

Determination of Selected Volatiles in Cigarette Mainstream Smoke. The CORESTA 2009 Collaborative Study and Recommended Method*

by

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SUMMARY

A recommended method has been developed and published by CORESTA, applicable to the quantification of selected volatiles (1,3-butadiene, isoprene, acrylonitrile, benzene, and toluene) in the gas phase of cigarette mainstream smoke. The method involved smoke collection in impinger traps and detection and measurement using gas chromatography/mass spectrometry techniques.

This report describes the final collaborative study applying the recommended method. It provides additional notes to inform other laboratories that might wish to adopt it, about some of the main features that need to be well controlled to provide data as robust and consistent as the data presented herein.

Data was provided by 15 industry-related and 4 independent laboratories and one governmental laboratory. Overall, 6 linear and 14 rotary smoking machines were used.

The joint experiments and collaborative work between the large number of participating laboratories has provided solutions to several methodological problems and reduced the high data variability that had initially been found particularly for 1,3-butadiene and acrylonitrile smoke yields.

Even so, the levels of reproducibility among laboratories are much greater than the levels found for 'tar', nicotine and carbon monoxide and given in the equivalent ISO standards. When expressing the reproducibility (R) value as a percentage of the mean yield among-laboratories and across all of the studied products, values ranged from 63–93% for 1,3-butadiene; from 36–62% for isoprene; from 41–110% for acrylonitrile; from 35–70% for benzene, and

from 27–116% for toluene. For the higher 'tar' yielding products, the lower levels of variability were in line with those previously evaluated during Task Force work on standard methods for benzo[a]pyrene and tobacco specific nitrosamines. As expected, the lowest 'tar' yielding product gave the most variable data. [Beitr. Tabakforsch. Int. 24 (2011) 243–251]

ZUSAMMENFASSUNG

Zur Bestimmung der ausgewählten flüchtigen organischen Verbindungen 1,3-Butadien, Isopren, Acrylonitril, Benzol und Toluol in der Gasphase des Hauptstromrauchs von Zigaretten wurde von der CORESTA eine Methode entwickelt und veröffentlicht. Die Methode basiert auf dem Sammeln des Rauchs in Flüssigfallen und dem anschließenden Nachweis und der quantitativen Messung mittels Gaschromatographie gekoppelt mit Massenspektrometrie.

Der Report beschreibt die Studie über die Anwendung der empfohlenen Methode. Er beschreibt umfänglich für Laboratorien, die diese Methode einführen wollen, die relevanten Einflussfaktoren, die zu beachten sind, um robuste und verlässliche Daten zu erhalten.

Die Studie umfasst Daten von 15 Industrielaboratorien, 4 Auftragslaboratorien und einem staatlichen Labor. Zum Abrauchen der Zigaretten wurden 6 lineare und 14 rotative Rauchmaschinen eingesetzt.

Die Durchführung verschiedener Studien, an denen zahlreiche Laboratorien beteiligt waren, führte zur Lösung von Methodenproblemen und reduzierte die anfangs beobachteten Streuungen, insbesondere für 1,3-Butadien

und Acrylonitril.

Dennoch sind die ermittelten Reproduzierbarkeiten zwischen den Laboratorien erheblich größer im Vergleich zu den Werten, die für Kondensat, Nikotin und Kohlenstoffmonoxid in den entsprechenden ISO Standardmethoden genannt werden. Die Reproduzierbarkeiten (R) ausgedrückt in Prozent der Mittelwerte liegen für alle untersuchten Produkte zwischen 63–93% bei 1,3-Butadien, 36–62% bei Isopren, 41–110% bei Acrylonitril, 35–70% bei Benzol und 27–116% bei Toluol. Für Produkte mit höheren Kondensatgehalten nach ISO wurden für diese Substanzen Reproduzierbarkeiten ermittelt, die vergleichbar zu denen waren, die für die Standardmethoden von Benzo[a]pyren und den tabak-spezifischen Nitrosaminen berichtet wurden. Wie erwartet waren die berechneten Reproduzierbarkeiten für Produkte mit sehr niedrigen Kondensatgehalten entsprechend sehr viel höher. [Beitr. Tabakforsch. Int. 24 (2011) 243–251]

RESUME

Une méthode recommandée a été élaborée et publiée par CORESTA. Cette méthode s'applique à la quantification de substances volatiles sélectionnées (1,3-butadiène, isoprène, acrylonitrile, benzène, et toluène) lors de la phase gazeuse de la fumée principale de cigarette. La méthode a associé le captage de la fumée dans des pièges à impact, la détection et la quantification en utilisant les techniques de la chromatographie en phase gazeuse et la spectrométrie de masse.

Ce rapport décrit l'étude collective finale appliquant la méthode recommandée. Il fournit des notes supplémentaires pour informer d'autres laboratoires qui souhaiteraient adopter cette méthode des principales caractéristiques devant être bien contrôlés pour fournir des données qui soient aussi solides et cohérentes que les données présentées ci-dessous.

Les données ont été fournies par 15 laboratoires liés à l'industrie et 4 laboratoires indépendants et un laboratoire gouvernemental. Au total, 6 machines à fumer linéaires et 14 machines à fumer rotatives ont été employées.

Les expériences communes et le travail collectif d'un nombre important de laboratoires participants ont résolu de nombreux problèmes méthodologiques et réduit la variabilité élevée entre les données qui avait été constatées dans un premier temps en particulier pour les teneurs en butadiène et en acrylonitrile dans la fumée.

Les niveaux de reproductibilité parmi les laboratoires restent quand même nettement supérieurs aux taux constatés et indiqués dans les normes ISO équivalentes pour le goudron, la nicotine et le monoxyde de carbone. Lorsque la valeur de la reproductibilité (R) est exprimée en pourcentage de la teneur moyenne entre les laboratoires sur l'ensemble des produits étudiés, les valeurs variaient de 63 à 93% pour le 1,3-butadiène; de 36 à 62% pour l'isoprène; de 41 à 110% pour l'acrylonitrile; de 35 à 70% pour le benzène et de 27 à 116% pour le toluène. Pour les produits à teneur plus forte en goudron, les niveaux inférieurs de variabilité étaient cohérents avec ceux préalablement évalués pendant les travaux du groupe d'étude sur les méthodes standard pour le benzo[a]pyrène et les nitrosami-

nes spécifiques au tabac. Comme on s'y attendait, le produit ayant la plus faible teneur en goudron a donné les résultats les plus variables. [Beitr. Tabakforsch. Int. 24 (2011) 243–251]

PARTICIPATING LABORATORIES

A summary of the twenty participating laboratories and their principal investigators is given below. The Task Force co-ordinators wish to acknowledge the contributions of each principal investigator and their colleagues without whom the work of the Task Force could not have been completed.

Participating laboratories and institutions	Principal investigators
Arista (USA)	Brad Norwood / Fraser Williamson
Arista Labs Europe (UK)	Tatiana Humphries
BAT – Souza Cruz (Brazil)	Waldenir Farias Braga
British American Tobacco (UK)	Tina Mead
CNTC Beijing (China)	Jun Zhou
CNTC QSTC (China)	Hongwei Hou
CNTC ZTRI (China)	Fuwei Xie
Chemisches und Veterinär-landesuntersuchungsamt Sigmaringen (Germany)	Jürgen Hahn
Filtrona International (UK)	Mike Taylor
Imperial Tobacco Group / ALTADIS (France)	Sandrine Destruhaut
Imperial Tobacco Group / Reemtsma (Germany)	Michael Intorp / Steve Purkis
Japan Tobacco (Japan)	Takatsugu Hyodo
Japan Tobacco International (Austria)	Jutta Pani
KT&G (Korea)	Hyo-Keun Kim
Labstat (Canada)	Pete Joza / Wendy Wagstaff
Lorillard (USA)	Rob Stevens
Philip Morris Research Laboratories (Germany)	Jörg Diekmann
Philip Morris International (Switzerland)	Michel Rotach
Philip Morris Sampoerna (Indonesia)	Bimo Haryo Progo / Rachmat Hidayat
Rothmans Benson and Hedges (Canada)	Jennifer DeGraaf / Ian Jenkins

The Task Force co-ordinators also wish to acknowledge the contributions made by other Task Force members, William M. Coleman III (R.J. Reynolds, USA), Linda Crumpler (Cerulean, USA), and Katrin Schade (Borgwaldt, Germany).

INTRODUCTION

A CORESTA study carried out in 2006 (1) described an investigation of some of the main parameters that may cause variability in smoke analyte yields collected from both the particulate and gas phase of mainstream cigarette smoke when machine smoking under ISO conditions (2). The laboratories had used their own in-house methods to

determine yields and the work had been carried out to obtain guidance towards a robust and practicable recommended method.

Initial work was followed by exploratory joint experiments on the best and necessary features that need to be incorporated in a recommended method. The Part 1 report (3) on the selected smoke volatiles (1,3-butadiene, benzene, toluene, isoprene, and acrylonitrile) had shown that similar yields were obtained when comparing data from Tedlar bag trapping with those from impinger traps, the latter method being used by the majority of laboratories. Several key parts of the methodology were investigated in detail to evaluate their effects on smoke yields and the learning exercise proved to be a further important directional step towards a recommended method.

The work described in this report provides details on a CORESTA collaborative study leading to a recommended method (4), and based on the Health Canada method (5) for the determination of selected volatiles in the gas phase of cigarette mainstream smoke. The statistical analysis of the yield data from 20 laboratories including repeatability and reproducibility data is also presented. The report describes key components of the recommended method and provides additional notes to inform other laboratories about some of the main features that need to be well controlled to provide data as robust and consistent as the data presented herein.

EXPERIMENTAL

Recommended method

A CORESTA recommended method (CRM 70) (4) was agreed upon by the Special Analytes Task Force based on the learning from the earlier joint experiment (3).

After conditioning (6), cigarettes were smoked under standardised conditions (2). The smoking machine was additionally equipped with impinger traps containing methanol cryogenically cooled to -70°C into which the gas phase components of cigarette mainstream smoke were trapped and collected, as shown, for example, in Figure 1. The impinger solutions were spiked with hexa-deuterated benzene standard; an aliquot of the solution was separated on a gas chromatograph and the selected volatiles (1,3-butadiene, benzene, toluene, acrylonitrile, and isoprene) were detected and quantified by mass spectrometry by the single ion monitoring (SIM) technique using the ions specified in CRM 70.

It should be noted that the minimal yields of selected volatiles that trap within the particulate phase on the Cambridge filter pad are not included in these measurements. An example chromatogram for the 3R4F Kentucky Reference cigarette is given in Figure 2. No internal standard is shown in the chromatogram.

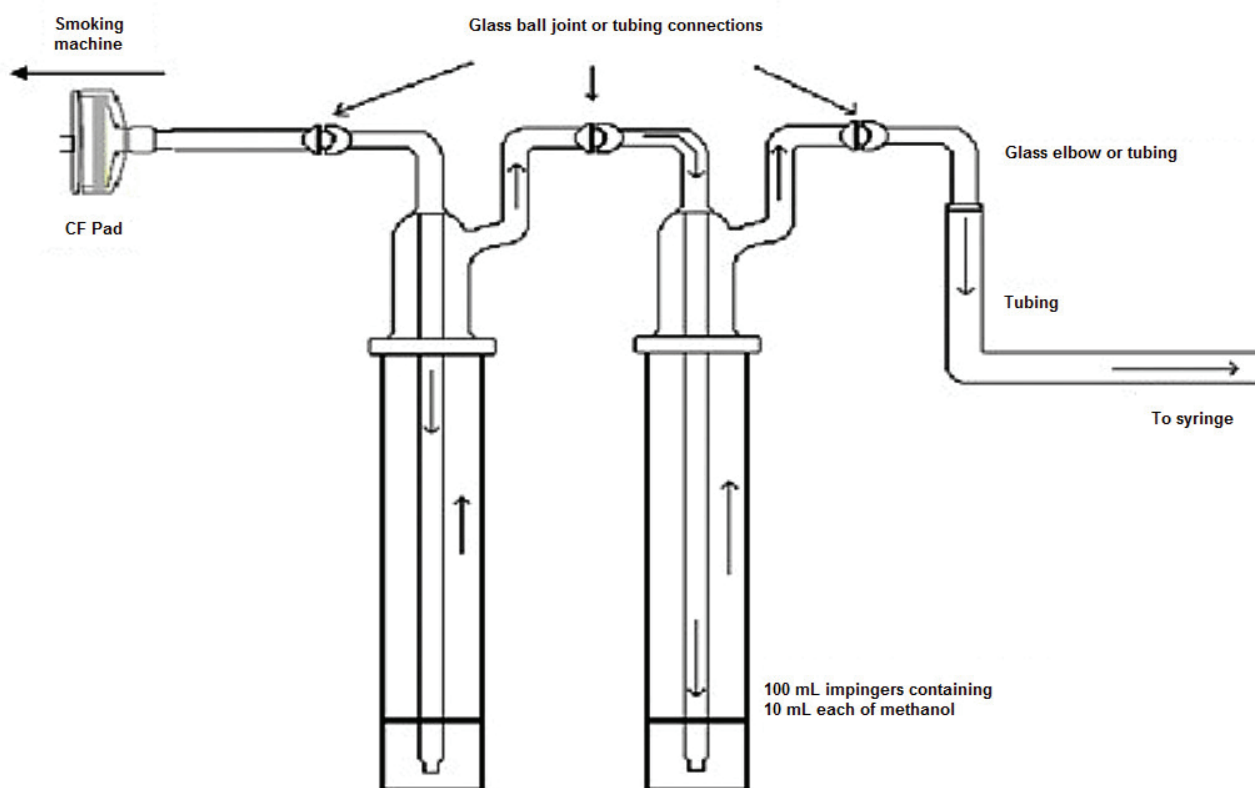


Figure 1. Example of an impinger setup for smoking machines.

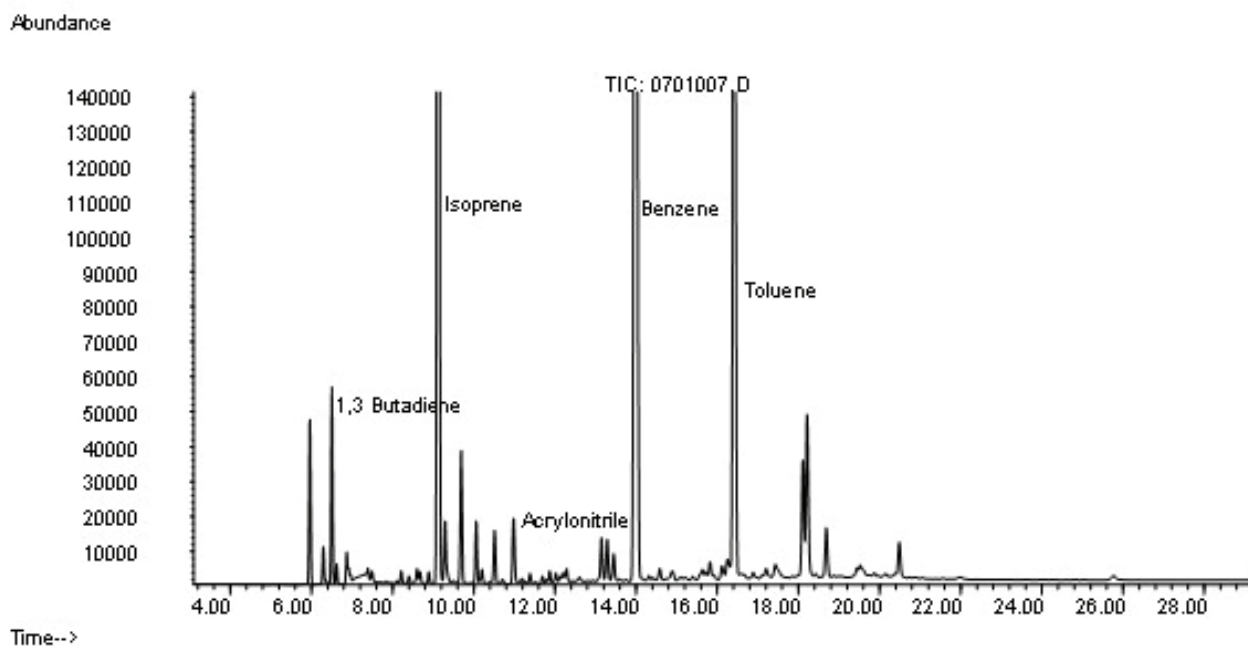


Figure 2. Example chromatogram for the 3R4F Kentucky Reference cigarette.

OVERVIEW OF THE PROTOCOL AND SAMPLE DESCRIPTIONS

The main objective of the collaborative study was to run the recommended method for the determination of selected volatiles (1,3-butadiene, benzene, toluene, acrylonitrile, and isoprene) in the gas phase of cigarette mainstream smoke to provide robust repeatability and reproducibility data.

The test samples listed in Table 1 were used in the collaborative study. Samples 1–5 were commercially available cigarettes provided by various manufacturers to represent a range of ‘tar’ yields, blend and cigarette design styles to complement the three reference cigarettes (CM6, 1R5F, and 3R4F).

Table 1. Cigarette test samples.

Sample code	Blend and design	NFDPM yield (mg)
Sample 1	Dark air cured blend / CA filter	10
Sample 2	American blend / CA filter	6
Sample 3	Virginia blend / CA filter	8
Sample 4	American blend / charcoal filter	1
Sample 5	American blend / charcoal filter	10
CM6	CORESTA monitor / Virginia blend / CA filter	15
1R5F	Kentucky reference / American blend / CA filter	2
3R4F	Kentucky reference / American blend / CA filter	8

The laboratories were asked to smoke the five replicates of the eight test samples using a randomized design within eight runs per experiment. In this way, each replicate was

part of a separate experiment as shown in the smoking plan given in Table 2.

Table 2. Smoking plan.

Run No.	Experiment number				
	1	2	3	4	5
1	CM6	Sample 5	Sample 3	CM6	3R4F
2	Sample 4	Sample 1	Sample 5	Sample 2	Sample 4
3	Sample 1	1R5F	Sample 1	1R5F	Sample 5
4	Sample 2	CM6	CM6	Sample 3	Sample 2
5	Sample 5	Sample 2	Sample 4	Sample 5	1R5F
6	3R4F	Sample 3	Sample 2	3R4F	Sample 3
7	1R5F	3R4F	3R4F	Sample 1	CM6
8	Sample 3	Sample 4	1R5F	Sample 4	Sample 1

RESULTS

Analytical yields of the five selected volatiles were obtained and mean yields are given in Table 3 (calculated after exclusion of outliers as described later). Each laboratory recorded the dates on which the analyses were performed and most laboratories provided information on the limits of detection (LOD) and limits of quantification (LOQ), with the range summarized as maximum and minimum values in Table 4. There was no requirement that LOQs or LODs were measured in the same way at different laboratories and this is reflected in the wide range given in Table 4.

However, there was no indication that the means of determining LODs / LOQs lead to lower or higher levels of outlier or excluded data. Excellent recovery rates were given by all laboratories for the selected volatiles as shown in Table 5.

Table 4. Limits of quantification (LOQ) and limits of detection (LOD).

	$\mu\text{g/cig}$	1,3-Butadiene	Benzene	Toluene	Acrylonitrile	Isoprene
LOQ	minimum	0.08	0.02	0.04	0.03	0.05
	maximum	7.44	4.63	8.32	3.97	31.8
LOD	minimum	0.03	0.01	0.01	0.01	0.02
	maximum	2.22	1.39	2.50	1.32	9.6
Recovery	minimum	89	90	95	85	91
	maximum	109	109	111	112	110

DATA ANALYSIS

Variability of standard smoke parameters

Total Particulate Matter (TPM) and puff count data were calculated (7) as received from all participating laboratories and are summarised in Table 5. The low puff count variability indicated good adherence to the ISO conditioning standard (6) by the participating laboratories. The highest variability in TPM yields was associated with the lowest yielding cigarette types, i.e. 1R5F and Sample 4.

Table 5. TPM and puff count data.

Sample code	Puffs		TPM	
	Mean	CoV%	Mean	CoV%
CM6	9.3	3.5	18.3	5.7
1R5F	6.9	2.7	2.5	13.8
3R4F	8.4	2.7	10.7	6.6
Sample 1	5.7	3.4	12.9	12.6
Sample 2	9.8	4.1	6.9	12.3
Sample 3	6.9	3.4	12.3	8.1
Sample 4	6.8	5.6	1.7	14.9
Sample 5	7.4	3.6	13.8	7.0

Where CoV = Coefficient of Variation

Table 6 shows the effect on volatile yields of the two different smoking machine types used in the laboratories. Six laboratories used “linear” and 14 used “rotary” smoking machines for smoke collection. No observable effect of smoking machine type was found for any of the studied analytes although this was not statistically tested.

General statistical analysis

Statistical calculations were based on ISO guidelines (8, 9) and applied directly to determine estimates of repeatability (r) and reproducibility (R) after smoking under defined conditions (2), where all of the participating laboratories used the same analytical method (4).

Determination of inter-laboratory repeatability and reproducibility variance

For each of the j test samples ($j = 1, \dots, 8$), the general mean (\bar{y}_j) was determined according to ISO 5725–2 section 7.4.4 (8) over the p participating laboratories, whose data remained following the removal of outliers. The outlier analysis methods are discussed in the following sections.

Table 6. Effect of smoking machine type on selected volatile yields.

Sample	Smoking machine	1,3-Butadiene ($\mu\text{g/cig}$)	Benzene ($\mu\text{g/cig}$)	Toluene ($\mu\text{g/cig}$)	Acrylonitrile ($\mu\text{g/cig}$)	Isoprene ($\mu\text{g/cig}$)
CM6	linear	61.6	59.7	88.6	12.0	570
	rotary	59.6	60.5	84.0	12.5	546
1R5F	linear	11.9	13.1	15.7	2.0	116
	rotary	12.4	14.8	19.5	2.2	122
3R4F	linear	38.8	39.9	64.4	8.4	355
	rotary	42.5	42.7	64.9	8.6	365
Sample 1	linear	31.4	37.1	57.6	10.0	206
	rotary	32.5	39.3	57.9	10.4	221
Sample 2	linear	30.3	30.2	44.7	5.1	255
	rotary	31.3	32.2	44.3	5.4	256
Sample 3	linear	31.7	33.4	49.0	7.4	243
	rotary	32.9	35.5	49.3	7.5	247
Sample 4	linear	7.1	6.1	5.9	0.8	57
	rotary	7.3	7.4	9.7	1.1	59
Sample 5	linear	37.9	27.7	37.5	6.3	276
	rotary	39.5	28.8	37.4	6.4	283

Determination of inter-laboratory repeatability and reproducibility variance

For each of the j test samples ($j = 1, \dots, 8$), the general mean ($\bar{\bar{y}}_j$) was determined according to ISO 5725–2 section 7.4.4 (8) over the p participating laboratories, whose data remained following the removal of outliers. The outlier analysis methods are discussed in the following sections.

$$\text{General mean} = (\bar{\bar{y}}_j) = \frac{\sum_{i=1}^p n_{ij} \bar{y}_{ij}}{\sum_{i=1}^p n_{ij}}$$

where \bar{y}_{ij} is the mean for test sample j as reported by laboratory i and n_{ij} is the number of observations.

Repeatability variance (s_{rj}^2) was determined as per ISO 5725–2 section 7.4.5.1 (9).

$$\text{Repeatability variance } s_{rj}^2 = \frac{\sum_{i=1}^p (n_{ij} - 1) s_i^2}{\sum_{i=1}^p (n_{ij} - 1)}$$

where s_{rj}^2 is the within-laboratory variance for test sample j as reported by laboratory i .

Reproducibility variance (s_{Rj}^2) was determined as per ISO 5725–2 section 7.4.5.5 (9).

Reproducibility variance $s_{Rj}^2 = s_{rj}^2 + s_{Lj}^2$ where s_{Lj}^2 is the between-laboratory variance and calculated as

$$s_{Lj}^2 = \frac{\left(\frac{\sum_{i=1}^p n_{ij} (\bar{y}_{ij} - \bar{\bar{y}}_j)^2}{p-1} \right) - s_{rj}^2}{\frac{\sum_{i=1}^p n_{ij} - \left(\frac{\sum_{i=1}^p (n_{ij})^2}{\sum_{i=1}^p n_{ij}} \right)}{p-1}}$$

Data excluded due to being below the limits of quantification

Some individual data points were reported as non-numeric (i.e. below the LOQ) and had to be removed prior to evaluation of numeric data for outliers. This applied

particularly to 1,3-butadiene and acrylonitrile. For 1,3-butadiene, 759 of the possible 800 observations and for acrylonitrile, 768 of the possible 800 observations were above the LOQ. A summary of exclusions is given in Table 7.

Data excluded within the context of among-laboratory variability (R)

For evaluation of agreement among laboratories, the Grubbs' test was applied as described (8) to detect single or multiple outlying laboratories with respect to the sample mean.

If the Grubbs statistic was significant at the 5% level, the sample data from that laboratory was removed. Six out of eight samples had significant Grubbs results (at 1%) for high outliers for acrylonitrile data from laboratory 18. Hence, all eight test samples were excluded from the analysis due to the expectation that there was a problem with the entire acrylonitrile analysis for this laboratory.

The Grubbs statistic for a single high outlier was significant for the following analytes, samples and laboratories. The data, as shown in Table 8, was removed prior to the calculations of the general mean, repeatability and reproducibility variance.

- 1,3-butadiene: laboratory 18 (Sample 3 and CM6)
- Isoprene: laboratory 13 (Sample 4)
- Benzene: laboratory 13 (Sample 4)
- Acrylonitrile: laboratory 18 (Samples 1, 2, 3, 4, 5, and CM6, 1R5F, 3R4F).

Data excluded within the context of within-laboratory variability (r)

The approach used was to consider of highest importance the removal of data from those laboratories whose means were not in agreement with other laboratories. The degree of agreement for within-laboratory variance was not evaluated with as much rigour as the among-laboratory variance. In this case, only those laboratories whose variation among five replicates was considered 'abnormally high' in comparison to the other laboratories, that is, with a standard deviation at least two times greater than that reported for any other laboratory, were examined for potential outliers. The determination of the outliers was made based on a comparison with the individual values reported by the other laboratories. In this way some data from laboratory 16 were excluded and are shown in Table 8.

Table 7. Excluded data below LOQ.

Laboratory	Sample 2	Sample 4	1R5F	All samples
4		all acrylonitrile data		
8		all acrylonitrile data	acrylonitrile data for single 1R5F data point	
12	all acrylonitrile data	all acrylonitrile data	all acrylonitrile data butadiene data for single 1R5F data point	
19		all acrylonitrile data	acrylonitrile data for single 1R5F data point	all butadiene data although LOQ not supplied

Table 8. Data exclusion after statistical evaluation.

Analyte	Lab	Outlier types for excluded laboratory data
1,3-Butadiene	18	Mean and variance outliers for sample 3 and CM6
	13	Mean outliers for sample 4
Benzene	16	Variance outlier for single replicate in 1R5F
Toluene	16	Variance outliers for 1R5F
	16	Variance outliers for 2 low 1R5F
Acrylonitrile	18	replicates
Isoprene		Mean outliers for all samples
	13	Mean outliers for sample 4

Summary of excluded data

A summary of the number of observations made and the number of observations excluded as outliers is given in Table 9. The results show that a very small percentage of observations were treated as outliers (0.6–5.5%) for each analyte.

Table 9. Overall summary of excluded data

Analyte	Outlier summary			
	Total number of observations	Total reported observations (%)	Number of excluded observations	Outliers (%)
1,3-Butadiene	759	94.9	10	1.3
Benzene	800	100	10	1.3
Toluene	800	100	5	0.6
Acrylonitrile	768	96.0	42	5.5
Isoprene	800	100	5	0.6

After exclusion of outlier data, the mean smoke yields and levels of repeatability and reproducibility of 1,3-butadiene, toluene, benzene acrylonitrile, and isoprene across each of the cigarette types were summarized in Table 3. The levels of variability found for the 3R4F and 1R5F products were improved when compared to previous studies, where laboratories either used their preferred methodology (1) or similar methods in joint experiment work (3). However, the levels of reproducibility among laboratories are still much worse than the levels found for ‘tar’, nicotine, and carbon monoxide and given ISO 8243 (10). When expressing the R value as a percentage of the mean across all of the studied products, values ranged from 63–93% for 1,3-butadiene; from 36–62% for isoprene; from 41–110% for acrylonitrile; from 35–70% for benzene, and from 27–116% for toluene. For the higher ‘tar’ yielding products the levels of variability were in line with those previously evaluated during work on CRMs for benzo[*a*]pyrene (11) and tobacco-specific nitrosamines (12). The lowest ‘tar’ yielding product (Sample 4) gave the most variable data.

Some recommendations on the methodology

The knowledge gained from the joint experiments and the practical experience shared within the Task Force has led to the following recommendations.

Tubing

Figure 1 shows glass tubing as being mainly used to connect the smoking machine to the impingers. However, tubing other than silicone can also be used, such as polyethylene, polypropylene, or polyvinyl chloride (e.g. Tygon). Adsorption of the analytes on to silicon tubing has been found to occur and its use is not recommended. Tubing should be as short as possible between connections to minimise the potential for any adsorption.

Trapping efficiency

It is recommended that the trapping efficiency should be checked when validating this method. A third impinger should be added to check the trapping efficiency and the method followed accordingly. Each impinger should be analysed individually for the volatile compounds of interest. If no compounds are detected in the third impinger then only two impingers are required to trap all the volatiles effectively.

Minimisation of 1,3-butadiene variability

Variability could be reduced by storage in a cooled auto-sampler, by thorough closure of and minimisation of headspace in the vial. A UV method (5) is recommended to measure 1,3-butadiene yields in stock solution.

Minimisation of acrylonitrile variability

A broad chromatographic peak is often found for this analyte due to combination of its low level and the choice of chromatographic stationary phase. Individual laboratories need to find their optimum conditions during their validation procedures.

Internal standards

Individual laboratories may not choose to use deuterated benzene-d6 as an internal standard (IS) and, for example, may wish to apply deuterated toluene-d5. However, the collaborative study for the CRM used benzene-d6 and the *r* and *R* values were based on this IS. Therefore, any laboratory that wishes to modify the calibration will need to carry out proper validation with the different IS. If there are commercially available calibration solutions then these can be used instead of preparation of fresh IS. Certified standards should be checked to ensure that they are of the correct concentration. All solutions should be checked for stability before use and the effect of local storage conditions. IS concentrations should be used that are within the range of the expected yields of the studied products.

CONCLUSIONS

The CORESTA Special Analytes Task Force has developed a CRM identifying and optimizing key components of the methodology that contribute significantly to variability of test results.

The relative levels of reproducibility among laboratories are much higher than the levels found for 'tar', nicotine and carbon monoxide given in ISO 4387 (7). When expressing the 'R' value as a percentage of the mean across all of the studied products, values ranged from 63–93% for 1,3-butadiene; from 36–62% for isoprene; from 41–110% for acrylonitrile; from 35–70% for benzene, and from 27–116% for toluene. For the higher 'tar' yielding products the levels of variability were in line with those previously evaluated during work on standard methods for benzo[a]pyrene and tobacco-specific nitrosamines. The lowest 'tar' yielding product (Sample 4) gave the most variable data.

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Table 3. Mean yields, repeatability and reproducibility data obtained using the CORESTA Recommended Method

Cigarette type	1,3-Butadiene				Isoprene				Acrylonitrile				Benzene				Toluene			
	NL	M	r	R	NL	M	r	R	NL	M	r	R	NL	M	r	R	NL	M	r	R
1	19	33.30	11.80	25.00	20	216	46	89	19	10.20	2.40	4.20	20	38.6	7.20	14.60	20	57.8	13.30	27.30
2	19	32.10	11.00	25.00	20	256	48	108	18	5.32	1.74	2.67	20	31.6	6.70	13.10	20	44.5	12.40	20.70
3	18	32.50	13.30	22.40	20	245	43	102	19	7.48	1.92	3.45	20	34.9	5.90	13.70	20	49.3	9.70	23.50
4	19	7.53	4.89	6.98	19	58	15	31	15	0.98	0.37	1.08	19	6.73	1.98	4.69	20	8.53	3.53	9.93
5	19	39.00	10.90	28.00	20	281	52	122	19	6.34	1.74	3.08	20	28.5	5.70	13.70	20	37.4	9.70	20.50
CM 6	18	60.30	21.20	37.90	20	553	98	198	19	12.30	3.20	5.90	20	60.3	10.60	21.00	20	85.4	19.40	41.00
1R5F	19	12.20	5.30	8.20	20	120	38	74	18	2.10	0.69	1.27	19	14.3	2.50	7.20	19	18.5	4.70	9.90
3R4F	19	41.40	13.30	29.60	20	362	57	134	19	8.56	2.11	3.56	20	41.9	6.30	15.30	20	64.8	12.20	30.80

Where NL = number of laboratories used to provide mean, repeatability and reproducibility data

M = mean yield (µg/cigarette)

r = repeatability (µg/cigarette at 95% confidence interval)

R = reproducibility (µg/cigarette at 95% confidence interval)