% Reported	99,1						
% Removed	0,9						

*Abbreviations: *N - number of data sets taken for statistical analysis after removing of outliers;*
***sr - repeatability standard deviation; ***sR - reproducibility standard deviation*

Table 12. 2-butyraldehyde (ISO 3308 Smoking)

Sample description	NFDPM yield (mg/cigarette)	N*	Mean	sr**	r	sR***	R
			(µg/cigarette)				
1	10	14	23,4	2	5,8	3,8	11
2	6	13	18,4	1,5	4,3	2,4	7
3	8	13	24,1	2,5	5,7	3,2	9
4	1	14	4,4	0,5	1,4	0,9	3
5	10	12	23,3	1,5	4,3	4,1	12
CM 6	15	15	36,5	3,0	8,6	5,4	15
1R5F	2	14	7,6	0,7	1,9	1,6	5
3R4F	8	14	26,9	1,6	4,6	4,4	12
% Reported	93,7						
% Removed	6,3						

*Abbreviations: *N - number of data sets taken for statistical analysis after removing of outliers;*
***sr - repeatability standard deviation; ***sR - reproducibility standard deviation*

13.2 Results from the 2012 Collaborative Study

Calculated statistical data for the individual selected carbonyls are given in the Tables 13-20.

Table 13. Formaldehyde

Sample description	ISO smoking				Intense smoking			
	N*	Mean	r	R	N*	Mean	r	R
		(µg/cigarette)				(µg/cigarette)		
CM6	17	47,2	8,5	22	17	104,2	21,1	50
1R5F	18	3,7	1,2	3	19	38,7	11,6	27
3R4F	19	20,9	4,5	10	19	76,0	15,5	38
1	14	14,2	3.96	9	15	39,0	7,5	21
2	14	19,0	5,5	10	15	64,0	15,5	39
3	13	15,7	3,5	8	14	71,9	18,4	37
4	15	15,5	4,6	11	14	140,3	33,6	107
5	15	6,6	2,5	6	15	87,9	24,0	87
6	14	70,5	16,3	43	15	164,2	35,4	84
7	14	3,2	2,1	3	14	72,8	16,0	34

*Abbreviation: *N - number of data sets taken for statistical analysis after removing of outliers*

Table 14. Acetaldehyde

Sample description	ISO smoking				Intense smoking			
	N*	Mean	r	R	N*	Mean	r	R
		(µg/cigarette)				(µg/cigarette)		
CM6	17	694,2	81,5	193	17	1309,3	159,3	365
1R5F	19	143,6	35,9	102	16	1363,6	225,4	434
3R4F	18	552,4	75,6	147	18	1605,8	185,5	389
1	14	489,2	86,4	171	14	1191,9	150,8	221
2	14	508,0	77,2	151	14	1341,5	200,3	315
3	14	396,1	62,4	130	13	1361,3	137,5	325
4	14	189,4	44,0	79	14	1054,6	157,6	444
5	14	100,0	23,9	52	15	836,5	169,4	595
6	15	577,4	121,0	172	14	1209,6	112,9	222
7	15	93,2	37,8	49	14	1175,4	159,0	321

*Abbreviation: *N - number of data sets taken for statistical analysis after removing of outliers*

Table 15. Acetone

Sample description	ISO smoking				Intense smoking			
	N*	Mean	r	R	N*	Mean	r	R
		(µg/cigarette)				(µg/cigarette)		
CM6	18	269,0	38,3	95	18	517,3	64,8	172
1R5F	18	63,5	16,0	54	19	488,6	103,1	258
3R4F	19	209,7	33,1	91	18	596,8	81,3	180
1	15	193,7	42,6	93	15	473,2	69,8	149
2	14	190,8	31,8	100	14	490,8	67,4	179
3	14	154,4	23,8	68	13	496,3	50,9	192
4	15	77,0	21,4	50	14	369,1	71,3	179
5	13	38,7	10,8	20	14	281,9	51,2	227
6	15	227,2	50,4	95	15	475,6	62,1	161
7	14	42,8	21,1	39	13	415,5	57,5	156

*Abbreviation: *N - number of data sets taken for statistical analysis after removing of outliers*

Table 16. Acrolein

Sample description	ISO smoking				Intense smoking			
	N*	Mean	r	R	N*	Mean	r	R
		(µg/cigarette)				(µg/cigarette)		
CM6	17	68,5	10,7	19	17	133,4	16,6	37
1R5F	17	9,3	2,3	6	17	121,6	23,6	53
3R4F	18	48,3	8,9	17	18	155,4	21,9	41
1	14	36,4	7,5	17	14	95,4	15,0	21
2	15	43,6	10,2	18	14	125,9	20,4	41
3	14	31,8	6,7	14	13	125,6	15,1	39
4	15	18,4	5,4	10	14	127,6	25,7	66
5	14	9,5	3,1	7	14	95,3	20,6	80
6	15	62,6	15,7	23	14	138,8	19,8	31
7	15	7,0	3,4	5	14	125,6	21,7	36

*Abbreviation: *N - number of data sets taken for statistical analysis after removing of outliers*

Table 17. Propionaldehyde

Sample description	ISO smoking				Intense smoking			
	N*	Mean	r	R	N*	Mean	r	R
		(µg/cigarette)				(µg/cigarette)		
CM6	17	53,1	6,8	17	18	104,5	16,1	39
1R5F	19	12,0	2,7	7	18	98,5	17,9	47
3R4F	19	42,1	7,1	14	18	122,5	15,8	39
1	15	36,1	9,1	16	15	87,3	12,1	31
2	14	38,4	6,3	12	14	99,4	16,9	35
3	14	29,6	5,4	9	13	100,5	10,3	26
4	15	14,8	4,4	8	14	80,5	13,7	34
5	15	8,1	2,3	5	15	60,0	13,5	44
6	15	44,6	9,6	18	15	95,3	13,0	43
7	15	7,8	2,8	5	15	83,7	12,4	55

*Abbreviation: *N - number of data sets taken for statistical analysis after removing of outliers*

Table 18. Crotonaldehyde

Sample description	ISO smoking				Intense smoking			
	N*	Mean	r	R	N*	Mean	r	R
		(µg/cigarette)				(µg/cigarette)		
CM6	18	20,5	3,6	11	17	47,9	8,1	17
1R5F	15	2,4	1,1	2	18	35,6	8,6	24
3R4F	18	11,0	2,9	6	18	49,9	10,4	22
1	14	13,9	3,6	8	14	40,2	6,8	12
2	14	11,1	2,8	6	14	41,6	8,6	18
3	13	7,8	2,2	4	14	43,9	9,0	19
4	14	4,4	1,5	3	14	40,2	10,0	21
5	14	2,3	1,1	2	14	30,4	8,1	25
6	14	18,4	4,8	10	15	46,0	8,1	13
7	14	1,9	0,9	2	14	35,8	8,2	15

*Abbreviation: *N - number of data sets taken for statistical analysis after removing of outliers*

Table 19. 2-butanone

Sample description	ISO smoking				Intense smoking			
	N*	Mean	r	R	N*	Mean	r	R
		(µg/cigarette)				(µg/cigarette)		
CM6	18	62,1	12,7	36	18	131,4	27,7	69
1R5F	19	13,6	3,8	14	19	111,6	25,7	85
3R4F	18	51,7	8,3	24	18	147,0	30,3	79
1	15	45,3	12,4	31	14	112,7	21,6	57
2	14	47,3	10,2	16	14	118,5	22,8	62
3	14	35,8	7,9	25	14	121,2	22,2	65
4	14	17,1	4,3	13	15	89,9	22,7	66
5	15	10,0	3,8	10	14	69,6	18,2	32
6	14	54,6	15,3	39	15	116,2	24,6	66
7	15	9,1	3,8	10	13	95,7	19,3	47

*Abbreviation: *N - number of data sets taken for statistical analysis after removing of outliers*

Table 20. 2-butyraldehyde

Sample description	ISO smoking				Intense smoking			
	N*	Mean	r	R	N*	Mean	r	R
		(µg/cigarette)				(µg/cigarette)		
CM6	17	36,9	4,5	20	16	69,8	14,1	33
1R5F	18	7,7	2,1	6	17	58,9	15,1	40
3R4F	18	25,3	3,4	9	17	71,8	11,3	31
1	13	21,9	4,7	12	13	52,4	7,0	14
2	13	24,3	3,7	11	13	62,0	13,1	26
3	14	19,6	3,7	10	14	65,7	9,4	30
4	14	9,7	2,6	6	14	48,9	12,3	29
5	13	6,1	1,6	3	13	40,2	9,0	34
6	13	30,2	5,3	12	14	62,4	11,3	21
7	14	5,0	1.9	3	14	46,8	9,2	34

*Abbreviation:*N - number of data sets taken for statistical analysis after removing of outliers*

14. REPORT

14.1 Test Results

- 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.

Carbonyl yields in the mainstream smoke of cigarette in units of microgram per cigarette (µg/cigarette) should be reported rounded to the nearest 0,1 µg/cigarette.

15. REFERENCES

[1] Intorp M., Purkis S.W., Wagstaff W., Determination of carbonyl compounds in cigarette mainstream smoke: the CORESTA 2010 Collaborative Study and Recommended Method. Beiträge zur Tabakforsch., 25(2), p. 361-374, June 2012.

[2] *Health Canada Official Method T-115*: Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke, December 1999.

[3] "CORESTA Smoke Analytes Sub-Group Technical Report – 2012 Collaborative Study on B[a]P, VOCs, and Carbonyls in Mainstream Cigarette Smoke", August 2019.

Appendix 1

Recrystallisation of 2,4-Dinitrophenylhydrazine

- Weigh approximately 35 g of DNPH into a weighing boat. Transfer the DNPH into a clean 2 l Erlenmeyer flask and add a stirrer.
- Add 750 ml of anhydrous reagent grade ethanol to the flask. Place the flask on a hot plate equipped with a stirrer. Gently heat the solution with constant stirring.
- When the solution is warm, slowly add 1000 ml of ethyl acetate. Continue to heat and stir (making sure not to boil) until all of the DNPH is completely dissolved. The solution should be clear and a very dark red.
- Vacuum filter the hot solution.
- Transfer the filtrate to a 2 l Erlenmeyer flask.
- If crystallization does not start to occur, scratch the inside of the flask with a glass rod. Cover the Erlenmeyer flask with a watch glass and allow the solution to cool overnight in a cupboard.
- Vacuum filter the recrystallized DNPH.
- Transfer the crystals into a clean weighing boat that is labelled with the date of recrystallization and the Lot# of the DNPH. Weigh the recrystallized DNPH. Place the crystals in a desiccator to remove any moisture.
- The filtrate can be evaporated down with a rotovap and vacuum filtered again to recover more crystals.

Note: If recrystallizing a larger quantity of DNPH (more than 2 days requirement), the DNPH must be hydrated to approximately 30 % with water. After adding the water, place in an airtight container and label it as containing 30 % water.

Appendix 2

Example of calibration standards preparation

(a): Stock Standards

Carbonyl Hydrazone	Formula Wt Hydrazone	Primary Standards					Secondary Standard *		
		Formula Wt Carbonyl	Weight	Purity	Volume	Stock	Vol of 1° Stock	Dilute to Volume	Stock
			(mg)	(%)	(mL)	(µg/mL)	(µL)	(mL)	(µg/mL)
Formaldehyde	210,15	30,03	50,6	99,9	25	288,9	500	25	5,779
Acetaldehyde	224,18	44,05	33,9	99,9	25	266,2	1800	25	19,16
Acetone	238,21	58,08	40,6	99,9	25	395,6	800	25	12,66
Acrolein	236,18	56,06	17,7	99,9	25	167,9	850	25	5,708
Propionaldehyde	238,21	58,08	35,8	99,9	25	348,8	500	25	6,976
Crotonaldehyde	250,22	70,09	36,1	99,9	25	404,1	500	25	8,082
MEK	252,23	72,11	33,4	99,9	25	381,6	500	25	7,631
Butyraldehyde	252,23	72,11	42,8	99,9	25	489,0	1000	25	19,56

* In a single 25mL volumetric flask, made to volume with acetonitrile

(b): Carbonyl Working Standards **

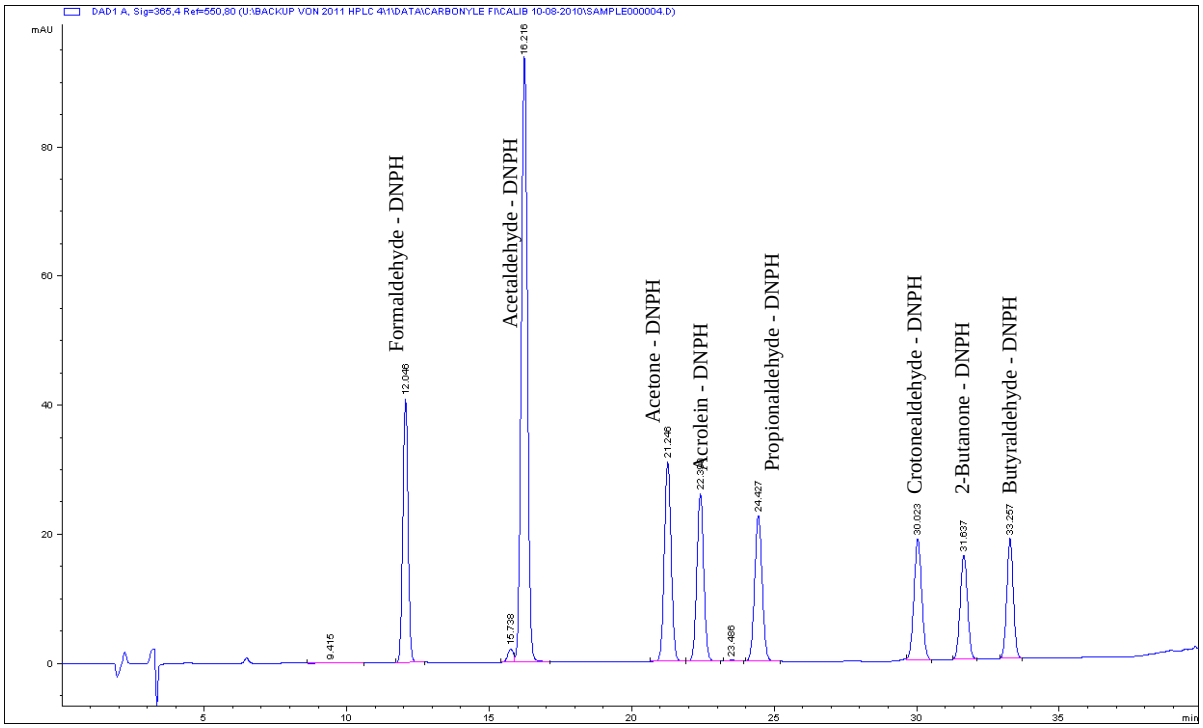
Carbonyl	*Volume of Secondary Standard (mL)*							
	10,0	*7,0*	*4,0*	*2,0*	*0,80*	*0,40*	*0,20*	*0,05*
	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)
Formaldehyde	5,779	4,045	2,311	1,156	0,4623	0,2311	0,1156	0,0289
Acetaldehyde	19,16	13,42	7,666	3,833	1,533	0,7666	0,3833	0,0958
Acetone	12,66	8,861	5,063	2,532	1,013	0,5063	0,2532	0,0633
Acrolein	5,708	3,996	2,283	1,142	0,4566	0,2283	0,1142	0,0285
Propionaldehyde	6,976	4,883	2,790	1,395	0,5581	0,2790	0,1395	0,0349
Crotonaldehyde	8,082	5,657	3,233	1,616	0,6465	0,3233	0,1616	0,0404
MEK	7,631	5,342	3,053	1,526	0,6105	0,3053	0,1526	0,0382
Butyraldehyde	19,56	13,69	7,823	3,912	1,565	0,7823	0,3912	0,0978

** In a single 10mL volumetric flask, made to volume with acetonitrile

Note: MEK = methyl ethyl ketone = 2-butanone

Appendix 3

HPLC chromatogram of a typical combined carbonyl calibration standard using column AQ RP C 18 5µm, 250 mm × 4,6 mm



Appendix 4

HPLC chromatogram of carbonyls in DNPH extract of mainstream tobacco smoke of 3R4F using column AQ RP C18, 5 µm, 250 mm × 4,6 mm