Application note | 001566

Food safety

Sensitive and reproducible analysis of 16 polyaromatic hydrocarbons (PAHs) using gas chromatography – triple quadrupole mass spectrometry (GC-MS/MS)

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Keywords

Polyaromatic hydrocarbons, PAH, GC-MS/MS, gas chromatography, triple quadrupole mass spectrometry, TSQ 9610 mass spectrometer

Goal

To demonstrate the performance of the Thermo Scientific™ TRACE™ 1610 GC system and Thermo Scientific™ TSQ™ 9610 triple quadrupole GC-MS system under daily routine high-throughput conditions for sensitive and reproducible PAHs analysis

Introduction

Polyaromatic hydrocarbons (PAHs) contain two or more benzene rings in various configurations with no heteroatoms. There are more than more than 200 known structures, commonly non-polar and uncharged. PAHs are formed as a result of incomplete combustion of organic compounds, for example in energy production or fossil fuel-based mobility. They can be present in soil, air, and water[1,2] and are classified as health harming chemicals, potentially as human carcinogens, and endocrine disrupters.[3] Therefore, most countries across the world monitor and regulate the presence of PAHs in the environment, food products, and drinking water.[4]

Analysis of PAHs is usually performed by gas chromatography (GC) coupled with flame ionization detection (FID) or mass spectrometry (MS) or, alternatively, using high-performance liquid chromatography (HPLC) with UV, fluorescence detectors (FLD),

or mass spectrometry-based detection. One of the main challenges associated with PAH analysis is achieving adequate chromatographic resolution for many targeted compounds and meeting sensitivity requirements without long analysis times, which can limit instrument sample capacity. Although liquid chromatography offers the advantage of shorter run times, the analysis of PAHs is generally carried out using GC as this technique allows greater selectivity, better chromatographic resolution, and improved sensitivity.[5]

In this application note, we highlight the suitability of the TSQ 9610 triple quadrupole GC-MS for the analysis of PAHs. One of the main benefits of the TSQ 9610 GC-MS system is the Thermo Scientific™ Advance Electron Ionization (AEI) ion source, which provides greater sensitivity and improved detection limits. The AEI source also enables improved robustness, allowing more samples to be analyzed with less interruptions. Downtime when performing routine maintenance of the system is greatly reduced with Thermo Scientific™ NeverVent™ technology, which allows the removal of the ionization source and analytical column without breaking instrument vacuum.

Experimental

Standard and sample preparation

The calibration curve was prepared in nonane by a serial dilution of a mixed standard solution containing 16 highly regulated PAHs at a concentration of 1 mg/mL each. Each vial was spiked with a solution containing nine deuterated PAHs to be used as internal standards. The final concentration of the internal standards in each vial was 0.010 µg/mL. Table 1 summarizes the analytes and corresponding isotopically labeled internal standards.

Instrument and method setup

The study was performed using a TRACE 1610 GC system coupled to a TSQ 9610 triple quadrupole GC-MS/MS equipped with an AEI source. The samples were injected using a Thermo Scientific™ TriPlus™ RSH autosampler. Samples were injected using a Thermo Scientific™ iConnect™ Programmable Temperature Vaporizing (PTV) injector. The detailed information on the GC and MS settings is shown in Table 2.

Table 1. Evaluated polyaromatic hydrocarbons and internal standards

Native compound	Isotopically labeled internal standard
5-Methylchrysene	Chrysene D_{12}
Benzo[*a*]anthracene	Benzo[*a*]anthracene D_{12}
Benzo[*a*]pyrene	Benzo[*a*]pyrene D_{12}
Benzo[*b*]fluoranthene	Benzo[*b*]fluoranthene D_{12}
Benzo[*c*]fluorene	Pyrene D_{10}
Benzo[*g,h,i*]perylene	Benzo[*g,h,i*]perylene D_{12}
Benzo[*j*]fluoranthene	Benzo[*b*]fluoranthene D_{12}
Benzo[*k*]fluoranthene	Benzo[*k*]fluoranthene D_{12}
Chrysene	Chrysene D_{12}
Cyclopenta[*c,d*]pyrene	Benzo[*a*]anthracene D_{12}
Dibenzo[*a,e*]pyrene	Benzo[*g,h,i*]perylene D_{12}
Dibenzo[*a,h*]anthracene	Dibenzo[*a,h*]anthracene D_{14}
Dibenzo[*a,h*]pyrene	Benzo[*g,h,i*]perylene D_{12}
Dibenzo[*a,i*]pyrene	Benzo[*g,h,i*]perylene D_{12}
Dibenzo[*a,l*]pyrene	Benzo[*g,h,i*]perylene D_{12}
Indeno[1,2,3,*c,d*]pyrene	Indeno[1,2,3,*c,d*]pyrene D_{12}

Table 2A. GC parameters

TRACE 1610 GC parameters	
Injector	
Injector type	iConnect Programmable Temperature Vaporizer (PTV) Injector Module with integrated Backflush for TRACE 1600 Series GC
Liner	PTV Straight Liner (P/N 45352057)
Operating mode	Splitless
Split flow [mL/min]	5
Split ratio	-
Purge flow [mL/min]	1.25
Vacuum compensation	On
Temperature [°C]	80
Injection volume	2.5 µL
PTV ramp settings	
Injection time [min]	0.1
Transfer rate [°C/s]	12
Transfer temperature [°C]	335
Transfer time [min]	10
Cleaning rate [°C/s]	14
Cleaning temperature [°C]	340
Cleaning time [min]	26.5
Cleaning flow	50
Oven	
Analytical column	Thermo Scientific™ TraceGOLD™ TG-PAH GC column, 40 m × 0.18 mm × 0.07 µm (P/N 26055-3570)
Carrier gas	He
Carrier gas flow [mL/min]	1.2
Oven temperature program	
Temperature 1 [°C]	60
Hold [min]	1
Rate [°C/min]	40
Temperature 2 [°C]	210
Hold [min]	0
Rate [°C/min]	3
Temperature 3 [°C]	260
Hold [min]	0
Rate [°C/min]	8
Temperature 4 [°C]	310
Hold [min]	0
Rate [°C/min]	60
Temperature 5 [°C]	340
Hold [min]	9

Table 2B.MS parameters

TSQ 9610 triple quadrupole GC-MS/MS parameters	
Ion source	Advanced EI
Transfer line temperature [°C]	280
Ion source temperature [°C]	280
Acquisition threshold	500
Emission current [µA]	50
Electron energy voltage [eV]	50
Data acquisition mode	Timed SRM

A selective reaction monitoring (SRM) mode was used for quantification of different PAHs and related internal standards. At least three transitions were acquired for each of the analytes and isotopically labeled standards. Some compounds allowed monitoring of up to eight specific transitions. The transitions together with the optimized collision energies can be found in Table 3. The table mentions only evaluated pesticides, however the acquisition method covered additionally nine native PAHs and nine internal standards.

Data acquisition, processing, and reporting

Data was acquired, processed, and reported using the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.3.1. Integrated instrument control ensures full automation from instrument set up to data processing, reporting, and storage. Simplified e-workflows deliver effective data management, ensuring ease of use, sample integrity, and traceability. Chromeleon CDS software also offers the option to scale up the entire data handling from a single workstation to an enterprise environment.

Results and discussion

Chromatographic resolution

The TraceGOLD TG-PAH column (P/N 26055-3570) and applied oven temperature program provided good separation for all analytes. Figure 1 shows the (total ion current) chromatogram obtained for an injection of all compounds at a concentration of 0.100 µg/mL. The most challenging pair was benzo[*k*] fluoranthene/benzo[*j*]fluoranthene, which typically elute close together and may cause an overlap. However, even for this pair, the resolution was >1 (calculated automatically in Chromeleon CDS software applying the European Pharmacopoeia formula[7]). Figure 2 shows the separation.

Table 3. SRM transitions

Compound	Retention time (min)	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision energy (V)
Pyrene D_{10}	12.04	212	208	20
	12.04	212	210	10
	12.04	212	212	15
Benzo[*c*]fluorene	13.73	215	215	15
	13.73	215	215	20
	13.73	216	189	40
	13.73	216	213	40
	13.73	216	215	25
	13.73	216	216	10
	13.73	216	216	15
	13.73	216	216	15
Benzo[*a*] anthracene D_{12}	18.14	236	232	40
	18.14	240	236	30
	18.14	240	236	35
	18.14	240	240	20
Benzo[*a*] anthracene	18.3	226	224	40
	18.3	228	202	35
	18.3	228	226	30
	18.3	228	226	35
	18.3	228	228	20
Cyclopenta[*c,d*] pyrene	18.56	226	200	30
	18.56	226	224	40
	18.56	226	226	20
Chrysene D_{12}	18.57	236	232	40
	18.57	240	236	30
	18.57	240	236	25
	18.57	240	240	20
Chrysene	18.74	226	224	40
	18.74	228	202	35
	18.74	228	226	30
	18.74	228	226	25
5-Methylchrysene	21.48	242	215	22
	21.48	242	226	30
	21.48	242	239	32
	21.48	242	242	10
	21.48	242	242	15
Benzo[*b*] fluoranthene D_{12}	24.56	264	236	30
	24.56	264	260	30
	24.56	264	264	20
Benzo[*b*] fluoranthene	24.69	252	226	30
	24.69	252	250	30
	24.69	252	252	20

Table 3 (continued). SRM transitions

Compound	Retention time (min)	Precursor ion (*m/z*)	Product ion (*m/z*)	Collision energy (V)
Benzo[*k*] fluoranthene D_{12}	24.7	264	236	30
	24.7	264	260	30
	24.7	264	264	20
Benzo[*k*] fluoranthene	24.83	252	226	30
	24.83	252	226	20
	24.83	252	250	30
	24.83	252	252	20
Benzo[*j*] fluoranthene	24.87	252	226	30
	24.87	252	250	30
	24.87	252	252	20
Benzo[*a*]pyrene D_{12}	26.23	264	236	30
	26.23	264	260	30
	26.23	264	264	20
Benzo[*a*]pyrene	26.34	252	226	30
	26.34	252	250	30
	26.34	252	252	20
Indeno[1,2,3,*c,d*] pyrene D_{12}	29.31	288	256	40
	29.31	288	284	40
	29.31	288	288	20
Dibenz[*a,h*] anthracene D_{14}	29.32	292	264	30
	29.32	292	288	30
	29.32	292	292	20
Indeno[1,2,3,*c,d*] pyrene	29.38	276	248	40
	29.38	276	274	40
	29.38	276	276	20
Dibenzo[a,h] anthracene	29.4	278	252	30
	29.4	278	276	30
	29.4	278	278	20
Benzo[*g,h,i*] perylene D_{12}	30	288	256	40
	30	288	284	40
	30	288	288	20
Benzo[*g,h,i*] perylene	30.07	276	248	40
	30.07	276	274	40
	30.07	276	276	20
Dibenzo[*a,l*]pyrene	32.8	302	298	60
	32.8	302	300	35
	32.8	302	302	20
Dibenzo[*a,i*]pyrene	33.86	302	298	60
	33.86	302	300	35
	33.86	302	302	20
Dibenzo[*a,h*]pyrene	34.49	302	298	60
	34.49	302	300	35
	34.49	302	302	20
Dibenzo[*a,e*]pyrene	34.81	302	298	60
	34.81	302	300	35
	34.81	302	302	20

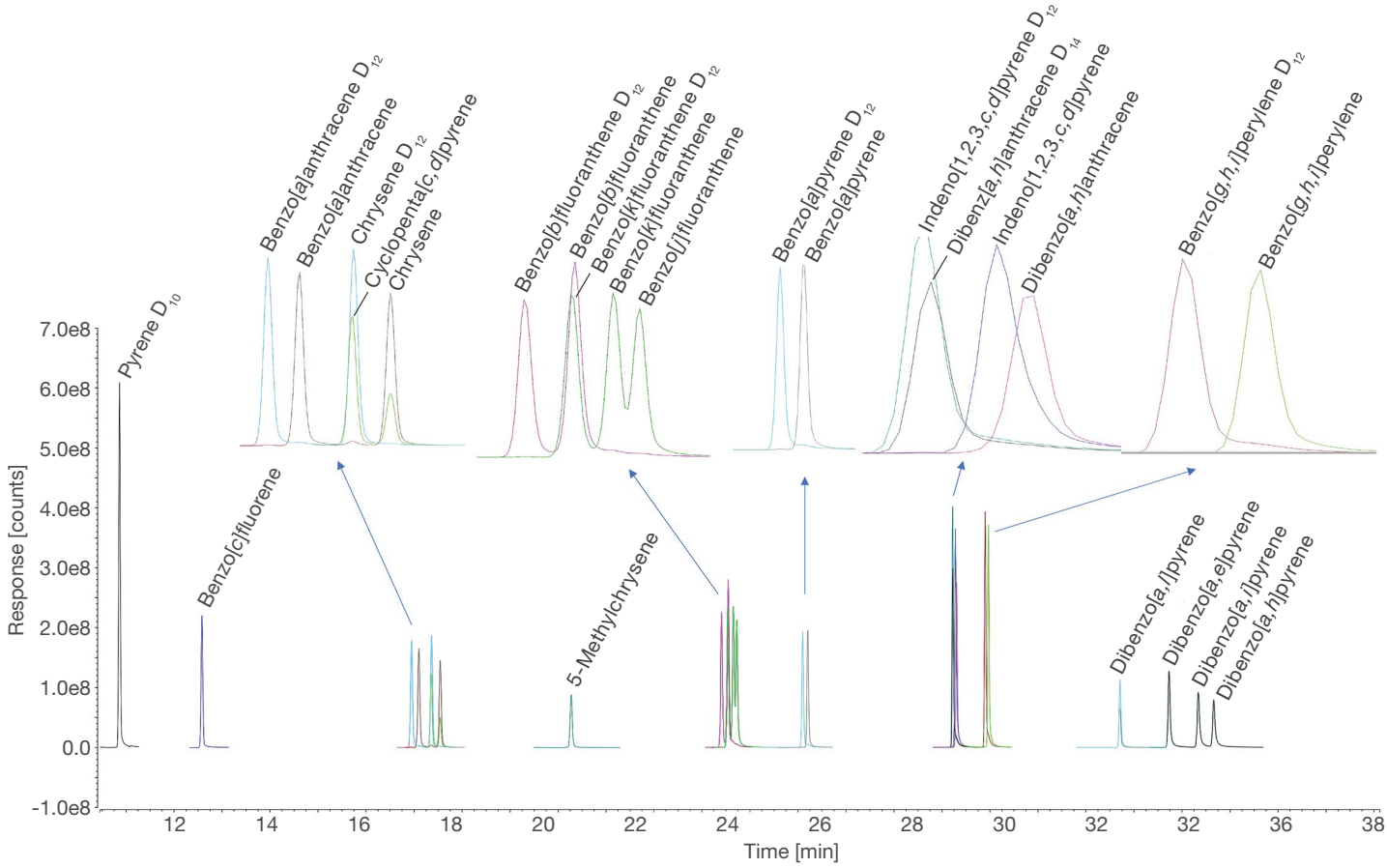

Figure 1. Total ion current chromatogram at 0.100 mg/mL

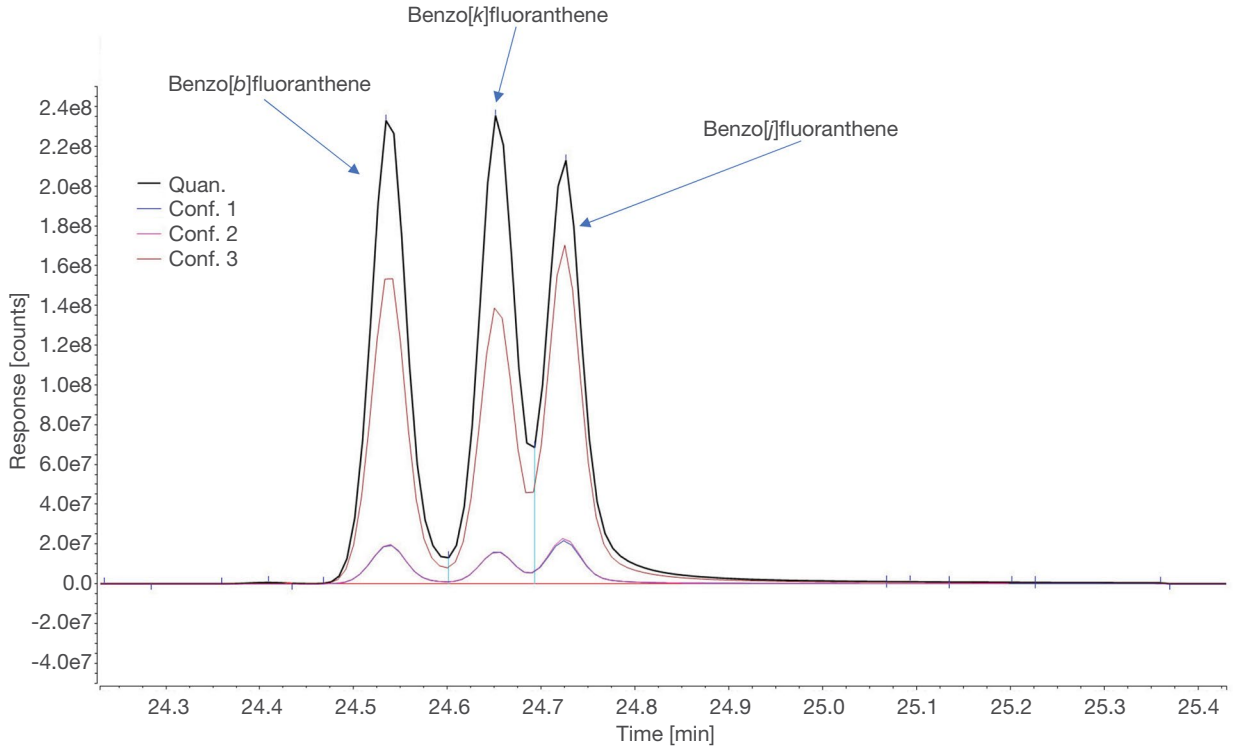

Figure 2. Separation of benzo[*k*]fluoranthene from benzo[*j*]fluoranthene. The resolution calculated by applying the European Pharmacopoeia formula was equal to 1.08.

Linearity and sensitivity

To evaluate the linearity of the method, an eleven-point calibration curve was injected. The concentration range covered in the calibration curve aimed to achieve a linear range of four orders of magnitude between 0.0005 µg/mL and 1.0000 µg/mL. All the analytes were found to show linear behavior in the investigated range. The coefficient of determination ranged between 0.9955 and 0.9996 across all compounds under investigation. Detailed results of the linearity studies can be found in Table 4.

The instrument detection limit reflects the true detection limit of an instrument, based on the precision of a measurement at low analyte levels in solvent-based standard.[6]

The IDL was determined using the following equation:

IDL = t · Concentration · % RSD where:

t is Student's *t*-value for a one-tailed distribution at 99% confidence with 5 degrees (n-1) of freedom corresponding to n=6 injections: *t*=3.365; Concentration is the concentration of the analyte; % RSD is relative standard deviation of the response.

The instrument´s detection limit was assessed from n=6 consecutive solvent standard injections obtained by serially diluting the PAHs standard mix to a concentration of 0.001 µg/mL. The IDLs were calculated applying the equation above and were in the range of 0.0001 to 0.0005 µg/mL as reported in Table 4.

Table 4. Calculated coefficients of determination (R^2) and corresponding linearity ranges

Compound	R^2	Range [µg/mL]	IDL [µg/mL]
5-Methylchrysene	0.9991	0.0005–1.0000	0.0002
Benzo[*a*]anthracene	0.9996	0.0005–1.0000	0.0001
Benzo[*a*]pyrene	0.9981	0.0005–1.0000	0.0001
Benzo[*b*]fluoranthene	0.9995	0.0005–1.0000	0.0001
Benzo[*c*]fluorene	0.9992	0.0005–1.0000	0.0002
Benzo[*g,h,i*]perylene	0.9996	0.0005–1.0000	0.0002
Benzo[*j*]fluoranthene	0.9996	0.0005–1.0000	0.0001
Benzo[*k*]fluoranthene	0.9996	0.0005–1.0000	0.0001
Chrysene	0.9995	0.0005–1.0000	0.0001
Cyclopenta[*c,d*]pyrene	0.9965	0.0005–1.0000	0.0002
Dibenzo[*a,e*]pyrene	0.9987	0.0005–1.0000	0.0005
Dibenzo[*a,h*]anthracene	0.9981	0.0005–1.0000	0.0001
Dibenzo[*a,h*]pyrene	0.9973	0.0005–1.0000	0.0002
Dibenzo[*a,i*]pyrene	0.9977	0.0005–1.0000	0.0002
Dibenzo[*a,l*]pyrene	0.9955	0.0005–1.0000	0.0002
Indeno[1,2,3,*c,d*]pyrene	0.9992	0.0005–1.0000	0.0002

Repeatability

Repeatability was tested at three concentration levels: 0.0010 µg/mL, 0.0200 µg/mL, 0.1000 µg/mL. Each of the vials was injected six times. The stability of the Advanced Electron Ionization ion source allowed for excellent precision. Relative standard deviation of internal standard corrected concentrations exceeded 10% only in one case at the lowest level tested for (0.0010 µg/mL), namely for dibenzo[*a,e*] pyrene. Detailed RSD values are shown in Figure 3.

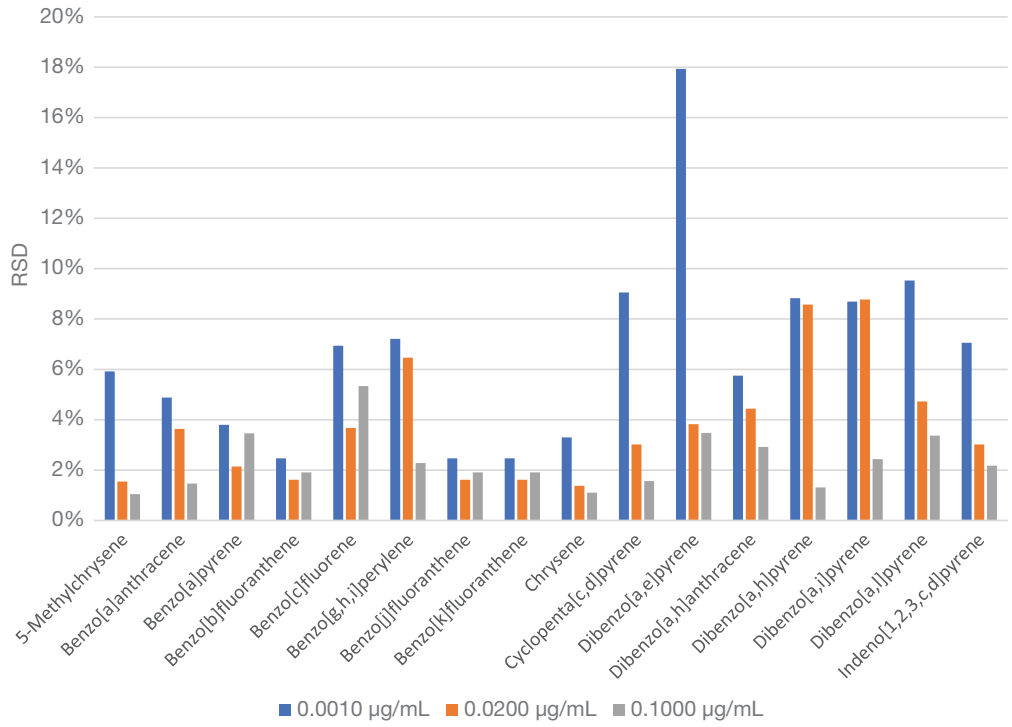

Figure 3. Relative standard deviation (n = 6) internal standard corrected concentrations of 0.1, 0.02, and 0.001 µg/mL

Conclusion

The results described in this technical note clearly demonstrate the performance of the TRACE 1610 GC and TSQ 9610 GC triple quadrupole GC/MS system for sensitive and reproducible PAHs analysis.

- The method provided good chromatographic separation of the critical compounds.
- Detection limits in the range of 0.0001–0.0005 µg/mL were achieved.
- Repeatability was tested at three concentration levels and showed low relative standard deviation.
- The XLXR™ detector assured linear response across four orders of magnitude.

The design of the AEI ion source in combination with NeverVent technology provides laboratories performing PAH analysis with a robust and reliable analytical tool for sample screening at a large scale with reduced interruptions.

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