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Analysis of volatiles and semi-volatiles in Platform 1 Aerosol

PRODUCT TESTING LABORATORY AND GOVERNANCE

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1. ANALYSIS OF VOLATILES AND SEMI-VOLATILES IN PLATFORM 1 AEROSOL

1.1. Abstract

The aerosols are generated on a linear smoking machine and collected using Cambridge filter pad (CFP) followed by two micro impingers at 0°C and -70°C respectively, filled with 10 mL of methanol. The content of the CFP is then combined and extracted with the content of the impingers and delivered to the analytical laboratory. After filtration, the internal standards are added to the samples.

The extracts are then analyzed by Gas Chromatography with Mass Spectrometry detection (GC-MS) using a column DB-WAXETR, 30m x 0.25mm ID x 0.5µm (film thickness).

Results are expressed as µg/item for P1.

1.1. Applicability

The method described is used to determine five volatile compounds (1,3-butadiene, isoprene, benzene, acrylonitrile and toluene) and four semi-volatile compounds (pyridine, styrene, naphthalene and quinoline) in aerosol from Platform 1 (P1) under Health Canada (HC) and International Organization for Standardization (ISO) smoking conditions, as well as under alternative smoking regimens.

1.1. Reagents

- Benzene
- 1,3-butadiene standard
- \Isoprene
- Acrylonitrile
- Toluene
- Pyridine
- Styrene
- Naphtalene
- Quinoline
- Benzene-d6
- Naphtalene-d8
- Quinoline-d7
- Pyridine-d5
- Styrene-d8
- Methanol
- Helium

1.2. Aerosol generation

P1 items are conditioned in climatized chamber for at least 48 hours at target conditions of 22 ± 1°C and relative humidity of 60 ± 3% before used for aerosol generation.

Cambridge filters are conditioned for at least 12 hours at target conditions of 22 ± 1°C and relative humidity of 60 ± 3% before used for aerosol generation.

The aerosol samples are generated on a linear smoking machine under ISO and HC smoking regimens and collected using a Cambridge filter pad and two micro impinger filled with 10 mL of methanol. The first impinger is immersed into a water-ice bath at 0°C, while the second one is immersed into a dry ice-isopropanol bath at about -70°C. The collection conditions for the different smoking regimes are summarized in Table **1**.

At the end of the smoking process, the glass fiber Cambridge filter pad is combined with the extraction solution of the two impingers and sent to the analytical lab for analysis.

Four replicates for each sample are generated. Two blanks are smoked each smoking day to ensure that no contamination is carried out. The first blank is smoked before the first aerosol collection, while the second one is smoked at the end of the smoking day.

Table 1: Aerosol Collection Condition

Regimen	Accumulation number	Puff number	Regimen Condition [puff volume/Puff duration/Puff Interval] [ml/s/s]
ISO	5	6	35/2/60
HC	5	12	55/2/30

1.3. Samples preparation

The samples are shaked for at least 10 minutes before to be filtered by a disposable syringe through a 0.45 µm disposable filter. Successively, 1000 µL of sample are mixed with 50 µL of internal standard solution into a glass vial before to be analyzed.

1.4. Internal standard solution

A 2 mg/mL benzene-d6 solution is prepared by dissolution of the deuterated compound in methanol. Similarly, a 0.4 mg/mL naphthalene-d8, 2 mg/mL pyridine-d5, 0.48 mg/mL styrene-d8 and 0.4 mg/mL quinolone-d7 solution is prepared by dissolution of the deuterated compounds in methanol.
The internal standard solution is then prepared by mixing equal amounts of these two solutions and diluting with methanol.

1.5. Stock solutions preparation

A 0.4 mg/mL naphthalene, 7 mg/mL pyridine, 4.8 mg/mL toluene, 0.4 mg/mL quinolone and 0.6 mg/mL styrene solution is prepared by dissolution of the compounds in methanol. Similarly, a 2.6 mg/mL benzene and 4 mg/mL acrylonitrile solution and a 16 mg/mL isoprene solution are prepared by dissolution of the compounds in methanol.
The standard mix solution is then prepared by mixing aliquots of these three solutions with an aliquot of 12 mg/mL 1,3-butadiene standard solution and diluted in methanol.

1.6. Calibration solutions preparation

Calibration standards (STD) 3 to 6 are prepared by dilution of the standard mix solution in methanol. Calibration standard 1 and 2 are prepared by dilution of calibration standard 6 in methanol. The ranges of concentrations cover the ranges relevant for analysis and are provided in Table 2. Two standard levels 1 are prepared: one for analysis of Volatile Organic Compounds (STD1 VOC) and the other one for analysis of Semi Volatile organic Compounds (STD1 SVC).

Table 2: Volatile and semi-volatile compounds calibration standards typical concentrations

	STD1 SVC (µg/mL)	STD1 VOC (µg/mL)	STD2 (µg/mL)	STD3 (µg/mL)	STD4 (µg/mL)	STD5 (µg/mL)	STD6 (µg/mL)
1,3-butadiene	0.024	0.048	0.095	0.950	2.376	3.802	4.752
Isoprene	0.040	0.080	0.160	1.600	4.000	6.400	8.000
Benzene	0.007	0.013	0.026	0.260	0.650	1.040	1.300
Acrylonitrile	0.010	0.020	0.040	0.400	1.000	1.600	2.000
Toluene	0.012	0.024	0.048	0.480	1.200	1.920	2.400
Pyridine	0.018	0.035	0.070	0.700	1.750	2.800	3.500
Styrene	0.002	0.003	0.006	0.060	0.150	0.240	0.300
Naphtalene	0.001	0.002	0.004	0.040	0.100	0.160	0.200
Quinoline	0.001	0.002	0.004	0.040	0.100	0.160	0.200

The standard level 4 is also used as quality check.

1.7. Instrumental Conditions

The samples are analyzed by Gas Chromatography (GC) with mass spectrometry detection (MS) following tables below:

Table 3: Instrumental Conditions for Determination of volatile and semi-volatile compounds

	VOC	SVC
Column	DB-WAXETR, 30m x 0.25mm ID x 0.5µm (film thickness)	
Total program time	15.50 min	27.67 min
Injection	1µL	
Injection temperature	250 °C	
Column temperature	40 °C	
Column flow	1.49 mL/min	
Tansfer line temperature	240 °C	
Ion source temperature	230 °C	
Gas	Helium	

Table 4: GC temperature Program

	Rate (°C/min)	Final Temperature (°C/min)	Hold Time (min)
VOC analysis	NA	40	2.5
	30	250	6
SVC analysis	NA	40	2
	15	150	0
	30	250	15

1.8. Testing procedure

The following typical analytical sequence, is used for the determination of VOC and SVC:

- Methanol;
- Calibration curve (STD 1 to 6)
- Two methanol injections
- smoked blanks
- Samples
- After every 5 samples, inject a quality check (STD level 4)

- Always end an analytical sequence with a quality check and a methanol injection

1.9. Verification of results

1.9.1. Calibration curve verification

A calibration curve is used to quantify the unknown samples using the response ratio of analyte to the internal standard. The peak area ratio is applied to generate the curve. The linear regression is calculated automatically by the GCMS solution software. Specific information about regressions are provided in Table 5:

Table 5: Parameters used for calibration curves

Compound	Internal standard	Regression type	Weighting factor
1,3-butadiene	Benzene-d6	linear	$1/x^2$
Isoprene	Benzene-d6	linear	$1/x^2$
Benzene	Benzene-d6	linear	$1/x^2$
Acrylonitrile	Benzene-d6	linear	$1/x^2$
Toluene	Benzene-d6	linear	$1/x^2$
Pyridine	Pyridine-d5	linear	$1/x^2$
Styrene	Styrene-d8	linear	$1/x^2$
Naphtalene	Naphtalene-d8	linear	$1/x^2$
Quinoline	Quinoline-d7	linear	$1/x^2$

1.9.2. Quality check

The validity of the calibration is continuously verified during the batch analysis by ensuring that the injected quality check (STD level 4) is within ±15% of its theoretical value.

1.10. Example Chromatograms

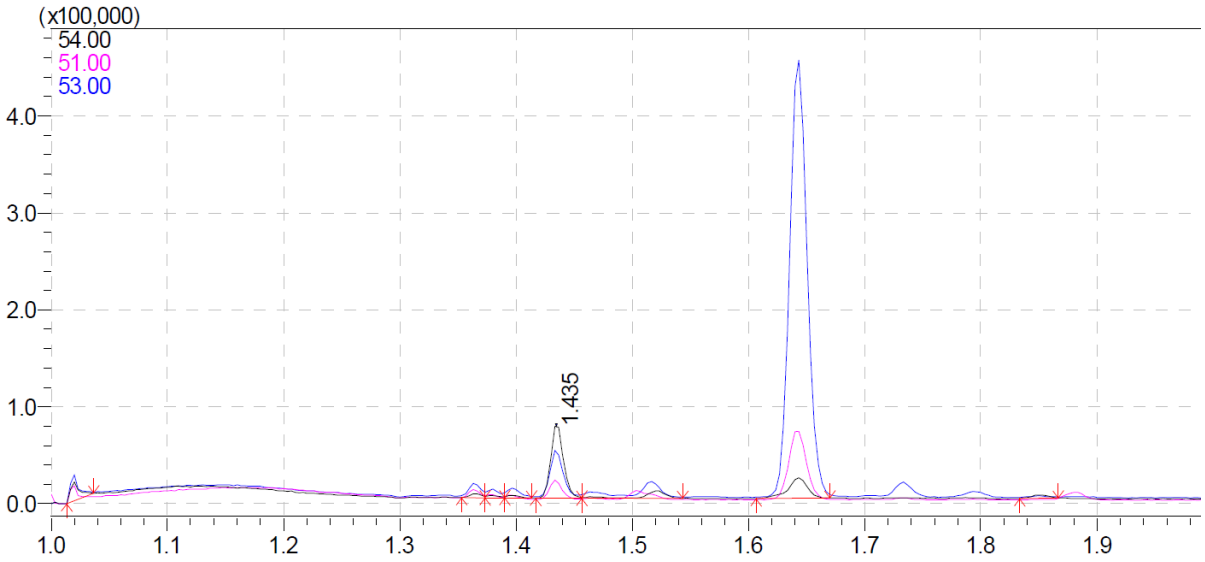

Figure 1: Extracted ion chromatograms for 1,2-butadiene in P1 sample

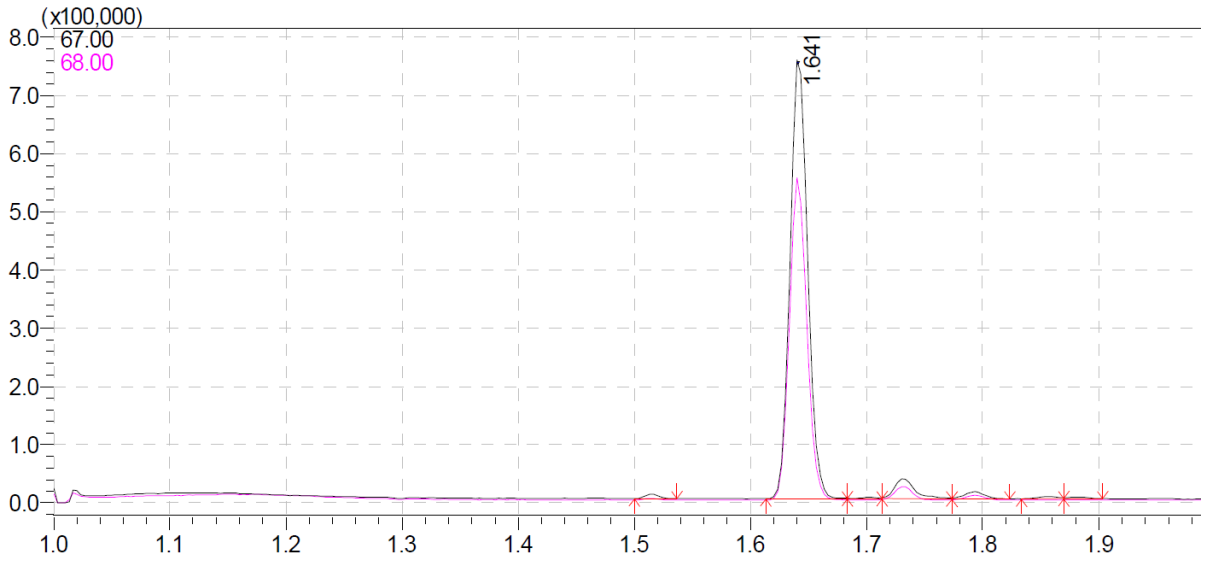

Figure 2: Extracted ion chromatograms for isoprene in P1 sample

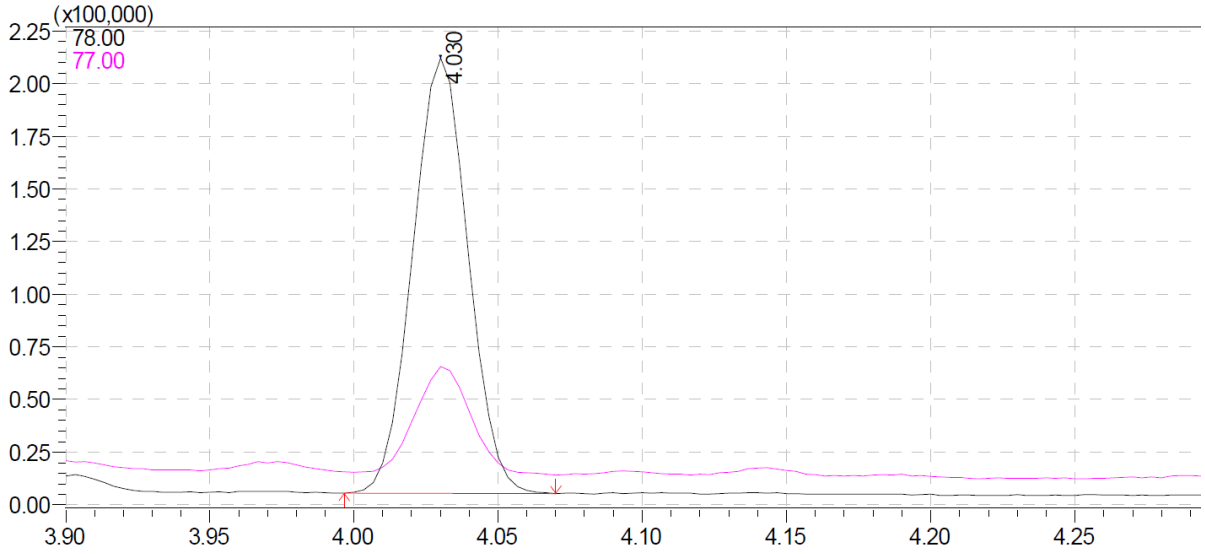

Figure 3: Extracted ion chromatograms for benzene in P1 sample

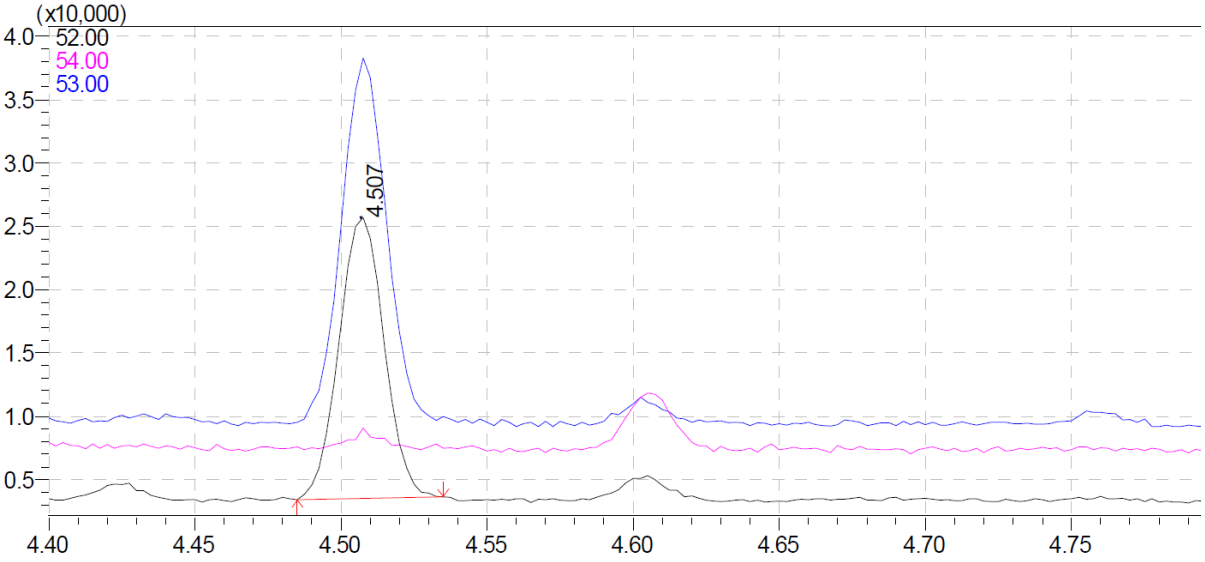

Figure 4: Extracted ion chromatograms for acrylonitrile in P1 sample

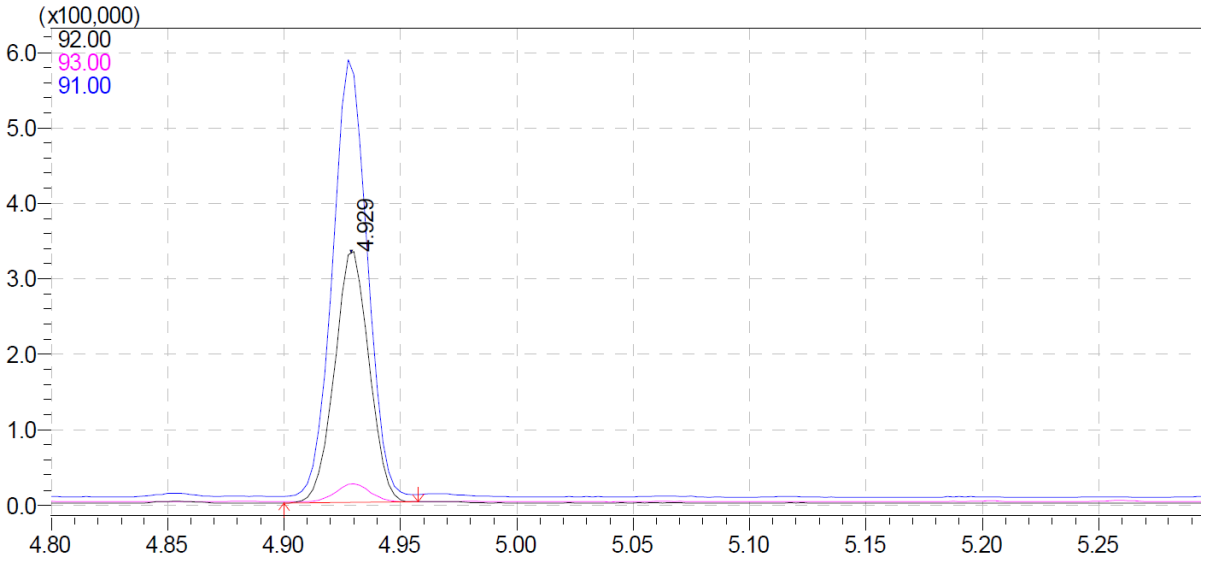

Figure 5: Extracted ion chromatograms for toluene in P1 sample

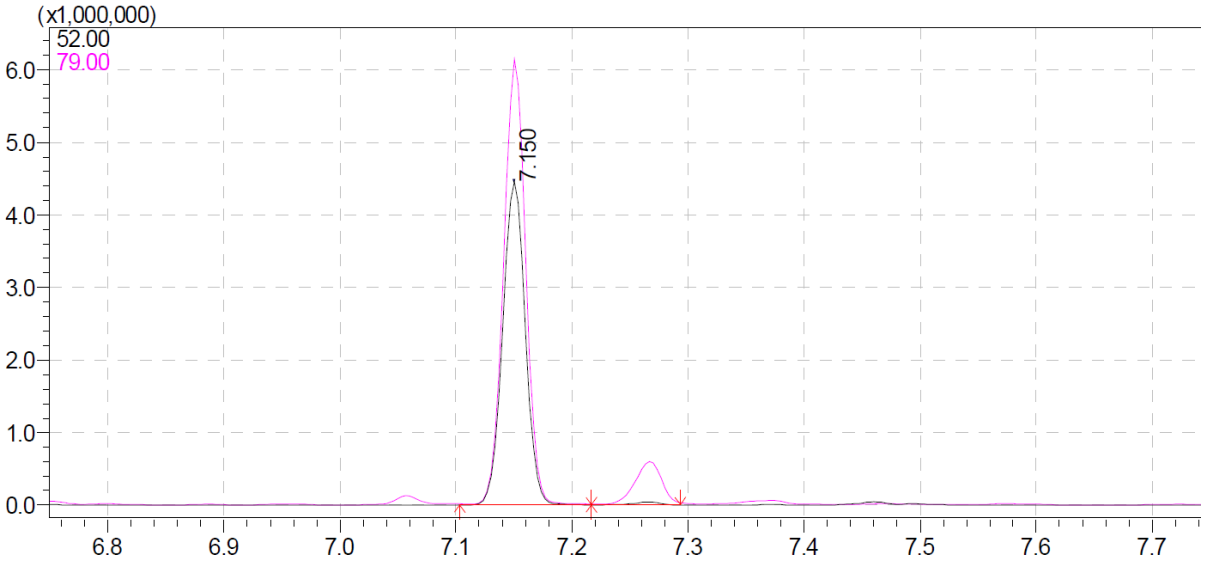

Figure 6: Extracted ion chromatograms for pyridine in P1 sample

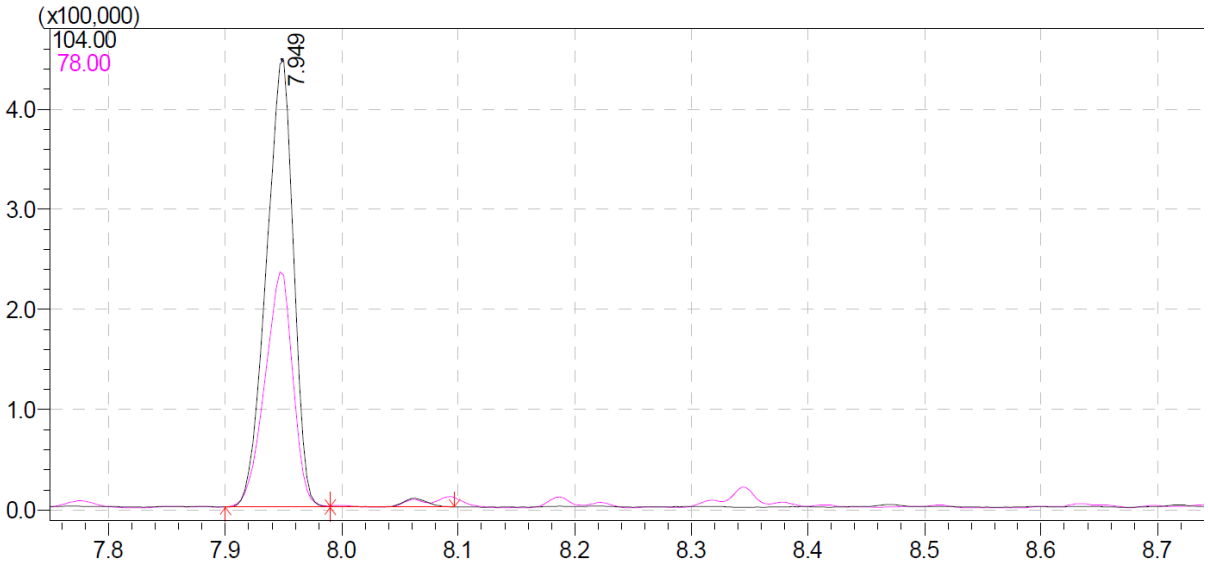

Figure 7: Extracted ion chromatograms for styrene in P1 sample

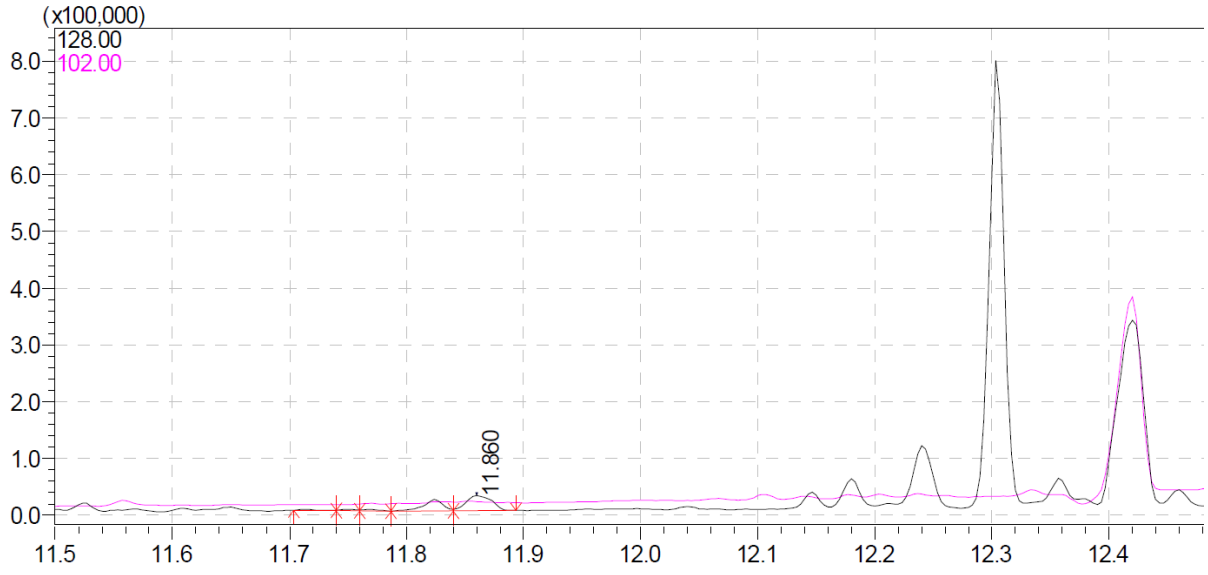

Figure 8: Extracted ion chromatograms for naphtalene in P1 sample

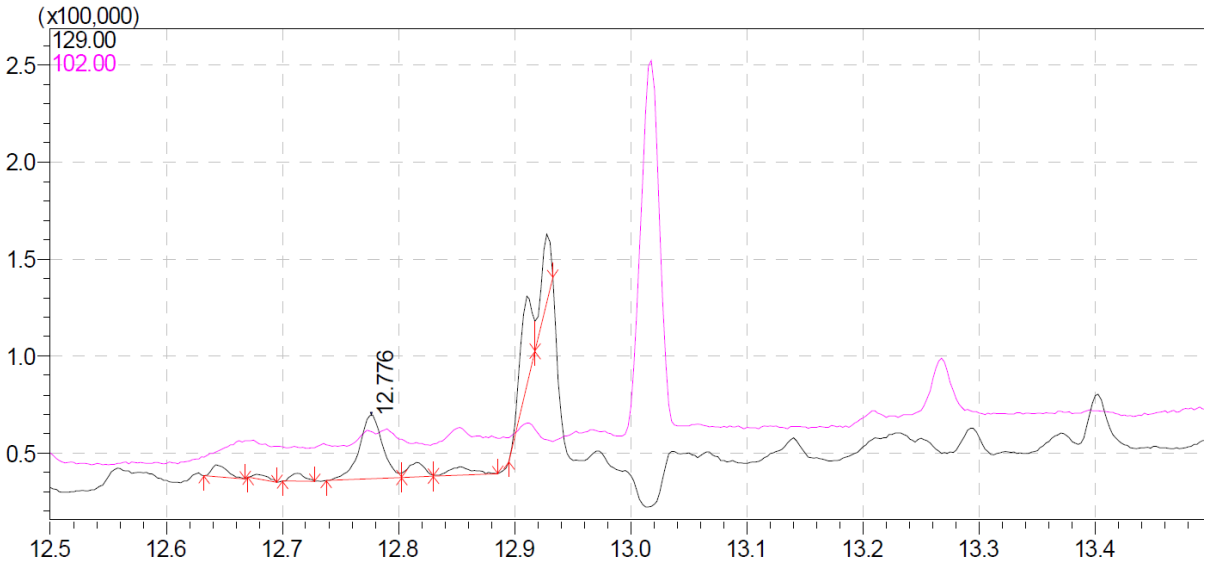

Figure 9: Extracted ion chromatograms for quinoline in P1 sample

1.11. Limit of Detection (LOD) / Lower Limit of Quantitation (LLOQ)

The LOD and LLOQ were both calculated in terms of the standard deviation of five different measurements of the lowest calibration standard (STD1 VOC for volatile compounds and STD1 SVC for semi-volatile compounds) for all other analytes produced under intermediate precision conditions (five different preparations from at least two different operators and analyzed on five different days).

$$LOD = 3 \times SD_{level1}$$

$$LLOQ = 10 \times SD_{level1}$$

Level 1 is the lowest calibration level.

Detailed results are provided in Table 6.

Table 6: Limits of Detection and Quantitation (HC and ISO regimens)

Compound	P1, HC smoking regimen			P1, ISO smoking regimen		
	LOD	LLOQ	STD 1	LOD	LLOQ	STD 1
	(µg/item)	(µg/item)	(µg/item)	(µg/item)	(µg/item)	(µg/item)
1,3-butadiene	0.013	0.045	0.190	0.020	0.067	0.196
Isoprene	0.011	0.037	0.328	0.021	0.070	0.353
Benzene	3.86E-03	0.013	0.048	3.79E-03	0.013	0.050
Acrylonitrile	0.015	0.049	0.074	9.17E-03	0.031	0.086
Toluene	8.20E-03	0.027	0.096	0.020	0.066	0.104
Pyridine	4.34E-03	0.014	0.069	6.61E-03	0.022	0.069
Styrene	1.44E-04	4.81E-04	5.67E-03	3.03E-04	1.01E-03	6.51E-03
Naphtalene	4.89E-04	1.63E-03	3.80E-03	3.85E-04	1.28E-03	3.88E-03
Quinoline	6.71E-04	2.24E-03	4.51E-03	7.57E-04	2.52E-03	4.26E-03

1.12. Repeatability limit (r) and Intermediate precision limit (IP)

$$r = 2 \cdot \sqrt{2} \cdot s_r$$

$$IP = 2 \cdot \sqrt{2} \cdot s_{IP}$$

s_r is the standard deviation of repeatability.

s_{IP} is the standard deviation of intermedidate precision.

For ISO regimen, repeatability limit is determined within a single smoking day using four different replicates.

For HC regimen, repeatability limit and intermediate precision limit are determined over three smoking days using different smoking machines, working standard preparations,analytical columns and instruments. Different operators are involved in the aerosol generation and analytical tests.

Table 7: Repeatability r and Intermediate precision IP (HC and ISO regimens)

Compound	P1, HC smoking regimen			P1, ISO smoking regimen		
	r	IP	Mean	r	IP	Mean
	(µg/item)	(µg/item)	(µg/item)	(µg/item)	(µg/item)	(µg/item)
1,3-butadiene	0.10	0.20	0.31	0.079	NA	0.220
Isoprene	0.50	0.55	2.15	1.09	NA	2.01
Benzene	0.09	0.11	0.67	0.097	NA	0.422
Acrylonitrile	0.04	0.05	0.20	0.020	NA	0.128
Toluene	0.47	0.74	2.25	0.605	NA	1.64
Pyridine	1.28	2.01	10.80	1.16	NA	6.45
Styrene	0.12	0.16	0.73	0.176	NA	0.581
Naphtalene	0.01	0.01	0.02	2.39E-03	NA	9.12E-03
Quinoline	0.01	0.03	0.03	1.84E-03	NA	5.73E-03

1.13. NORMATIVE REFERENCES

- ISO 3308:2000 – Routine analytical cigarette smoking machine – definitions and standard conditions
- ISO 3402:1999 – Tobacco and tobacco products – atmospheres for conditioning and testing