

# CORESTA RECOMMENDED METHOD N° 70

## **DETERMINATION OF SELECTED VOLATILE ORGANIC COMPOUNDS IN THE MAINSTREAM SMOKE OF CIGARETTES - GAS CHROMATOGRAPHY-MASS SPECTROMETRY METHOD**

*(June 2010)*

### **0. INTRODUCTION**

The CORESTA Special Analytes Task Force carried out a study in 2005 to compare various smoke analyte yield data obtained from different laboratories using their own preferred methodologies. This study had shown significant and unacceptable differences in volatile yields, especially for butadiene and acrylonitrile, and suggested that further work was required to understand the key features and influencing factors on their yield variability. The Task Force reviewed the key features of existing methodologies and further studies were carried out on selected volatiles between 2008 and 2009. These studies investigated the best and necessary features that needed to be incorporated in a recommended method for the analysis of selected smoke volatiles (1,3-butadiene, benzene, toluene, isoprene and acrylonitrile).

These studies had shown that similar yields were obtained when comparing data from Tedlar bag trapping with those from cooled impinger traps, the latter method being used by the majority of laboratories. The Task Force decided that the recommended method would be based on collecting the selected volatiles from cigarette mainstream smoke in cryogenically cooled impinger traps containing methanol. The impinger solutions were spiked with benzene-D6 standard, separated on a gas chromatograph and detected and quantified by mass spectrometry. In the process, several key parts of the methodology were investigated in detail to evaluate their effects on smoke yields and the learning exercise proved to be an important directional step towards a recommended method.

The recommended method was produced through a final collaborative experiment involving 20 laboratories from many countries. The method includes some 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 repeatability and reproducibility data provided. Statistical evaluations were made according to ISO recommendations and are included.

### **1. FIELD OF APPLICATION**

This method is applicable to the quantitation of volatiles (1, 3-butadiene, isoprene, acrylonitrile, benzene, and toluene) in mainstream tobacco smoke using gas chromatographic mass spectrometry (GC/MS).

The described method is specified using ISO 3308: 2000 smoking parameters, but is technically compatible with other smoking regimes.

## 2. REFERENCES

*ISO 3308 : 2000*

Routine analytical cigarette-smoking machine – Definition and standard conditions.

*ISO 8243 : 2003*

Cigarettes – Sampling

*ISO 3402 : 1999*

Tobacco and tobacco products – Atmosphere for conditioning and testing

*ISO 4387 : 2000*

Cigarettes – Determination of Total and Nicotine-free Dry Particulate Matter Using a Routine Analytical Smoking Machine

*ISO 57025-1: 1994-12-15*

Accuracy (trueness and precision) of measurement methods and results – Part 1: General principles and definitions.

*ISO 57025-2: 1994-12-15*

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.

## 3. PRINCIPLE

Selected Volatiles are collected by passing the mainstream smoke of cigarettes\* through a glass fibre filter disc (pad) and into cryogenic traps containing methanol. The impinger solutions are spiked with benzene-D6 and injected onto a gas chromatograph/mass spectrometer (GC/MS) for quantitation.

## 4. APPARATUS

Normal laboratory apparatus and equipment and in particular following items:

- 4.1 A standard smoking machine complying with ISO 3308. In order to trap volatile organic compounds present in the vapour phase of mainstream smoke efficiently a cooled impinger system is needed.
- 4.2. A gas chromatograph - mass spectrometer system to obtain chromatographic data to quantify specific ions (e.g. selected ion mode). The gas chromatograph must be configured to perform split injections on a capillary column. It is recommended to equip the gas chromatograph with an auto sampler for sample injection.
- 4.3. Gas tight syringes
- 4.4. A fused silica capillary column like a DB-624, length 60 m, I.D. 0.25 mm, 1.4 µm film (temperature limits -20 °C to 260 °C) was found to be applicable for the separation of volatile organic compounds.
- 4.5. The necessary general laboratory equipment for the preparation of samples, standards, and reagents. All glassware shall be cleaned before use to avoid any contamination.

## 5. REAGENTS

All reagents shall be of analytical grade quality.

- 5.1. Dry ice
- 5.2. Isopropanol (for Dewar flasks)
- 5.3. Methanol – HPLC grade
- 5.4. Ethanol - reagent grade or equivalent
- 5.5. Benzene-D6 – greater than or equal to 99 % purity
- 5.6. 1,3-Butadiene – 99 % minimum
- 5.7. Isoprene – 99 % minimum
- 5.8. Acrylonitrile – 99 % minimum
- 5.9. Benzene – 99 % minimum
- 5.10. Toluene – 99 % minimum

**Warning notice:** The solvents and chemicals to be used for 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.

## 6. STANDARDS

### 6.1. Preparation of Internal Standard Spiking Solution

#### 6.1.1. Benzene-D6 Stock Solution

Transfer the contents of a 1 g ampoule of Benzene-D6 into a 10 mL amber volumetric flask. Dilute to volume with methanol.

#### 6.1.2 Benzene-D6 Spiking Solution

Using a glass volumetric pipette, transfer 4 mL of the Benzene-D6 stock solution into a 100 mL volumetric flask and dilute to volume with methanol. This solution has a concentration of 4000 µg/mL.

#### 6.1.3 Storage

Store the diluted solutions in 25 mL vials with Teflon-lined caps at -20 °C.

### 6.2. Preparation of Working Standards for Isoprene, Acrylonitrile, Benzene, and Toluene

#### 6.2.1. Primary Isoprene, Acrylonitrile, Benzene and Toluene Stock Solutions

Using gas tight syringes, weigh accurately 100 mg of isoprene, acrylonitrile, benzene, and toluene into separate 10 mL amber volumetric flasks that are half filled with methanol. Dilute each compound to volume with methanol. Each solution contains a concentration of 10,000 µg/mL

**Note:** Approximate volumes corresponding to 100 mg are: isoprene = 150 µL, acrylonitrile = 140 µL, benzene = 130 µL, toluene = 120 µL.

**6.2.2. Secondary Stock Solution (Mixture of isoprene, acrylonitrile, benzene, and toluene primary stock solutions)**

A combined secondary stock solution is prepared by transferring appropriate amounts (see Table 1) of isoprene, acrylonitrile, benzene, and toluene primary stock solutions into a 50 mL volumetric flask that is a third full with methanol. Dilute to volume with methanol.

**Table 1**

<b>Compound</b>	<b>Volume of Primary Stock (mL)</b>	<b>Concentration (µg/mL)</b>
Isoprene	3.0	600
Acrylonitrile	1.0	200
Benzene	1.0	200
Toluene	1.0	200

**6.2.3. Calibration Standard Solutions (for isoprene, acrylonitrile, benzene, and toluene)**

Prepare 7 working standard solutions that cover the concentration range of interest. For example, transfer 200 to 10000 µL of the Secondary Stock Solution into 10 mL amber volumetric flasks and dilute to volume with methanol. These solutions have concentrations of approximately 12 to 600 µg/mL of isoprene; 4 to 200 µg/mL of acrylonitrile; 4 to 200 µg/mL of benzene and 4 to 200 µg/mL of toluene.

Spike each Calibration Standard Solution with 100 µL of the Benzene-D6 Spiking Solution. These solutions have a concentration of approximately 40 µg/mL of Benzene-D6 (ISTD).

**Note: It is best to spike the standards at the same time as the impinger samples are spiked to ensure that there is consistency in the addition of the ISTD. Consistency in pipetting technique will help reduce the variability that may be seen from one sample to another.**

Transfer aliquots of each Calibration Standard Solution into amber GC vials and analyze using the GC/MS. Fill each vial up to the shoulder of the vial to minimize headspace.

Adjust standard concentrations accordingly to reflect levels of volatiles found in smoke samples.

**Note: Concentration ranges recommended can vary depending on whether modified ISO conditions are conducted. The highest concentration found for 3R4F in the 2008 Special Analytes Task Force study was used to determine concentration ranges. For example, 3R4F has maximum concentration levels of 400 µg/mL for isoprene; 25 µg/mL for acrylonitrile; 60 µg/mL for benzene and 90 µg/mL for toluene under ISO conditions. Calculation was done based on smoke from 10 cigs/10 mL methanol.**

**6.2.4. Storage**

Store all Calibration Standard Solutions at -20°C until use.

### 6.3. Preparation of Working Standards for 1,3-Butadiene

#### 6.3.1. Primary 1,3-Butadiene Stock Solution

Attach a piece of Tygon tubing to the valve of a 1,3-butadiene cylinder. Place a Pasteur pipette on the other end of the tubing and immerse the tip of the pipette into a 100 mL volumetric flask containing methanol up to the base of the neck of the flask. Open the valve and gently bubble the 1,3-butadiene into the methanol for approximately 5 minutes. Dilute to volume using methanol and mix well.

#### 6.3.2. Secondary 1,3-Butadiene Stock Solution

Pipette 1 mL of the primary 1,3-butadiene stock solution into a 100 mL volumetric flask and dilute to volume with methanol. Mix well.

#### 6.3.3. Determination of Secondary 1,3-Butadiene Stock Concentration

Pipette 1 mL of the Secondary 1,3-butadiene Stock Solution into a 100 mL volumetric flask and dilute to volume using ethanol. This solution is used only to check the concentration of the secondary stock solution and must not be used to prepare the working standards.

Measure the absorbance of the solution against an ethanol blank on a spectrophotometer. Conduct a wave scan at 200 nm to 250 nm to determine the wavelength of maximum absorbance. 1,3-butadiene in hexane absorbs at 217 nm whereas 1,3-butadiene in ethanol may have a peak shift. Measure the absorbance at the peak maximum.

Repeat the above measurement three more times and calculate the average absorbance ( $A$ , at least three significant figures). The absorbance should be between 0.2 and 0.6 extinction units. If it is higher, make a new secondary stock solution using a smaller volume of the primary stock solution and repeat the spectrophotometer measurements to determine the concentration of the secondary stock. If the absorbance is lower, make a new secondary stock solution using a larger volume of the primary stock solution and repeat the spectrometer measurements to determine the concentration of the secondary stock.

The concentration of the secondary stock solution is calculated as follows:

$$\text{Conc.}(\mu\text{g} / \text{mL}) = \frac{A}{20893 \text{ L} / \text{mol}} \times 54 \text{ g} / \text{mol} \times \frac{1000 \text{ mg} / \text{g}}{1000 \text{ mL} / \text{L}} \times \frac{100 \text{ mL}}{1 \text{ mL}} \times 1000 \mu\text{g} / \text{mg}$$

Where  $20893 \text{ L} / \text{mol}^{-1} / \text{cm}^{-1}$  represents the molar absorption coefficient of 1,3-butadiene.

#### 6.3.4. 1,3-Butadiene Calibration Standard Solutions

Prepare 5 working standard solutions that cover the concentration range of interest. For example, transfer 1000 to 10000  $\mu\text{L}$  of the Secondary Stock Solution into 10 mL amber volumetric flasks and dilute to volume with methanol. These solutions have concentrations of approximately 5 to 50  $\mu\text{g} / \text{mL}$  of 1,3-butadiene.

Spike each Calibration Standard Solution with 100  $\mu\text{L}$  of the Benzene-D6 Spiking Solution. These solutions have a concentration of approximately 40  $\mu\text{g} / \text{mL}$  of Benzene-D6.

**Note:** It is best to spike the standards at the same time the impinger samples are spiked to ensure that there is consistency in the addition of the ISTD. Consistency in pipetting technique will help reduce the variability that may be seen from one sample to another.

Transfer aliquots of each Calibration Standard Solution into amber GC vials and analyze using the GC/MS. Fill each vial up to the shoulder of the vial to minimize headspace.

**Note:** Certified concentrations of 1,3-butadiene in methanol can be purchased and used to prepare the standards.

#### 6.3.5. Storage

Store all Calibration Standard Solutions at -20°C until use.

**All Standard Solutions for all components should be checked for stability before use as stability is depending on storage conditions.**

## 7. SAMPLE PREPARATION

### 7.1. Sampling and Conditioning

Sampling is done in accordance with ISO 8243: 2003.

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

### 7.2. Smoking procedure

#### 7.2.1. Smoking Machine Setup

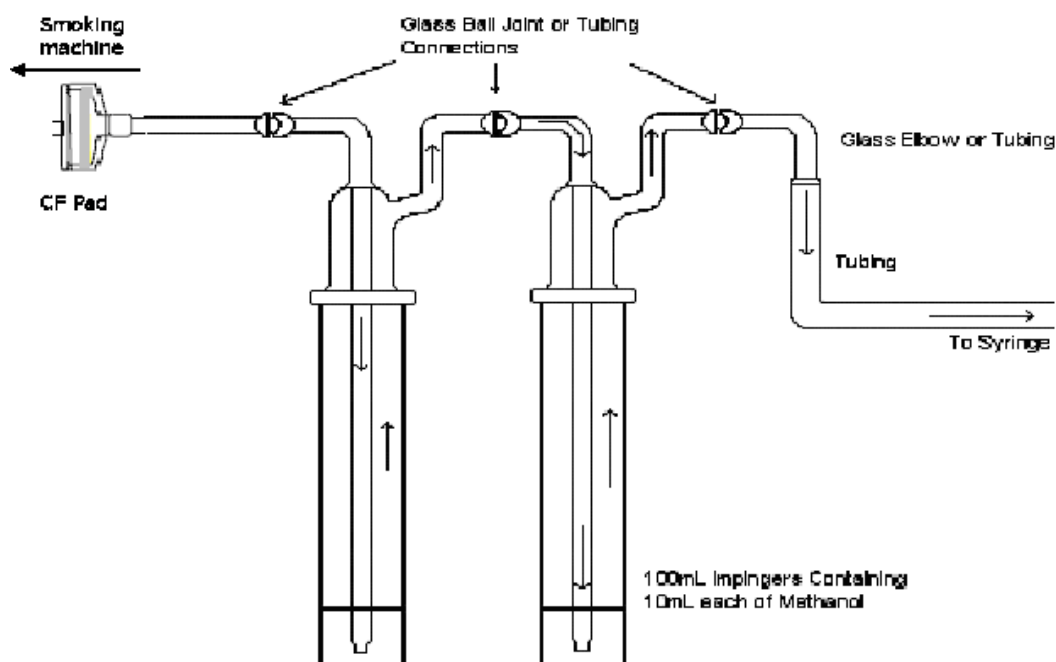
A routine analytical cigarette-smoking machine complying with the requirements of ISO 3308: 2000 is required but additionally equipped with a liquid trapping system as described in figure 1.

Fill all Dewar flasks one-third full with isopropanol and add dry ice until each flask is filled halfway. The number of flasks required is dependent on the impinger design and has to be optimized to ensure all volatiles are trapped effectively.

Add 10 mL of methanol to each impinger and place the impingers into the Dewar flasks containing the dry ice / isopropanol solution. Check each Dewar flask to ensure that the temperature is at or below -70 °C.

Hook up the impingers to the smoking machine. See Figure 1 for a typical impinger setup on the smoking machine.

**Figure 1** – Example of an Impinger Setup for Smoking Machines



### 7.2.2. Smoking

Cigarettes are smoked according to ISO 4387: 2000 with the following modifications:

#### Linear Smoking

- Check the puff volume of each port and adjust accordingly.
- Typically five or ten cigarettes are smoked per trap. Please note that if ten cigarettes are smoked then the Cambridge filter (CF) pad needs to be changed after five cigarettes to avoid total particulate matter (TPM) breakthrough.

#### Rotary Smoking

- Check the puff volume of the rotary smoking machine and adjust accordingly.
- Typically ten cigarettes are smoked per trap.

**Note:** To determine whether a leak has occurred in the smoking machine impinger setup, use a bubble meter. If the bubble does not maintain its position at the baseline and drops before puffing then there is a leak in the system.

It is recommended that tubing other than silicone tubing be used for connections between the smoking machine and the impingers (i.e. polyethylene, polyvinyl chloride (e.g. Tygon), polypropylene). Methyl silicone tubing is not recommended since adsorption of the analytes can occur. Tubing should be as short as possible to minimize the potential for any adsorption.

It is recommended that the trapping efficiency be checked when validating this method. To check the trapping efficiency of the method add a third impinger and follow the method accordingly. Analyse each impinger 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.

### 7.2.3. Final sample preparation

After all samples have been smoked, the TPM should be determined on the CF as quality control measure and the pad discarded. Connecting tubes to CF pad and impingers should be rinsed by using trapping solutions. It is good practice to rinse connecting tubes and as quickly as possible to avoid any loss of analytes. It is recommended that the trapping solution remains at the cold trap temperature at all times.

After all samples have been smoked each impinger is spiked with 100 µL of Benzene-D6 Spiking Solution. The impingers are stoppered and vortexed to ensure that the ISTD is well mixed. Then the trapping solutions are combined in such a way to ensure complete mixing of both impingers. The impingers should be kept in the Dewar flasks until sampling is complete. Transfer an aliquot of the combined impinger solutions into amber GC vial and analyze for volatiles using the GC/MS. Fill each vial up to the shoulder of the vial to minimise headspace and cap tightly. Prepare all samples in duplicate and keep a set in the freezer for re-injection purposes.

### 7.2.4. Storage

Samples are stable nominally at -20°C for at least 48 hours.

## 8. DETERMINATION

### 8.1. GC/MS operating conditions

Set up and operate the GC/MS system in accordance with the manufacturer's instruction.

The following parameters are found to be suitable for separation:

Injector temperature: 150 °C  
Column temperature: 40 °C hold for 6.0 minutes  
20 °C per minute to 225 °C hold for 6.0 minutes  
Injection mode: Split  
Injection split ratio: 30:1  
Injection split flow: 30 mL/min  
Injection volume: 3.0 µL

MS Parameters:

GC/MSD transfer line temperature: 240 °C  
MS-Source temperature: 240 °C  
Acquisition mode: SIM (or SCAN)  
*Solvent delay: Depends on column using*  
*Low mass: 40.0*  
*High mass: 200.0*

Ion Traces:	Quantification	Confirmation
1,3-Butadiene	54	53
Isoprene	67	68
Acrylonitrile	52	53
Benzene	78	77
Benzene-D6	84	83
Toluene	91	92

The choice of chromatographic conditions and quantitation ions may be different for different instrument configurations.

## 8.2. Calibration

Inject successively each working standard solution (6.2.3. and 6.3.4) in the GC-MS system. Record the area of each of the analysed compounds and the internal standard peaks. A calibration curve for each of the compounds is generated by calculating a linear equation regression of the area ratios of the analysed compounds to the internal standard peaks in function of the concentration. The intercept of these regression lines should be close to zero.

## 8.3. Calculation

The results are reported in µg/cig. To calculate the final results, the following calculation is used:

$$\text{Analyte } (\mu\text{g / cigarette}) = \frac{\text{Conc. of Analyte in Sample } (\mu\text{g / mL}) \times \text{Volume (mL)}}{\text{Number of Cigarettes}}$$

## 9. REPEATABILITY AND REPRODUCIBILITY

These were determined from a major international study involving 20 laboratories and eight cigarette samples including the reference cigarettes Ky3R4F and Ky1R5F (both available from the University of Kentucky) and the CORESTA Monitor Test CM 6. Smoke analysis was carried out in 2009 on these cigarettes that covered a wide range of blends and constructions. The statistical evaluation of the data from this collaborative study was conducted according the ISO 57025 part 1 and part 2.

### 9.1. Explanation of Outliers and Data Analysis for Selected Volatiles Collaborative Study

Calculations followed according to ISO 5725-2: 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.

In the case of this Selected Volatiles study, ISO 5725-2 can be applied directly in determining estimates of r and R since the data was collected under repeatability and reproducibility conditions as defined by ISO 5725-1 (i.e. the participating laboratories all used the same method as opposed to their own internal method, thus satisfying the reproducibility condition).

### 9.2. Data Removal

Individual data points that were reported as non-numeric (i.e. below the LOQ) had to be removed prior to evaluation of numeric data for outliers.

### 9.3. Among-Laboratory Variability

For evaluation of agreement among laboratories, the Grubbs' test was applied as per ISO 5725-2 section 7.3.4 to detect single or multiple outlying laboratories.

If the Grubbs statistic was significant at the 5% level, the sample data from that laboratory(s) was removed.

In case the Grubbs statistic for a single high outlier was significant for certain analytes, samples and laboratories the data were removed prior to the calculations of the general mean, repeatability and reproducibility variance.

#### 9.4. Determination of Inter-Laboratory Repeatability and Reproducibility Variance

For each of the  $j$  test samples ( $j = 1, \dots, 8$ ), the general mean ( $m_j$ ) was determined as per ISO 5725-2 section 7.4.4 across the  $p$  participating laboratories whose data remained following the removal of outliers.

Repeatability variance ( $sr_j$ ) was determined as per ISO 5725-2 section 7.4.5.1 and reproducibility variance ( $sR_j$ ) was determined as per ISO 5725-2 section 7.4.5.5.

Repeatability ( $r$ ) and Reproducibility ( $R$ ) figures calculated for 95% confidence level were also indicated for the individual compounds and sample cigarettes.

#### 9.5. Results

Calculated statistical data of the individual selected volatile compound are indicated in the tables below.

1,3 Butadiene [ $\mu\text{g}/\text{cigarette}$ ]						
Sample $j$	# laboratories	$m_j$	$sr_j$	$r$	$sR_j$	$R$
1	19	33.3	4.2	11.8	8.9	25.0
2	19	32.1	3.9	11.0	8.9	25.0
3	18	32.5	4.8	13.3	8.0	22.4
4	19	7.53	1.75	4.89	2.49	6.98
5	19	39.0	3.9	10.9	10.0	28.0
CM 6	18	60.3	7.6	21.2	13.5	37.9
Ky 1R5F	19	12.2	1.9	5.3	2.9	8.2
Ky 3R4F	19	41.4	4.7	13.3	10.6	29.6
% Reported	95%					
% Removed	1.3%					

Isoprene [ $\mu\text{g}/\text{cigarette}$ ]						
Sample $j$	# laboratories	$m_j$	$sr_j$	$r$	$sR_j$	$R$
1	20	216	16	46	32	89
2	20	256	17	48	39	108
3	20	245	15	43	36	102
4	19	58	5	15	11	31
5	20	281	18	52	44	122
CM 6	20	553	35	98	71	198
Ky 1R5F	20	120	14	38	27	74
Ky 3R4F	20	362	21	57	48	134
% Reported	100%					
% Removed	0.6%					

Acrylonitrile [ $\mu\text{g}/\text{cigarette}$ ]						
Sample j	# laboratories	$m_j$	$sr_j$	$r$	$sR_j$	$R$
1	19	10.2	0.9	2.4	1.5	4.2
2	18	5.32	0.62	1.74	0.96	2.67
3	19	7.48	0.69	1.92	1.23	3.45
4	15	0.98	0.13	0.37	0.39	1.08
5	19	6.34	0.62	1.74	1.10	3.08
CM 6	19	12.3	1.2	3.2	2.1	5.9
Ky 1R5F	18	2.10	0.25	0.69	0.45	1.27
Ky 3R4F	19	8.56	0.75	2.11	1.27	3.56
% Reported	96%					
% Removed	5.5%					

Benzene [ $\mu\text{g}/\text{cigarette}$ ]						
Sample j	# laboratories	$m_j$	$sr_j$	$r$	$sR_j$	$R$
1	20	38.6	2.6	7.2	5.2	14.6
2	20	31.6	2.4	6.7	4.7	13.1
3	20	34.9	2.1	5.9	4.9	13.7
4	19	6.73	0.71	1.98	1.68	4.69
5	20	28.5	2.0	5.7	4.9	13.7
CM 6	20	60.3	3.8	10.6	7.5	21.0
KR 1R5F	19	14.3	0.9	2.5	2.6	7.2
KR 3R4F	20	41.9	2.2	6.3	5.5	15.3
% Reported	100%					
% Removed	1.3%					

Toluene [ $\mu\text{g}/\text{cigarette}$ ]						
Sample j	# laboratories	$m_j$	$sr_j$	$r$	$sR_j$	$R$
1	20	57.8	4.7	13.3	9.7	27.3
2	20	44.5	4.4	12.4	7.4	20.7
3	20	49.3	3.5	9.7	8.4	23.5
4	20	8.53	1.26	3.53	3.54	9.93
5	20	37.4	3.5	9.7	7.3	20.5
CM 6	20	85.4	6.9	19.4	14.6	41.0
KR 1R5F	19	18.5	1.7	4.7	3.5	9.9
KR 3R4F	20	64.8	4.4	12.2	11.0	30.8
% Reported	100%					
% Removed	0.6%					

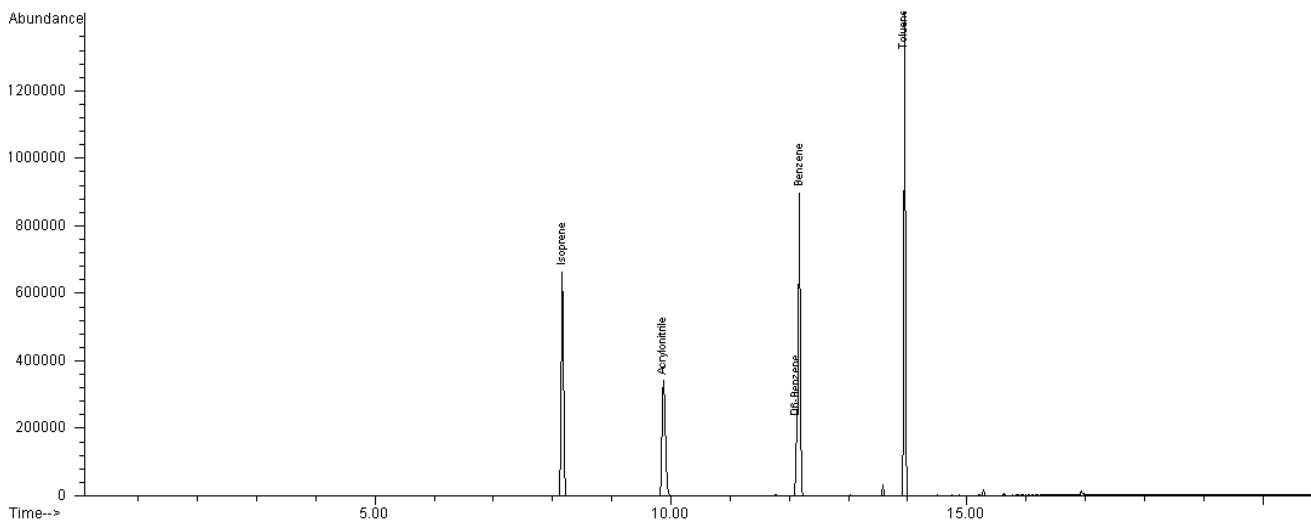
## 10. REPORT

### 10.1. Test results

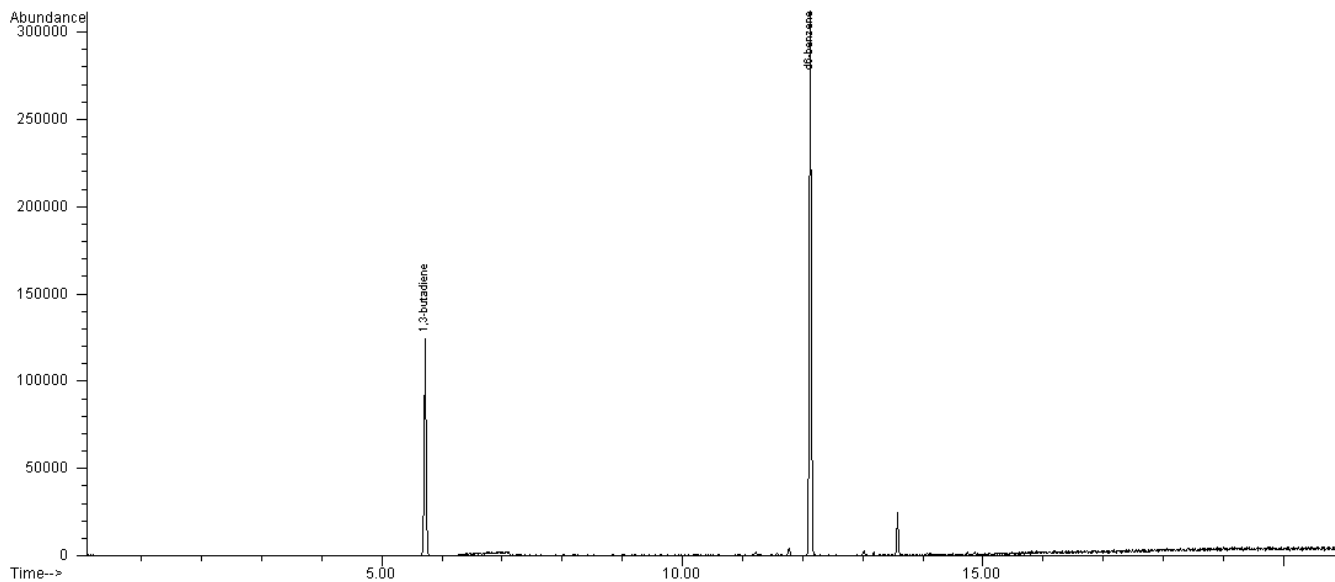
The expression of the laboratory data depends on the purpose for which the data are required, and the level of laboratory precision. Confidence limits shall be calculated and expressed on the basis of the laboratory data before any rounding has taken place.

The amount of individual selected volatile compounds in the mainstream smoke of cigarettes is reported in  $\mu\text{g}/\text{cig}$  to the nearest 0.1  $\mu\text{g}$ .

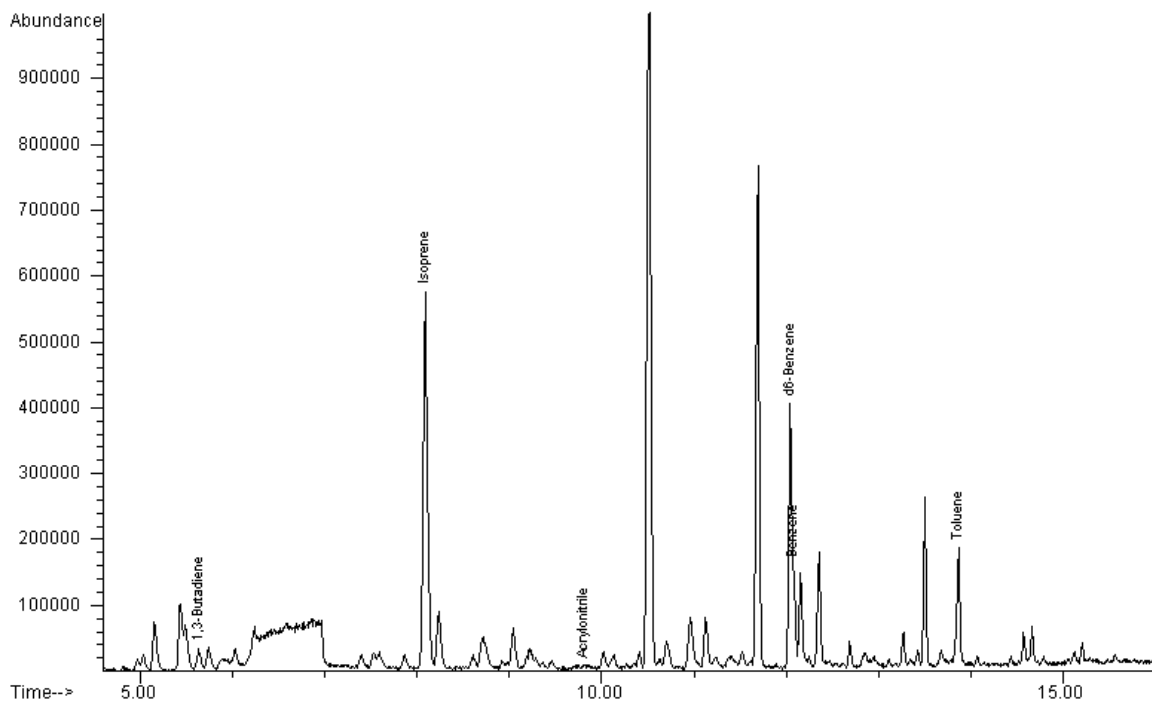
**Annex 1: Chromatogram of a Typical Calibration Standard for Toluene, Isoprene, Benzene and Acrylonitrile**



**Annex 2: Chromatogram of a Typical Calibration Standard for 1,3-butadiene**



### Annex 3: Chromatogram of 1R5F Kentucky Reference



### Annex 4: Chromatogram of 3R4F Kentucky Reference

