

Analysis of 12 polycyclic aromatic hydrocarbons in Platform 1 aerosol

PRODUCT TESTING LABORATORY AND GOVERNANCE

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1. ANALYSIS OF 12 POLYCYCLIC AROMATIC HYDROCARBONS IN PLATFORM 1 AEROSOL

1.1. Abstract

The aerosol samples are generated with a linear smoking machine and collected on a Cambridge filter pad. Polycyclic aromatic hydrocarbons (PAHs) are then extracted with hexane after addition of internal standards. The aerosol extract undergoes a two steps solid-phase extraction (SPE) clean-up procedure in order to remove some matrix co-extracted organic compounds that reduce the performance of the chromatographic system and affect the accuracy of PAHs determination.

The resulting extracts are analyzed by Gas Chromatography with Electron Impact Mass Spectrometry (GC-EI-MS). The GC system is equipped with a column DB-17MS column, 30m x 0.25mm ID x 0.15µm (film thickness).

Results are expressed as ng/item for P1.

1.2. Applicability

The method described is used for the determination of 12 polycyclic aromatic hydrocarbons in platform 1 (P1) aerosols generated under ISO and Health Canada (HC) conditions, as well as under alternative smoking regimens.

1.3. Reagents

- Pyrene-d10
- Dibenz(a,i)pyrene-d14
- PAH-MIX 156, containing benz(a)anthracene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, benz(a)pyrene, indeno(123cd)pyrene, dibenz(a,h)anthracene, dibenz(a,l)pyrene, dibenz(a,e)pyrene, dibenz(a,i)pyrene and dibenz(a,h)pyrene
- PAH-MIX 121: benz(a)anthracene-d12, benzo(b)fluoranthene-d12, benz(a)pyrene-d12, indeno(123cd)pyrene-d12 and dibenz(a,h)anthracene-d14
- 1-Octanol
- 1-Propanol
- Isooctane
- Hexane
- Acetonitrile
- Toluene
- Deionized water
- 1-Butanol
- Poly(ethylene glycol)
- Diethyl ether

1.4. Aerosol generation

P1 items are conditioned in opened pack for at least 48 hours at target conditions of $22 \pm 1^\circ\text{C}$ and relative humidity of $60 \pm 3\%$ before to be used for aerosol generation.

Cambridge filters are conditioned for at least 12 hours at target conditions of $22 \pm 1^\circ\text{C}$ and relative humidity of $60 \pm 3\%$ before to be used for aerosol generation.

The aerosol samples are generated on a linear smoking machine under ISO or HC smoking regimens and collected on a Cambridge filter pad (CFP). The collection conditions for the different smoking regimes are summarized in [Table 1](#).

Right after the end of the smoking step, the pad is transferred in a Filtrona tube and sent to the analytical laboratory for extraction and analysis.

Four replicates per sample are prepared. Two blank Cambridge filter pads are processed and analyzed to ensure that no contamination occurred throughout the sample preparation process.

[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

1.5. Samples preparation

Internal standard (ISTD) solution and hexane are added to the Filtrona tube containing the CFP and shaken for 30 ± 10 minutes to allow the extraction of the target compounds. The solution is successively evaporated at 60°C for 5-10 minutes.

The remaining solution is recovered with isooctane and cleaned-up by Solid Phase Extraction (SPE), using an aminopropyl cartridge previously conditioned with a hexane: diethyl ether 70:30 solution and successively with hexane. The solution is eluted through the SPE cartridge with a hexane:diethyl ether 70:30 solution and then evaporated at 60°C .

The remaining solution is recovered with 1-propanol and cleaned-up by a second SPE, using an octadecyl cartridge previously conditioned with acetonitrile, methanol and finally with a water:methanol solution 60:40. The solution is suspended with water and eluted through the SPE cartridge with a acetonitrile and then evaporated at 70°C . After addition of isooctane and analyte protectant solution, the remaining solution is transferred into a glass vial and analyzed by GC-MS.

1.6. Internal standard solution

PAH-MIX 121 solution does not contain dibenz(a,i)pyrene-d14 and pyrene-d10. As consequence, a 0.1 mg/mL dibenz(a,i)pyrene-d14 and 0.4 mg/mL pyrene-d10 solution is prepared by dissolution of the deuterated compounds in toluene and successively combined with the PAH-MIX 121 solution in isooctane.

1.7. Analyte protectant solution (PEG)

The analyte protectant solution is prepared by dissolution of poly(ethylene glycol) in 1-butanol.

1.8. Calibration solutions preparation

A stock solution is prepared by dilution of the PAH-MIX 156 solution in isooctane. Seven standard (STD) solutions are then prepared by dilution of the stock solution in isooctane. The ranges of concentrations cover the ranges relevant for analysis and is provided in [Table 2](#).

Table 2: Typical concentrations of calibration standards for the analysis of PAHs

Compound	STD1 (ng/mL)	STD2 (ng/mL)	STD3 (ng/mL)	STD4 (ng/mL)	STD5 (ng/mL)	STD6 (ng/mL)	STD7 (ng/mL)
Pyrene	100	250	750	3000	5000	7500	10000
Benz(a)anthracene	20	50	150	600	1000	1500	2000
Benzo(b)fluoranthene	10	25	75	300	500	750	1000
Benzo(k)fluoranthene	5	12.5	37.5	150	250	375	500
Benzo(j)fluoranthene	5	12.5	37.5	150	250	375	500
Benz(a)pyrene	20	50	150	600	1000	1500	2000
Indeno(123cd)pyrene	5	12.5	37.5	150	250	375	500
Dibenz(a,h)anthracene	2	5	15	60	100	150	200
Dibenz(a,l)pyrene	2	5	15	60	100	150	200
Dibenz(a,e)pyrene	2	5	15	60	100	150	200
Dibenz(a,i)pyrene	2	5	15	60	100	150	200
Dibenz(a,h)pyrene	2	5	15	60	100	150	200

Standard level 4 is also used as quality check.

The corresponding standard vials are prepared by mixing 100 μL of isooctane, 50 μL of internal standard solution, 50 μL of calibration standard solution and 10 μL of analyte protectant solution.

1.9. Instrumental Conditions

The samples are analyzed by Gas Chromatography with Electron Impact Mass Spectrometry following tables below:

Table 3: Chromatographic Conditions for Determination of twelve PAHs

Column	DB-17MS column, 30m x 0.25mm ID x 0.15 μ m (film thickness)
Filling volume	3 μ L
Injection speed	50 μ L/s
Injection volume	1 μ L
Injection type	Splitless
Injection temperature	320°C
Autosampler rack temperature	10°C
Carrier Gas	Helium
Mode	Constant flow
Column flow	2.00 mL/min
Run time	57.83 min
Initial temperature	70°C
Initial Hold Time	2.0 min
Ramp 1 Rate	40°C/min
Ramp 1 Temp	210°C
Ramp 1 Hold Time	0 min
Ramp 2 Rate	2.5°C/min
Ramp 2 Temp	270°C
Ramp 2 Hold Time	0 min
Ramp 3 Rate	3.0°C/min
Ramp 3 Temp	325°C
Ramp 3 Hold Time	10.0 min

Table 4 Mass spectrometer settings

Parameter	Description
Acquisition mode	SIM
Ion source temperature	300°C
Interface temperature	325°C
Ionization mode	EI

1.10. Testing procedure

The following typical analytical sequence is used for the determination of twelve PAHs:

- Three conditioning injections (old extract)
- Calibration curve (STD 1 to 8)
- Analytical blank (solvent with ISTD)
- Smoked blanks
- Samples
- After every 4 samples, inject a quality check (STD level 4)
- Always end the analytical sequence with a quality check injection

1.11. Verification of results

1.11.1. Calibration curve

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 regressions are calculated automatically by Thermo XCalibur software. Specific information about regressions are provided in [Table 5](#):

Table 5: Parameters used for calibration curves

Compound	Internal standard	Regression type	Weighting factor	R ²
Pyrene	Pyrene-d10	linear	1/x	≥ 0.995
Benz(a)anthracene	Benz(a)anthracene-d12	linear	1/x	≥ 0.995
Benzo(b)fluoranthene	Benzo(b)fluoranthene-d12	linear	1/x	≥ 0.995
Benzo(k)fluoranthene	Benzo(b)fluoranthene-d12	linear	1/x	≥ 0.995
Benzo(j)fluoranthene	Benzo(b)fluoranthene-d12	linear	1/x	≥ 0.995
Benz(a)pyrene	Benz(a)pyrene-d12	linear	1/x	≥ 0.995
Indeno(123cd)pyrene	Indeno(123cd)pyrene-d12	linear	1/x	≥ 0.995
Dibenz(a,h)anthracene	Dibenz(a,h)anthracene-d14	linear	1/x	≥ 0.995
Dibenz(a,l)pyrene	Dibenz(a,h)anthracene-d14	linear	1/x	≥ 0.995
Dibenz(a,e)pyrene	Dibenz(a,i)pyrene-d14	linear	1/x	≥ 0.995
Dibenz(a,i)pyrene	Dibenz(a,i)pyrene-d14	linear	1/x	≥ 0.995
Dibenz(a,h)pyrene	Dibenz(a,i)pyrene-d14	linear	1/x	≥ 0.995

1.11.2. Quality Check

The validity of the calibration is continuously verified during the batch analysis by analysing the calibration control standard injections. Each control standard must be within ±15% of its theoretical value.

1.12. Example Chromatograms

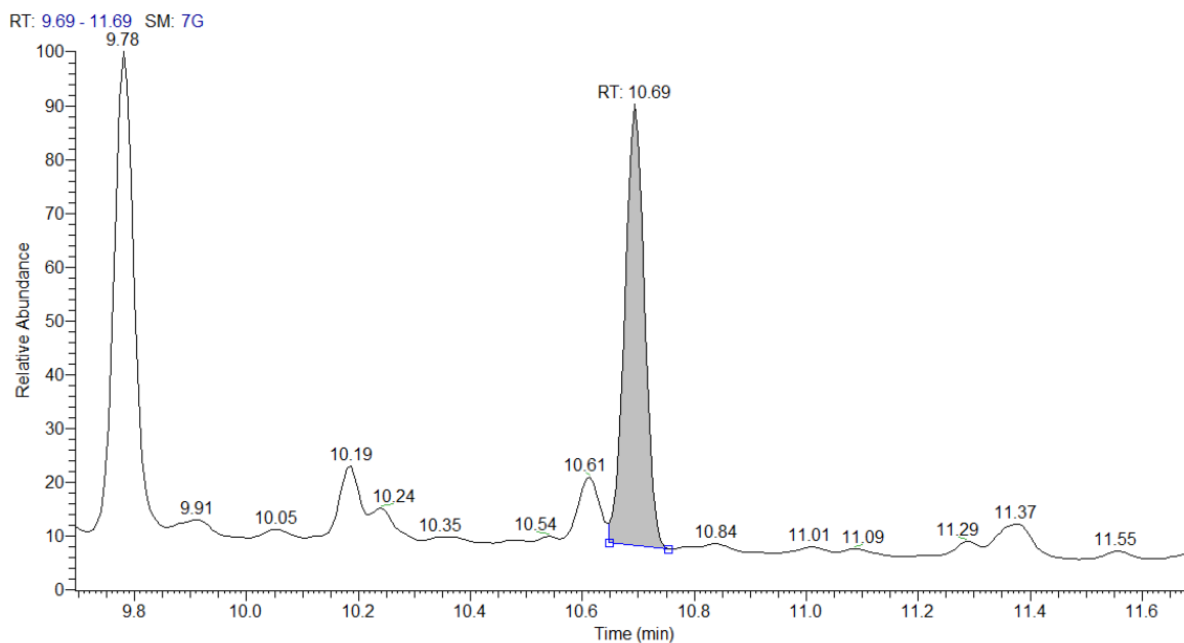


Figure 1: Extracted ion chromatogram for pyrene (202.00 amu) in P1 sample

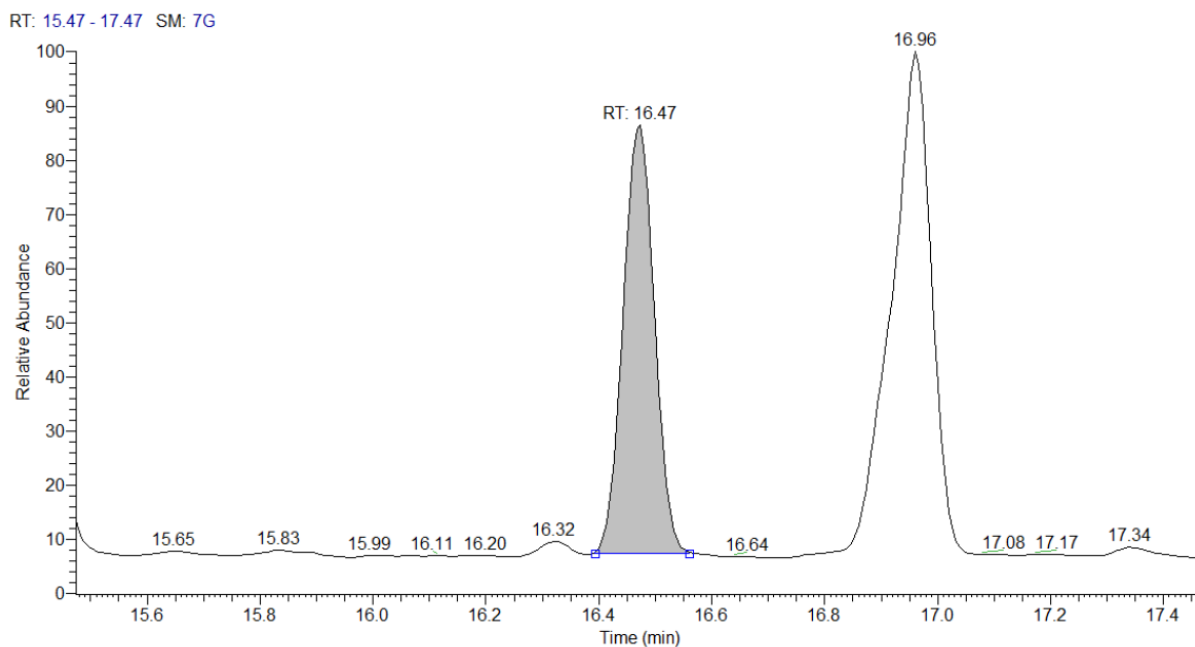


Figure 2: Extracted ion chromatogram for benz(a)anthracene (228.00 amu) in P1 sample

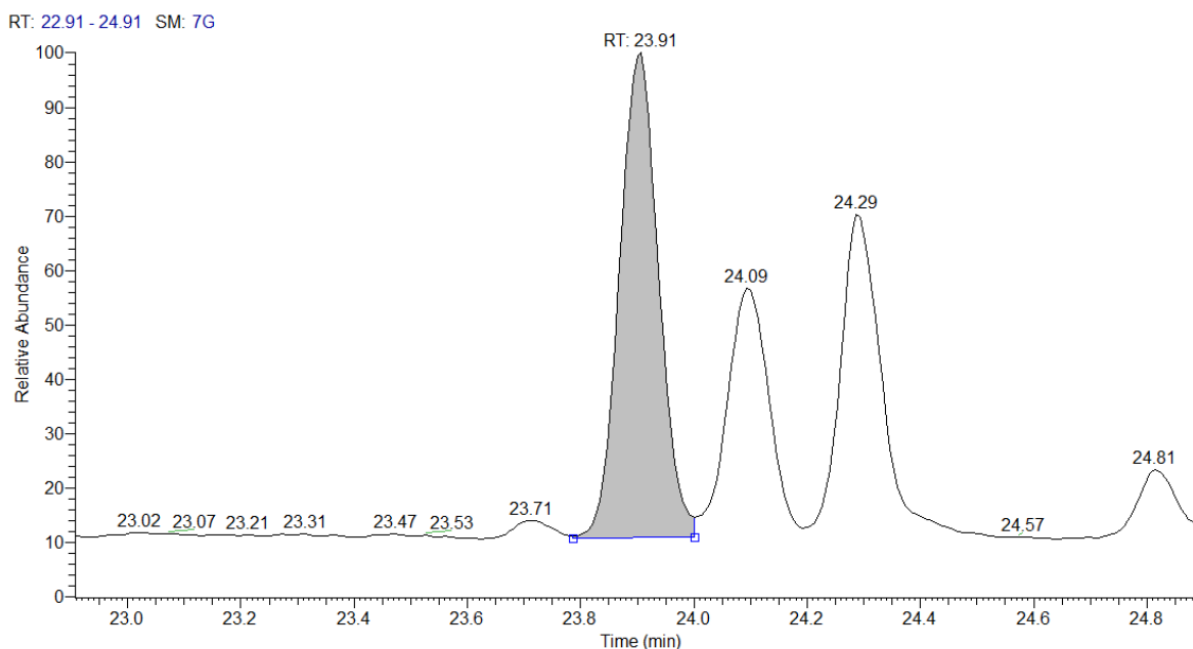


Figure 3: Extracted ion chromatogram for benz(b)fluoranthene (252.00 amu) in P1 sample

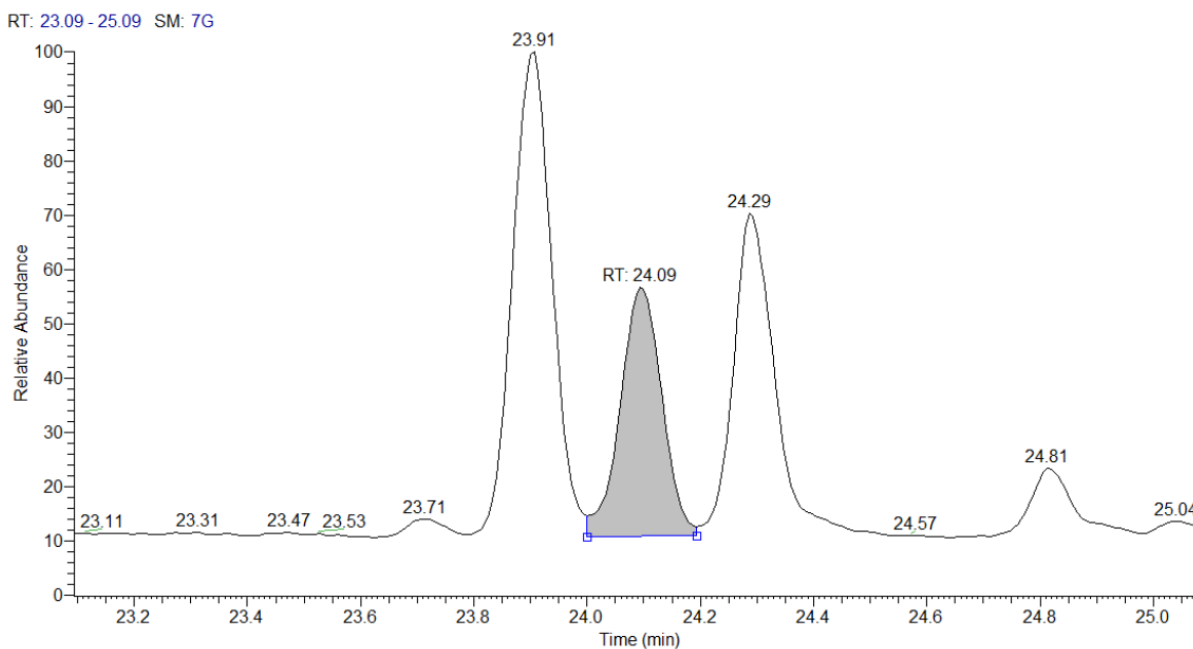


Figure 4: Extracted ion chromatogram for benz(k)fluoranthene (252.00 amu) in P1 sample

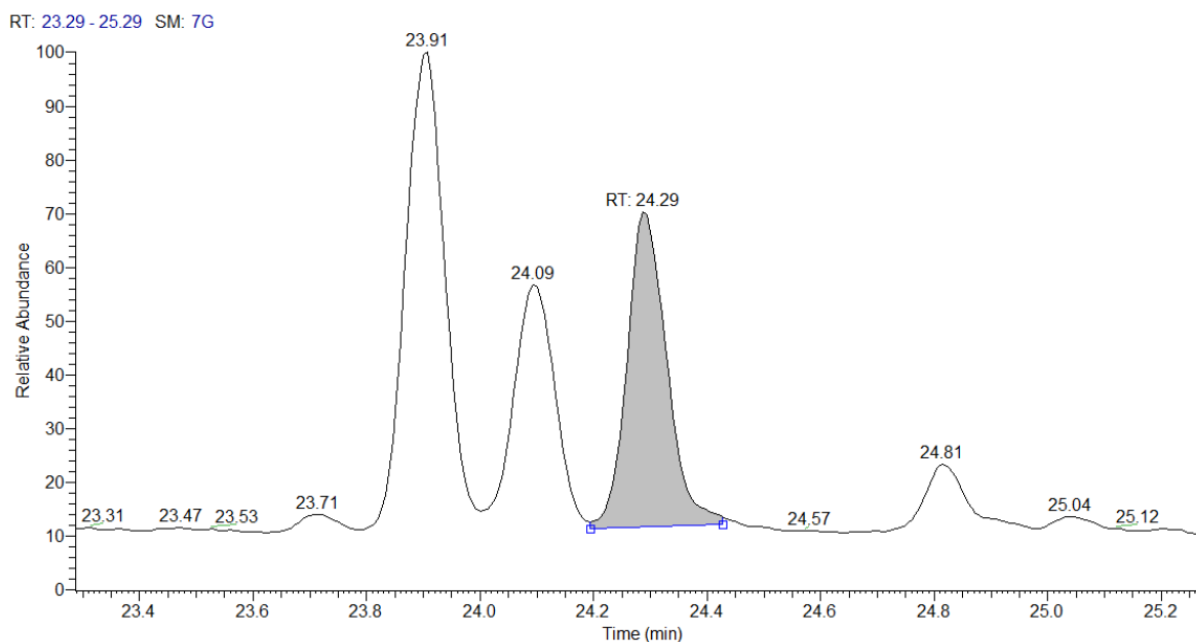


Figure 5: Extracted ion chromatogram for benz(j)fluoranthene (252.00 amu) in P1 sample

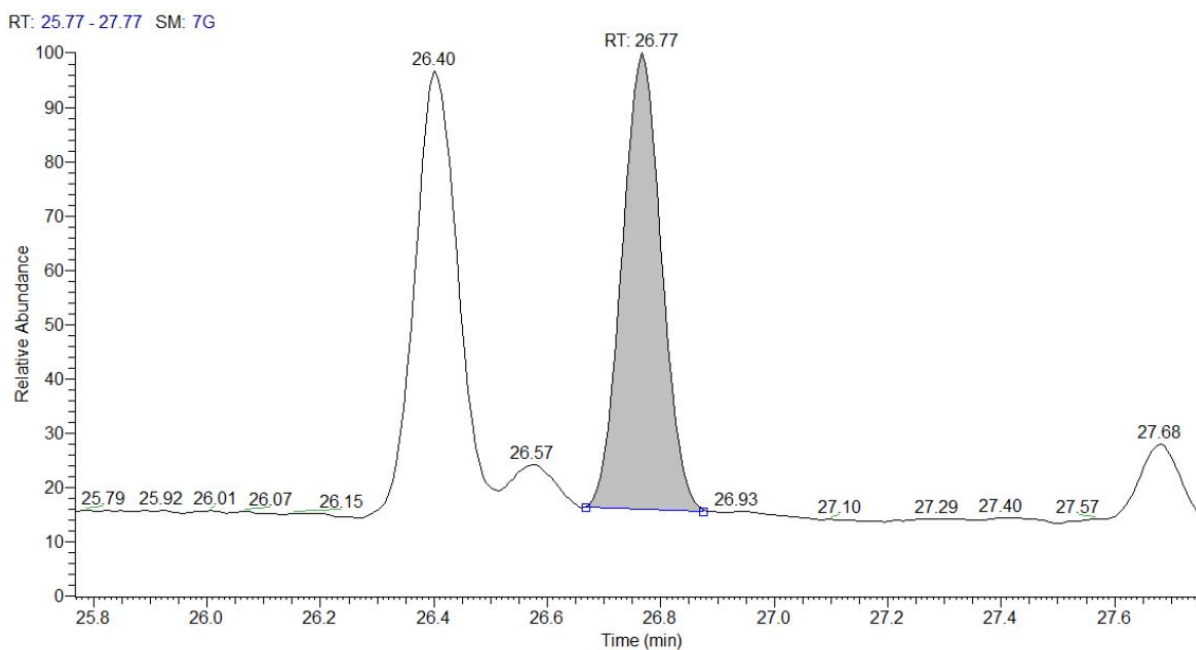


Figure 6: Extracted ion chromatogram for benz(a)pyrene (252.00 amu) in P1 sample

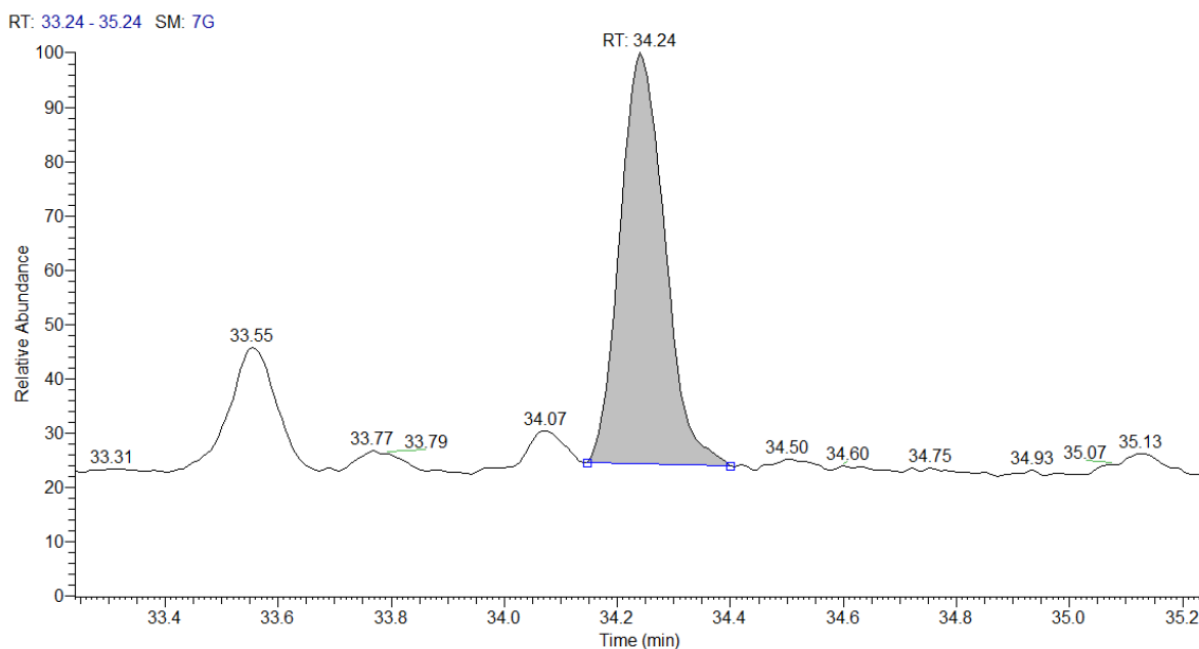


Figure 7: Extracted ion chromatogram for indeno(123cd)pyrene (288.00 amu) in P1 sample

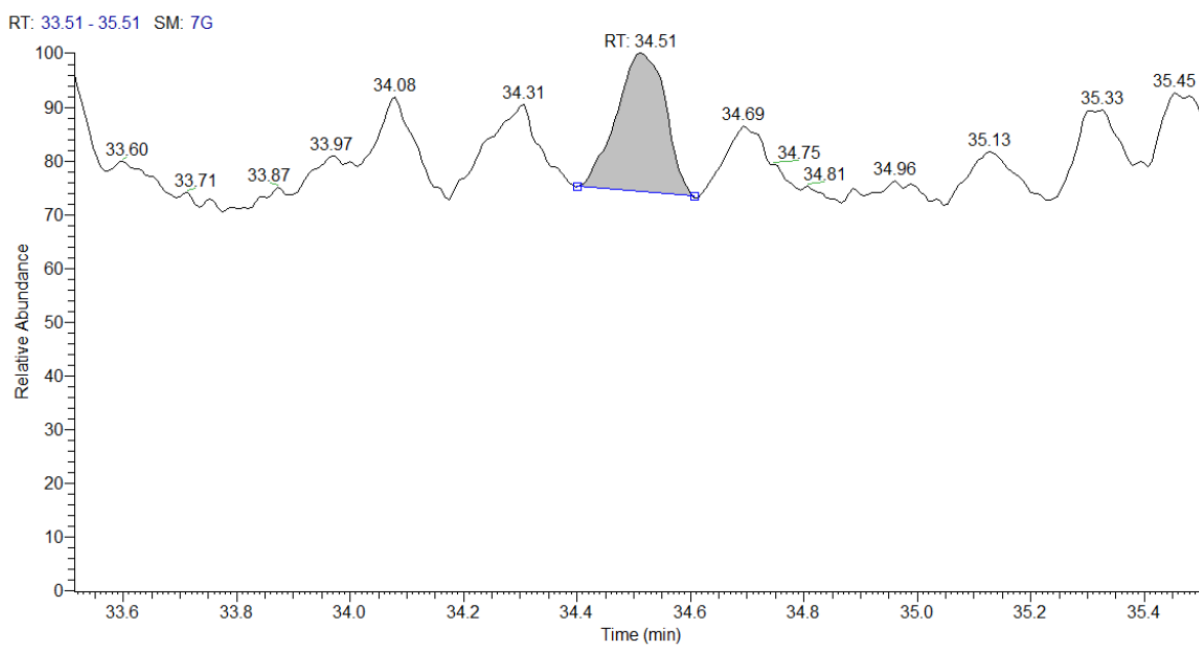


Figure 8: Extracted ion chromatogram for dibenz(a,h)anthracene (278.00 amu) in P1 sample

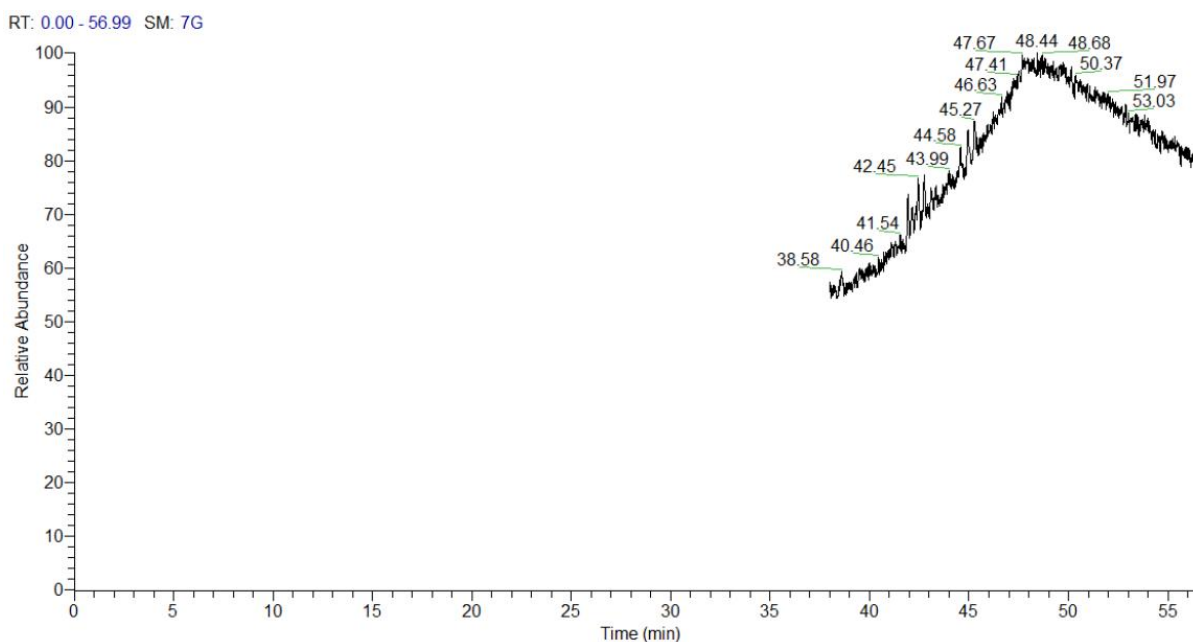


Figure 9: Extracted ion chromatogram for dibenz(a,l)pyrene (302.00 amu) in P1 sample

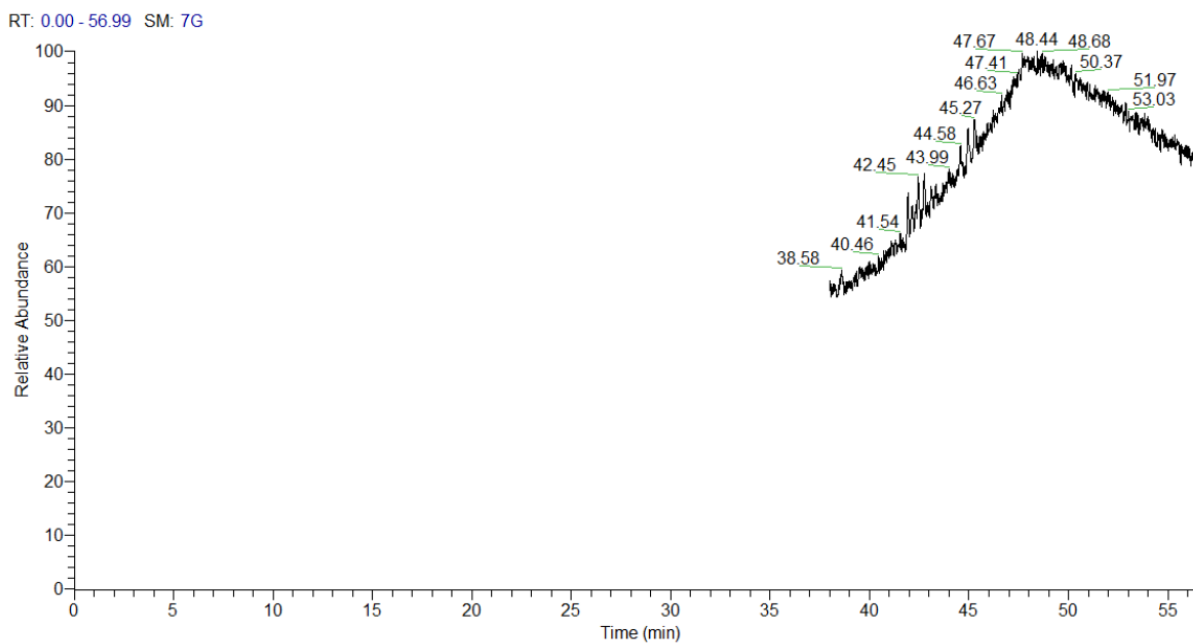


Figure 10: Extracted ion chromatogram for dibenz(a,e)pyrene (302.00 amu) in P1 sample

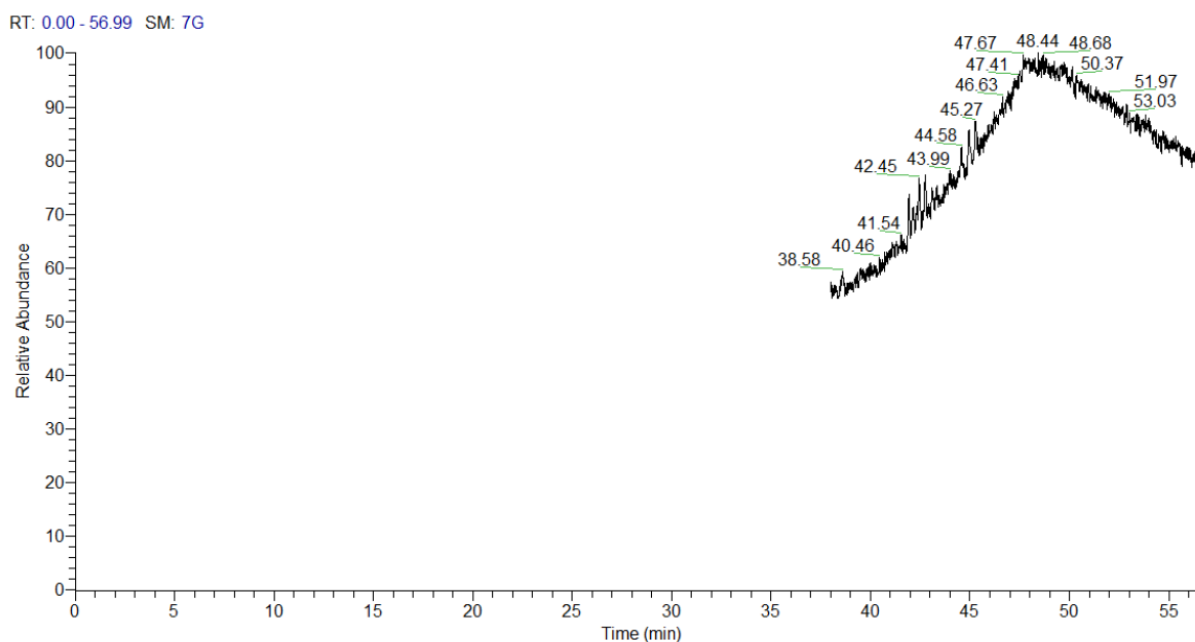


Figure 11: Extracted ion chromatogram for dibenz(a,i)pyrene (302.00 amu) in P1 sample

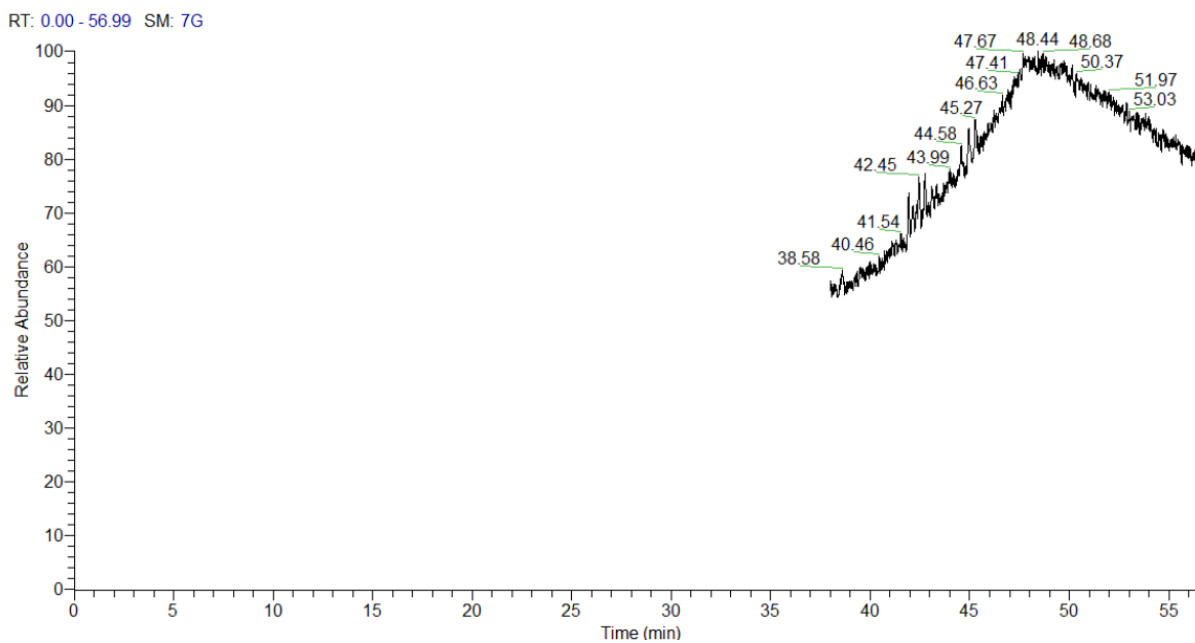


Figure 12: Extracted ion chromatogram for dibenz(a,h)pyrene (302.00 amu) in P1 sample

1.13. 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 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](#). The concentration level of PAHs in P1 aerosols generated under ISO smoking regime are extremely low and cannot be quantified.

[Table 6](#) LOD, LLOQ and ULOQ for P1 samples under HC regimen

Compound	P1, HC regimen		
	LOD [ng/item]	LLOQ [ng/item]	STD1 [ng/item]
Pyrene	0.054	0.181	2.00
Benz(a)anthracene	0.047	0.155	0.10
Benzo(b)fluoranthene	0.020	0.068	0.20
Benzo(k)fluoranthene	0.018	0.061	0.10
Benzo(j)fluoranthene	0.015	0.050	0.10
Benz(a)pyrene	0.054	0.181	0.40
Indeno(123cd)pyrene	0.021	0.071	0.10
Dibenz(a,h)anthracene	0.008	0.026	0.040
Dibenz(a,l)pyrene	0.008	0.025	0.040
Dibenz(a,e)pyrene	0.008	0.026	0.040
Dibenz(a,i)pyrene	0.007	0.022	0.040
Dibenz(a,h)pyrene	0.005	0.015	0.040

1.14. 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.

Repeatability limit and intermediate precision limit are determined during four different days using different smoking machines for the aerosol generation and standard solutions preparation. Different operators are involved in both aerosol generation and analysis.

IP and r values for P1 are depicted in [Table 7](#).

Table 7: Repeatability r and Intermediate precision IP for P1 samples (HC Regimen)

Compound	P1, HC regimen		
	Mean conc [ng/item]	r [ng/item]	IP [ng/item]
Pyrene	4.42	0.803	1.264
Benz(a)anthracene	1.32	0.227	0.270
Benzo(b)fluoranthene	0.72	0.130	0.130
Benzo(k)fluoranthene	0.40	0.068	0.077
Benzo(j)fluoranthene	0.56	0.101	0.101
Benz(a)pyrene	0.65	0.111	0.113
Indeno(123cd)pyrene	0.20	0.060	0.060
Dibenz(a,h)anthracene	<0.040	NA	NA
Dibenz(a,l)pyrene	<0.040	NA	NA
Dibenz(a,e)pyrene	<0.040	NA	NA
Dibenz(a,i)pyrene	<0.040	NA	NA
Dibenz(a,h)pyrene	<0.040	NA	NA

1.15. 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