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PMI Research & Development

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Analysis of seven phenolic compounds in Platform 1 aerosol

PRODUCT TESTING LABORATORY AND GOVERNANCE

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1. DETERMINATION OF SEVEN PHENOLIC COMPOUNDS IN PLATFORM 1 AEROSOL

1.1. Abstract

The aerosol samples are generated by mean of a linear smoking machine. The aerosol is collected on a 44mm Cambridge filter pad (CFP). Phenolic compounds are then extracted in a Filtrona tube with a 2-butanone solution containing the internal standards.

The extracts are analyzed by Gas Chromatography (GC) with Electron Impact Mass Spectrometry (EI-MS). The GC system is equipped with a column DB-624, 30m x 0.32mm ID x 1.8µm.

Results are expressed as µg/item for P1.

1.2. Applicability

The method described is used for the determination of seven phenolic compounds in Platform 1 (P1) aerosols generated under International Organization for Standardization (ISO) or Health Canada (HC) conditions, as well as under alternative smoking regimens.

1.3. Reagents

- phenol
- o-cresol
- m-cresol
- p-cresol
- catechol
- hydroquinone
- resorcinol
- phenol-d6
- catechol-d6
- hydroquinone-d6
- resorcinol-d6
- 2-butanone
- N,O-Bis(trimethylsilyl)-trifluoracetamide (BSTFA)

1.4. Aerosol generation

P1 items are conditioned in opened pack for at least 48 hours at target conditions of 22 ± 1°C and relative humidity of 60 ± 3% before to be 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 to be used for aerosol collection.

The aerosol samples are generated on a linear smoking machine under ISO or HC smoking regimens and collected on a Cambridge filter pad. The collection conditions for the different smoking regimes are summarized in Table **1**. After the aerosol collection, the CFP is placed in a Filtrona tube to perform the extraction step.

Four replicates for each sample are generated. Within each series of smoking runs, two smoked blanks and the internal standard (ISTD) extraction solution are processed and analyzed to verify that no contamination occurred throughout the 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

10 mL of internal standard (ISTD) extraction solution are added to the Filtrona tube containing the CFP and shaked for 30 minutes to allow the extraction of the target compounds. The solution is then filtered into a glass vial for analysis with a 0.45 µm filter using a disposable syringe. The samples are derivatized with BSTFA directly into the GC-MS vial prior to analysis.

1.6. ISTD Extraction solution

Individual 0.8 mg/mL phenol-d6, 5.0 mg/mL catechol-d6, 0.8 mg/mL resorcinol-d6 and 3.0 mg/mL hydroquione-d6 solutions are prepared by dissolution of the deuterated compounds in 2-butanone. Equal volumes of the individual solutions are then combined and the resulting solution is diluted 1000 times with 2-butanone.

1.7. Calibration solutions preparation

1.7.1. Target compounds stock solutions

Individual 4 mg/mL phenol, 8 mg/mL catechol, 1.8 mg/mL resorcinol, 1.8 mg/mL hydroquinone, 1.0 mg/mL o-cresol, 1.0 mg/mL m-cresol and 2.0 mg/mL p-cresol solutions are prepared by dissolution of the compounds in 2-butanone.
Successively, the target compounds stock solution is prepared by combining precise volumes of the individual target compounds and internal standard solutions and diluting the resulting solution with 2-butanone.

1.7.2. Calibration standards

Eight standard (STD) solutions are prepared in the extraction solution by dilution of the target compounds stock solution. The ranges of concentrations cover the ranges relevant for analysis and are provided in Table 2.

Table 2: Typical standard solution concentrations for seven phenolic compounds

Compound	STD1 [µg/mL]	STD2 [µg/mL]	STD3 [µg/mL]	STD4 [µg/mL]	STD5 [µg/mL]	STD6 [µg/mL]	STD7 [µg/mL]	STD8 [µg/mL]
Phenol	0.019	0.120	0.420	1.200	2.040	2.700	3.600	4.800
o-cresol	0.003	0.020	0.070	0.200	0.340	0.450	0.600	0.800
m-cresol	0.003	0.017	0.060	0.170	0.289	0.383	0.510	0.680
p-cresol	0.005	0.034	0.119	0.340	0.578	0.765	1.020	1.36
Catechol	0.077	0.480	1.680	4.800	8.160	10.80	140.4	19.20
Resorcinol	0.002	0.011	0.038	0.108	0.184	0.243	0.324	0.432
hydroquinone	0.043	0.270	0.945	2.700	4.590	60.75	8.100	10.80

The standard level 6 is also used as quality check.

Each calibration standard is derivatized with BSTFA directly into GC-MS vial prior to analysis.

1.8. Instrumental Conditions

The samples are analyzed by Gas Chromatography (GC) with Electron Impact Mass Spectrometry (EI-MS) following tables below:

Table 3: Chromatographic conditions for the determination of seven phenolic compounds

Column	DB-624, 30m x 0.32mm ID x 1.8µm
Run time	22.83 min
Injector	Split/Splitless (mode CAP/SSL)
Injectior temperature	225°C
Injection volume	1 µL
Injection mode	Splitless
Carrier Gas	Helium
Carrier Gas flow	2.8 mL/min
Flow mode	Constant pressure
Pressure	59 kPa

Table 4: GC oven temperature program

Rate (°C/min)	Temperature (°C)	Hold time (min)
-	50	2
30	150	0
4	200	0
Post-run temp	240°C	
Post-run time	5 min	

Table 5: Mass spectrometer settings

MS Quad temperature	150°C
Ion source temperature	230°C
Transfer line temperature	225°C
Solvent delay	6.5 min
Acquisition type	SIM

1.9. Testing procedure

The following typical analytical sequence is used for the determination of seven phenolic compounds:

- Calibration curve (STD 1 to 8)
- ISTD extraction solution
- Blanks and samples
- One quality check is included every five samples (STD level 6)
- Sequence ends with a quality check (STD level 6)

1.10. Verification of results

1.10.1. 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 curves. The linear regression is calculated automatically by Mass Hunter software. Specific information about regressions are provided in Table 6.

Table 6: Parameters used for calibration curves

Compound	Internal standard	Regression type	Weighting factor	R^2
Phenol	Phenol-d6	linear	1/x	≥ 0.995
o-cresol	Phenol-d6	linear	1/x	≥ 0.995
m-cresol	Phenol-d6	linear	1/x	≥ 0.995
p-cresol	Phenol-d6	linear	1/x	≥ 0.995
Hydroquinone	Hydroquinone-d6	linear	1/x	≥ 0.995
Catechol	Catechol-d6	linear	1/x	≥ 0.995
Resorcinol	Resorcinol-d6	linear	1/x	≥ 0.995

1.10.2. 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 ±10% of its theoretical value.

1.11. Example Chromatograms

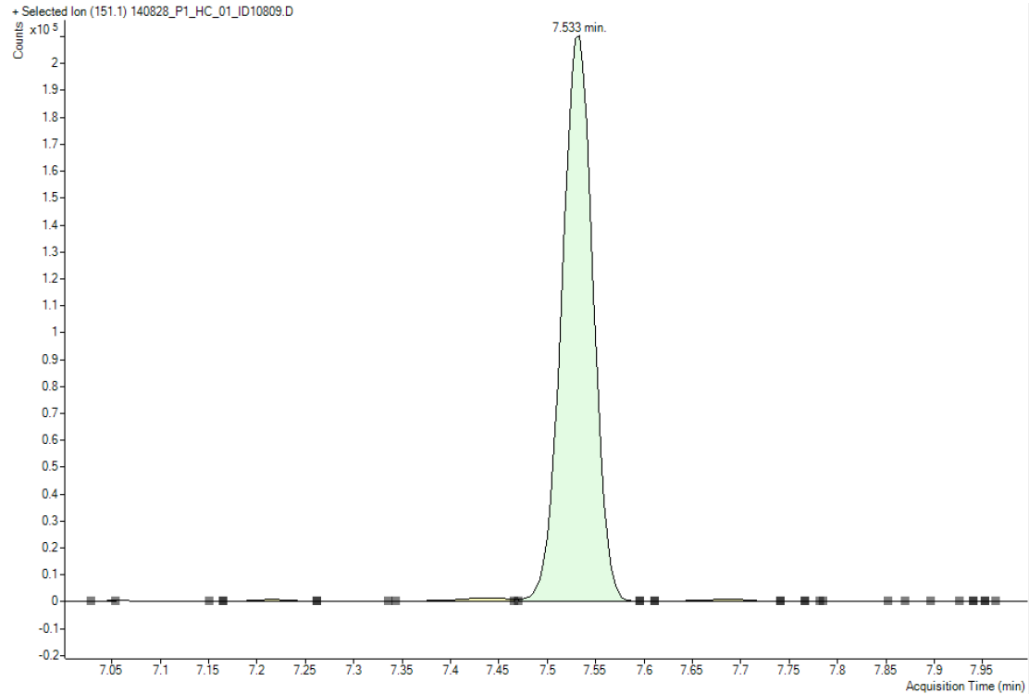

Figure 1: Extracted ion chromatogram for phenol (151.1 amu) in P1 sample

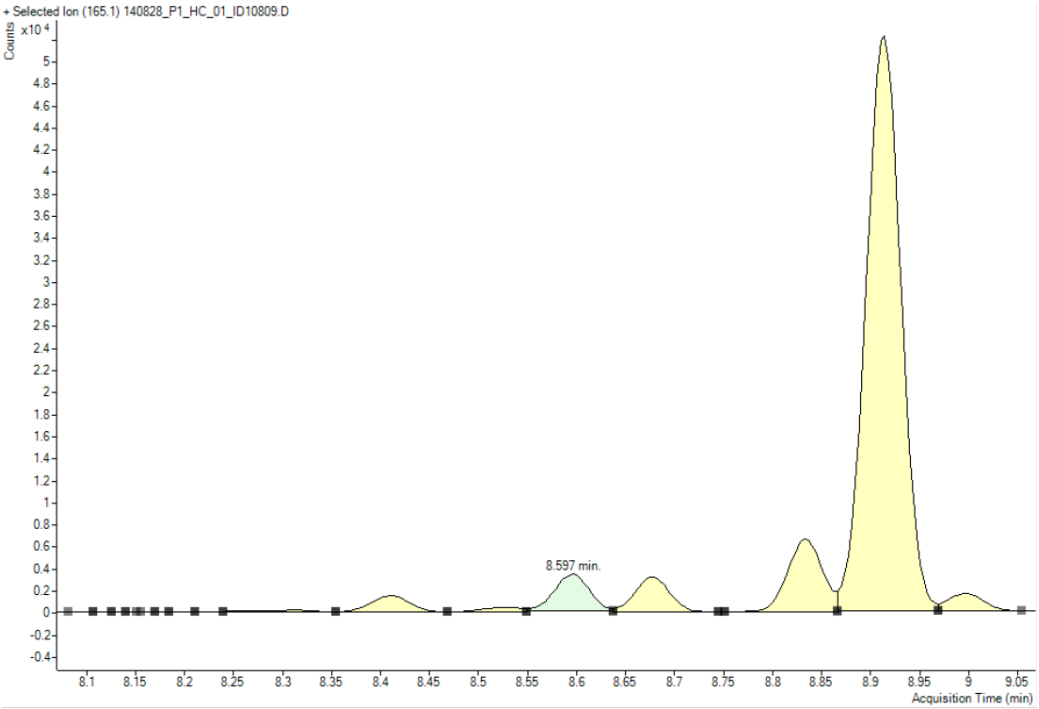

Figure 2: Extracted ion chromatogram for o-cresol (165.1 amu) in P1 sample

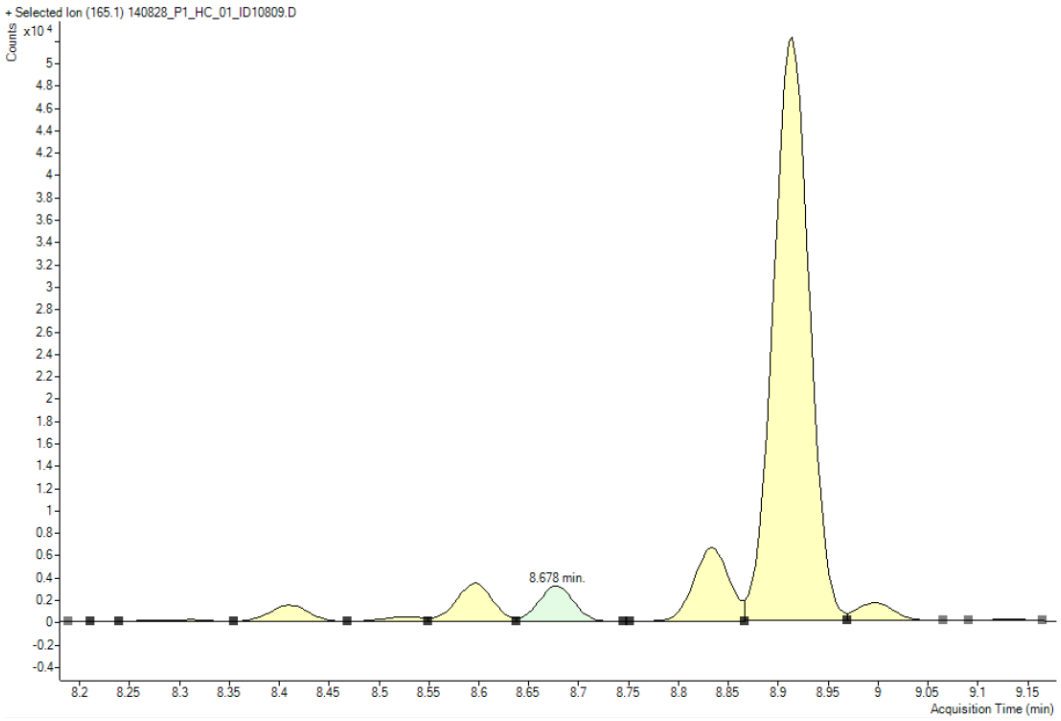

Figure 3: Extracted ion chromatogram for m-cresol (165.1 amu) in P1 sample

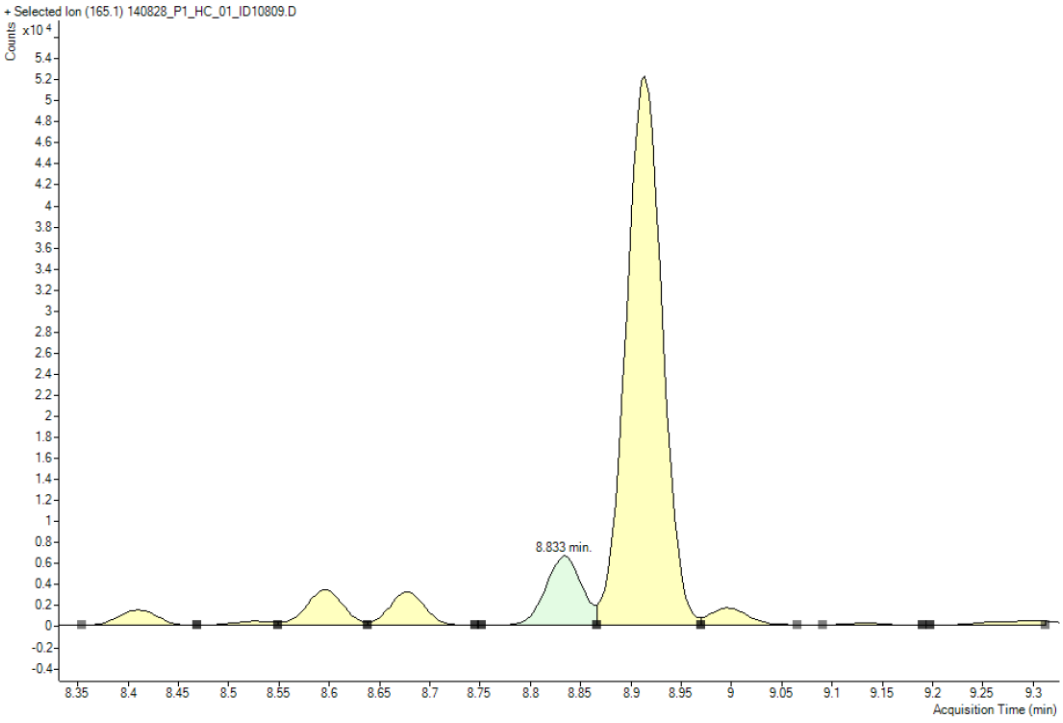

Figure 4: Extracted ion chromatogram for p-cresol (165.1 amu) in P1 sample

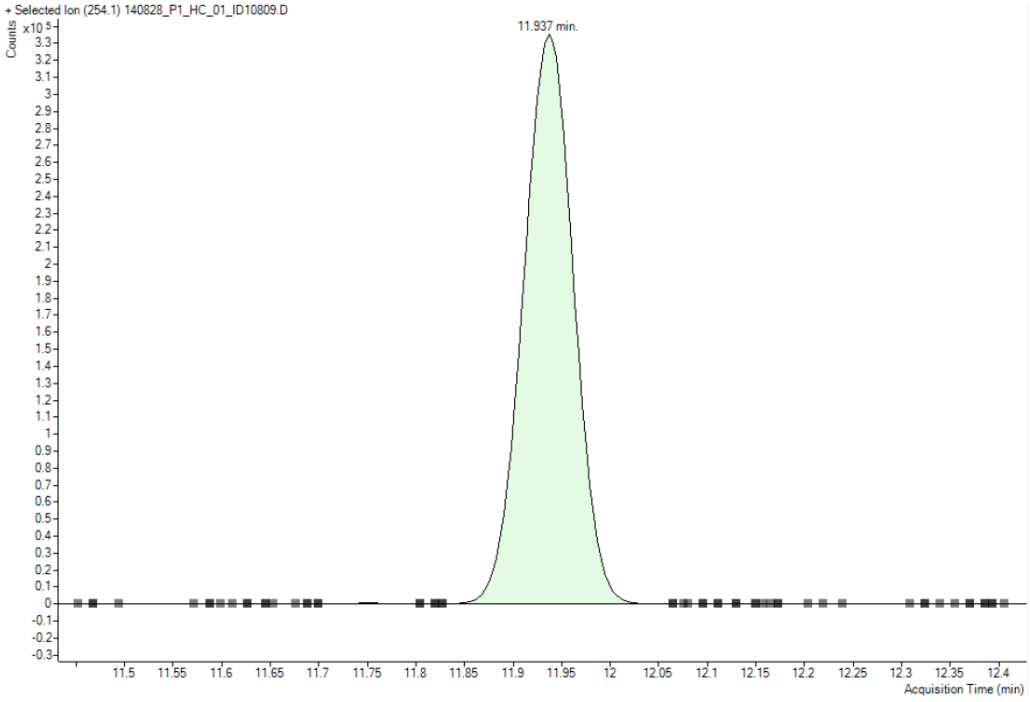

Figure 5: Extracted ion chromatogram for catechol (254.1 amu) in P1 sample

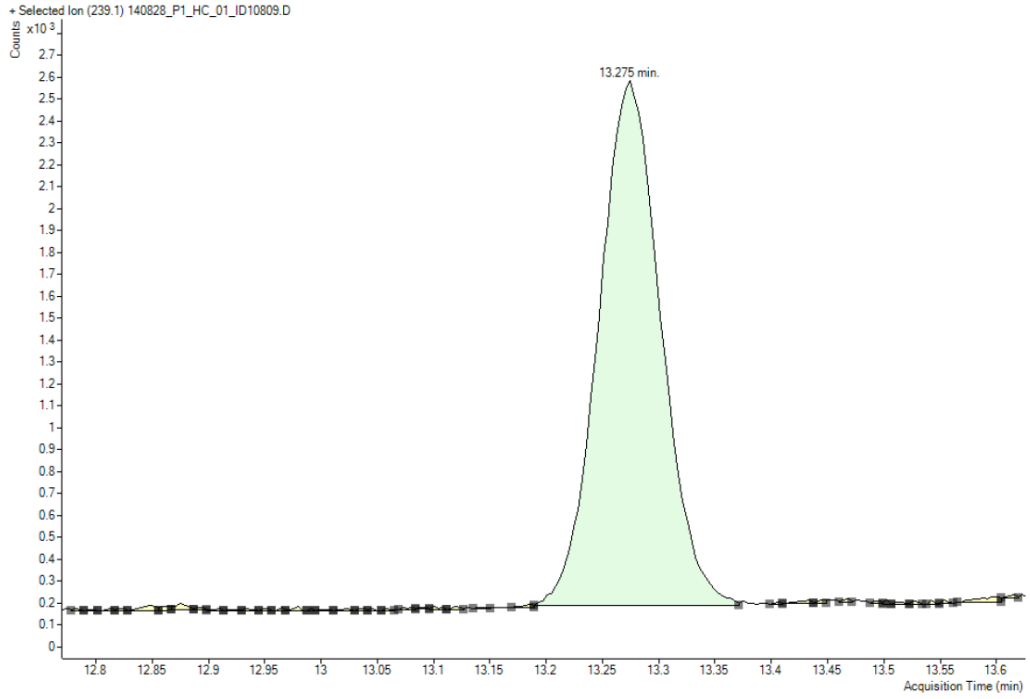

Figure 6: Extracted ion chromatogram for resorcinol (239.1 amu) in P1 sample

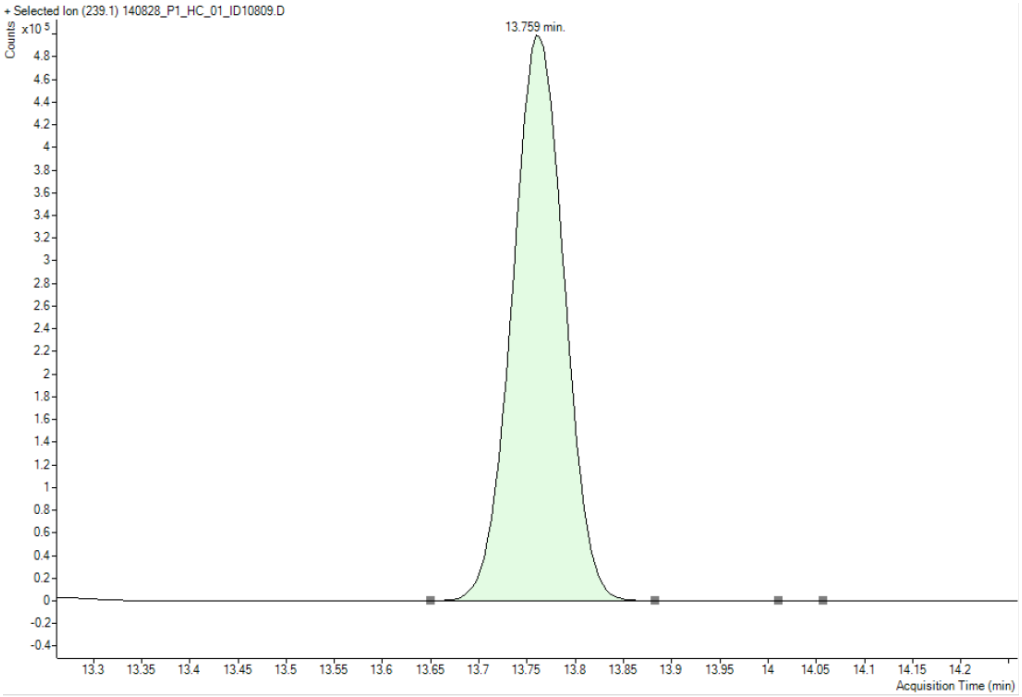

Figure 7: Extracted ion chromatogram for hydroquinone (239.1 amu) in P1 sample

1.12. 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 values are provided in Table 7.

Table 7: LOD and LLOQ for ISO and HC smoking regimens

Matrix	Compound	LOD [µg/item]	LLOQ [µg /item]	STD1 [µg /item]
P1	Phenol	0.008	0.026	0.038
	o-Cresol	0.001	0.003	0.006
	m-Cresol	0.001	0.003	0.006
	p-Cresol	0.002	0.006	0.012
	Catechol	0.008	0.026	0.162
	Resorcinol	0.001	0.002	0.004
	Hydroquinone	0.012	0.042	0.086

1.13. 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 and standard solutions preparation. Different operators are involved in the aerosol generation and analysis.

Table 8: Repeatability (r) and intermediate precision (IP) per matrix for HC smoking regimen

Matrix	Compound	Mean [µg/item]	r [µg /item]	IP [µg /item]
P1	Phenol	1.72	0.570	0.902
	o-Cresol	0.0870	0.0540	0.0605
	m-Cresol	0.0450	0.0143	0.0216
	p-Cresol	0.0960	0.0365	0.0489
	Catechol	15.5	2.05	2.37
	Resorcinol	0.0470	0.00990	0.00990
	Hydroquinone	7.86	1.53	1.79

Table 9: Repeatability (r) and intermediate precision (IP) per matrix for ISO smoking regimen

Matrix	Compound	Mean [µg/item]	r [µg /item]	IP [µg /item]
P1	Phenol	0.136	0.0607	0.0756
	o-Cresol	0.0160	0.00700	0.0218
	m-Cresol	0.00500	0.00180	0.00430
	p-Cresol	0.0110	0.00430	0.00990
	Catechol	5.59	1.30	1.30
	Resorcinol	0.0190	0.00630	0.0122
	Hydroquinone	3.19	0.942	1.66

1.14. 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