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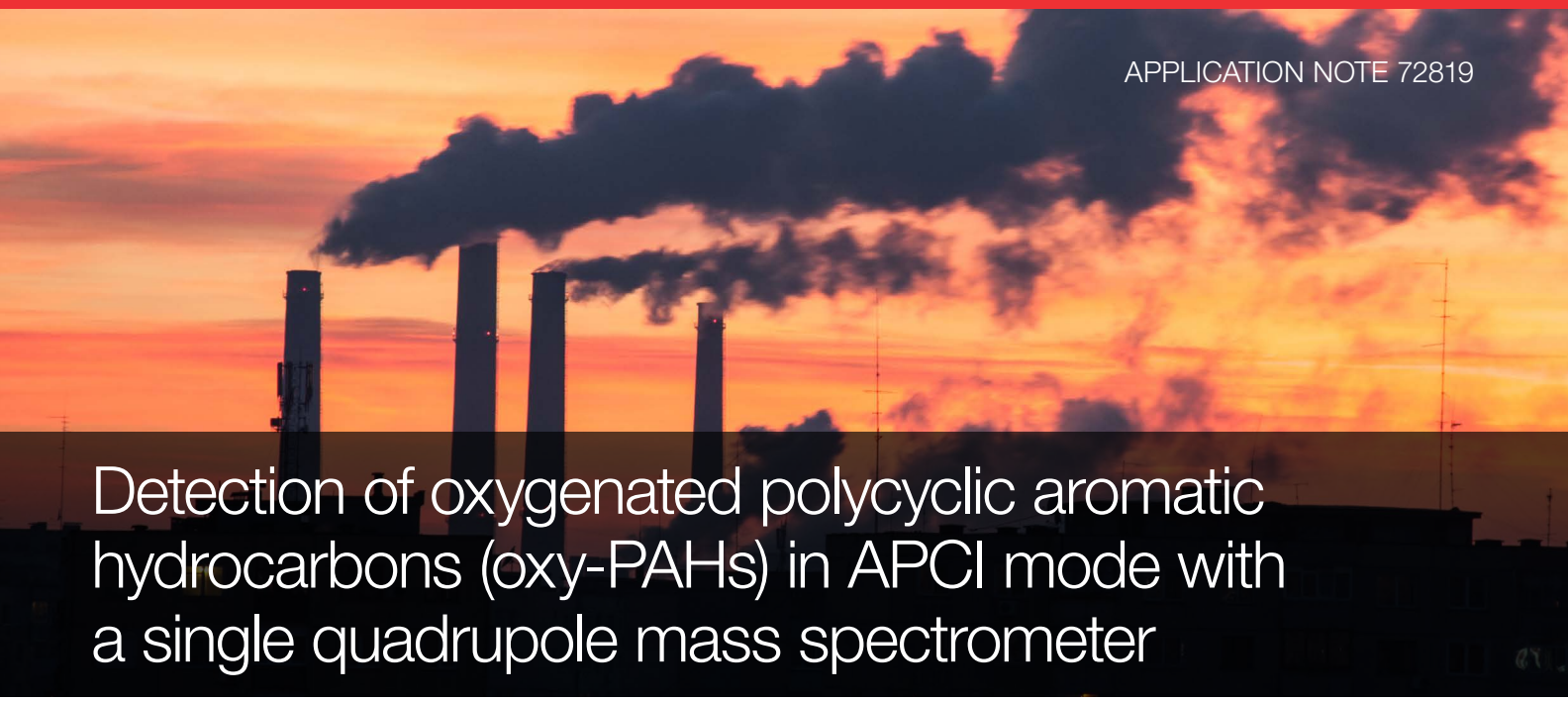
APPLICATION NOTE 72819



Detection of oxygenated polycyclic aromatic hydrocarbons (oxy-PAHs) in APCI mode with a single quadrupole mass spectrometer

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Keywords

APCI, atmospheric pressure chemical ionization, mass spectrometry, single quadrupole mass spectrometer, high-performance liquid chromatography, ESI, electrospray ionization, oxygenated polycyclic aromatic hydrocarbons, oxy-PAHs, ISQ EM, Vanquish Flex UHPLC, LC-MS, UHPLC

Goal

Demonstrate the benefits of APCI-MS detection of oxy-PAHs compared to ESI-MS detection with the Thermo Scientific™ ISQ™ EM single quadrupole mass spectrometer.

Application benefits

- APCI offers an alternative ionization mode to standard ESI for poorly ionizable compounds like oxy-PAHs.
- S/N values are mostly better with APCI and less adducts are formed.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants ubiquitous in water, plants, foods, soils, and the atmosphere.[1-3] These compounds comprise at least two fused aromatic rings and are generated by incomplete combustion processes of anthropogenic and natural origin. PAHs as well as accompanying derivatives like oxygenated PAHs (oxy-PAHs), which are also formed by combustion or in secondary reactions, exhibit severe toxicity and carcinogenicity. Harmfulness combined with inevitable human exposure by dermal adsorption, inhalation, and, most importantly, ingestion give reason for numerous environmental and nutritional studies focusing on the analysis of such compounds in various materials and risk assessment for people and nature.[1-3]

Gas chromatographic separation coupled to mass spectrometry (MS) is a frequently used technique for PAH analysis.[1,3,4] However, polar functional groups decrease the volatility of compounds so that oxy-PAHs are detected with less sensitivity or need to be derivatized.[1] Alternative techniques based

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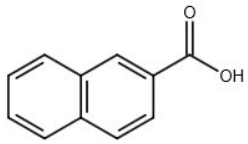
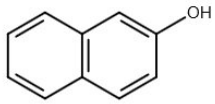
on high-performance liquid chromatography (HPLC) are applied either with optical (UV and/or fluorescence) or MS detection.[4,5] Although electrospray ionization (ESI) is generally the preferred ionization technique in most LC-MS applications, atmospheric pressure chemical ionization (APCI) was shown to be the superior mode in oxy-PAH analysis.[4,6]

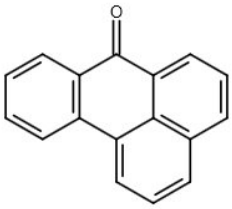
This application note demonstrates the capability of the ISQ EM single quadrupole mass spectrometer to perform APCI-MS detection of six oxy-PAH standard compounds (structures displayed in Figure 1), and a comparison to ESI-MS detection with the same instrument. Separation was performed with a Thermo Scientific™ Vanquish™ Flex Binary UHPLC system and a Thermo Scientific™ Hypersil™ Green PAH LC column with a stationary phase specially tailored for these kinds of analytes.



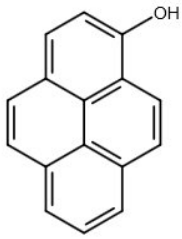
2-naphthoic acid



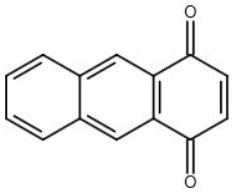
2-naphthol



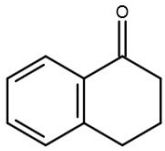
benzanthrone



1-hydroxypyrene



1,4-anthraquinone



1-tetralone

Figure 1. Structures of six oxy-PAHs analyzed in the current work

Experimental

Chemicals

- Deionized water, 18.2 MΩ•cm resistivity or higher (P/N N/A)
- Fisher Scientific™ Methanol, Optima™ LC/MS grade (P/N A456-212)
- Synthesized standards of 2-naphthoic acid, 2-naphthol, benzanthrone, 1-hydroxypyrene, 1,4-anthraquinone, and 1-tetralone were kindly provided by Technical University of Munich (Thomas Letzel)

Instrumentation

A Vanquish Flex Binary UHPLC system equipped with an ISQ EM single quadrupole mass spectrometer was used for the analysis.

- Thermo Scientific™ Vanquish™ System Base Vanquish Horizon/Flex (P/N VF-S01-A-02)
- Thermo Scientific™ Vanquish™ Binary Pump F (P/N VF-P10-A-01)
- Thermo Scientific™ Vanquish™ Split Sampler HT (P/N VH-A10-A-02)
- Thermo Scientific™ Vanquish™ Column Compartment H (P/N VH-C10-A-02)
- Thermo Scientific™ Vanquish™ Variable Wavelength Detector F (P/N VF-D40-A-01)
- Flow Cell Semi-Micro, 2.5 µL, 7 mm light path (SST) (P/N 6077.0360)
- ISQ EM Mass Spectrometer (P/N ISQEM-ESI-APCI)

Standard preparation

Stock solutions of the standard compounds were prepared in methanol at a concentration of 1 mg/mL. A working solution for injection into the UHPLC-MS system was prepared by mixing of stock solutions and further dilution with methanol, giving a final concentration of 10 µg/mL per analyte.

Liquid chromatographic conditions

Column	Hypersil Green PAH, 150 × 2.1 mm, 3 µm (PN 31103-152130)	
Mobile phase	A: Water B: Methanol	
Flow rate	0.35 mL/min	
Gradient	Time (min)	%B
	0	25
	6	100
	8	100
	8.1	25
	11	25
Mixer volume	50 + 150 µL	
Column temperature	40 °C (forced air mode, fan speed 5)	
Autosampler temperature	20 °C	
Injection volume	1 µL	
UV detection	254 nm, 10 Hz, 0.5 s response time	

Mass spectrometry settings in APCI and ESI mode

MS source parameters	*APCI setting*	*ESI setting*
Sheath gas pressure	30 psig	39.3 psig
Aux gas pressure	2 psig	4.4 psig
Sweep gas pressure	0 psig	0.5 psig
Vaporizer temperature	350 °C	200 °C
Ion transfer tube temperature	300 °C	300 °C
Source current/voltage	5 µA/-5 µA	3000 V/-2000 V
MS method parameters		
Method type	Full Scan and SIM scans	
Ion polarity	Positive and negative	
Mass range	*m/z* 100–400	
Dwell time	0.1 s	
Source CID voltage:	10 V	20 V

Data processing

The Thermo Scientific™ Chromeleon™ 7.2.9 Chromatography Data System (CDS) was used for data acquisition and processing.

Results and discussion

In the current work, the performance of MS detection in APCI and ESI mode was compared for six oxy-PAH standard compounds (Figure 1) under otherwise identical conditions. The separation was performed with a dedicated column giving baseline separation (resolution >2) for the test compounds as well as for some impurities visible in the UV chromatogram, which are probably some extant from synthesis (Figure 2).

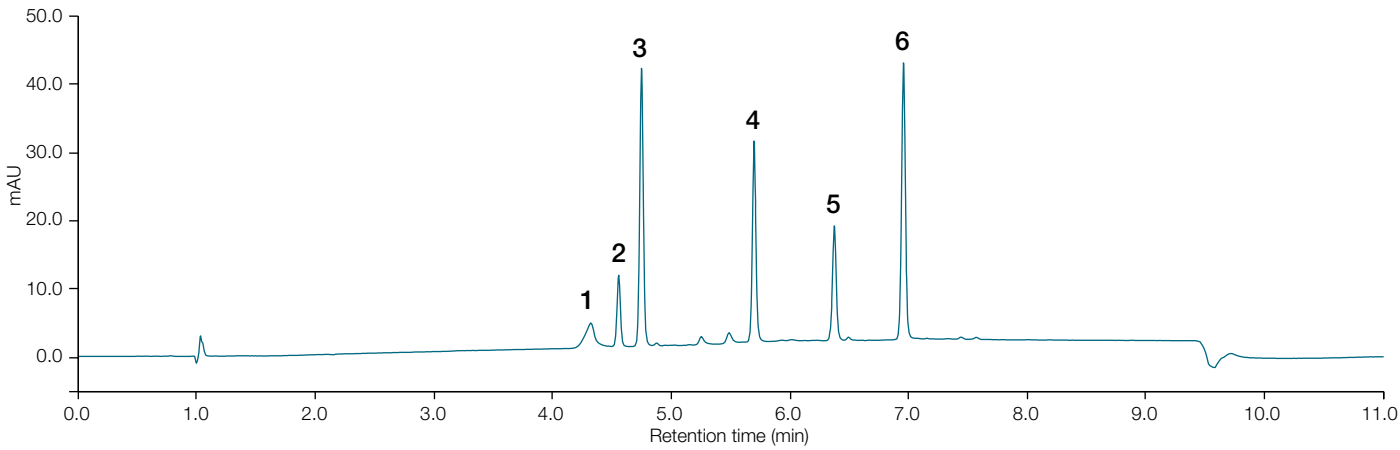


Figure 2. UV chromatogram of oxy-PAH separation (10 ng on column per analyte); for peak assignment see Table 1.

With the ISQ EM mass spectrometer, switching from ESI to APCI is easily done in a few minutes by changing the source probe and turning the APCI needle to the proper position with the respective lever. As the ionization mechanisms in both modes differ explicitly, in some cases the formed ions are not the same.

Table 1 gives an overview on molecular masses and the most abundant *m/z* detected of the six oxy-PAHs under the applied conditions. Most ions are formed as protonated molecular $[M+H]^+$ ions in positive mode and deprotonated molecular $[M-H]^-$ ions in negative mode, as one might expect from ESI-MS experience. However, 1-hydroxypyrene is detected as the molecular $[M]^+$ ion in positive ESI mode and 1,4-anthraquinone and benzanthrone are detected as molecular $[M]^-$ ions in negative APCI mode but are not detected in negative ESI mode. The latter is due to a well-known phenomenon called electron capture negative ionization, which is observed in APCI for molecules that lack an abstractable hydrogen.[6] Figures 3–6 show the spectra in APCI and ESI modes for the analyzed oxy-PAHs. Hydroxypyrene is well detected in both ionization modes and polarities, while 2-naphthoic acid, 2-naphthol, and 1-tetralone are detectable both in APCI and ESI modes but just in one polarity running full scans. With a SIM scan of the $[M+H]^+$ ion, naphthol is additionally detected in positive APCI mode. The $[M+H]^+$ ion of benzanthrone is easily formed in both positive ionization modes, but while APCI induces the $[M]^-$ ion in negative mode, detection is not possible in negative ESI mode. 1,4-anthraquinone provides good signals in positive and negative polarity with APCI, but is not observable in either of the full scans in ESI mode. Only with a SIM scan of the $[M+H]^+$ ion was it detectable as a small signal and thus is only barely detected under ESI conditions with the injected absolute amount of 10 ng on column. In addition to the described molecular ions, some further species, probably adducts, of substantial amounts can be observed in ESI spectra—in negative mode for naphthoic acid (*m/z* 365) and in positive mode for hydroxypyrene (*m/z* 247 and 301) and benzanthrone (*m/z* 253 and 285)—while in APCI mode only a single adduct was seen for tetralone (*m/z* 179).

Table 1. Overview of oxy-PAHs retention, molecular masses, and observed most abundant *m/z*; t_R(UV): retention time at UV detector (see Figure 2), MW: molecular weight, +/-: positive/negative mode, n.d.: not detected. *m/z* in parenthesis were hardly detectable from full scans but detectable in SIM scans, blue shaded fields indicate the best MS detection results for each compound (see also Figure 4).

#	Compound	t_R(UV) [min]	MW [Da]	*m/z* APCI +	*m/z* APCI -	*m/z* ESI +	*m/z* ESI -
1	2-naphthoic acid	4.33	172.2	n.d.	171 $[M-H]^-$	n.d.	171 $[M-H]^-$
2	2-naphthol	4.56	144.2	(145) $[M+H]^+$	143 $[M-H]^-$	n.d.	143 $[M-H]^-$
3	1-tetralone	4.75	146.2	147 $[M+H]^+$	n.d.	147 $[M+H]^+$	n.d.
4	1,4-anthraquinone	5.70	208.2	209 $[M+H]^+$	208 $[M]^-$	(209) $[M+H]^+$	n.d.
5	1-hydroxypyrene	6.37	218.3	219 $[M+H]^+$	217 $[M-H]^-$	218 $[M]^+$	217 $[M-H]^-$
6	benzanthrone	6.95	230.3	231 $[M+H]^+$	230 $[M]^-$	231 $[M+H]^+$	n.d.

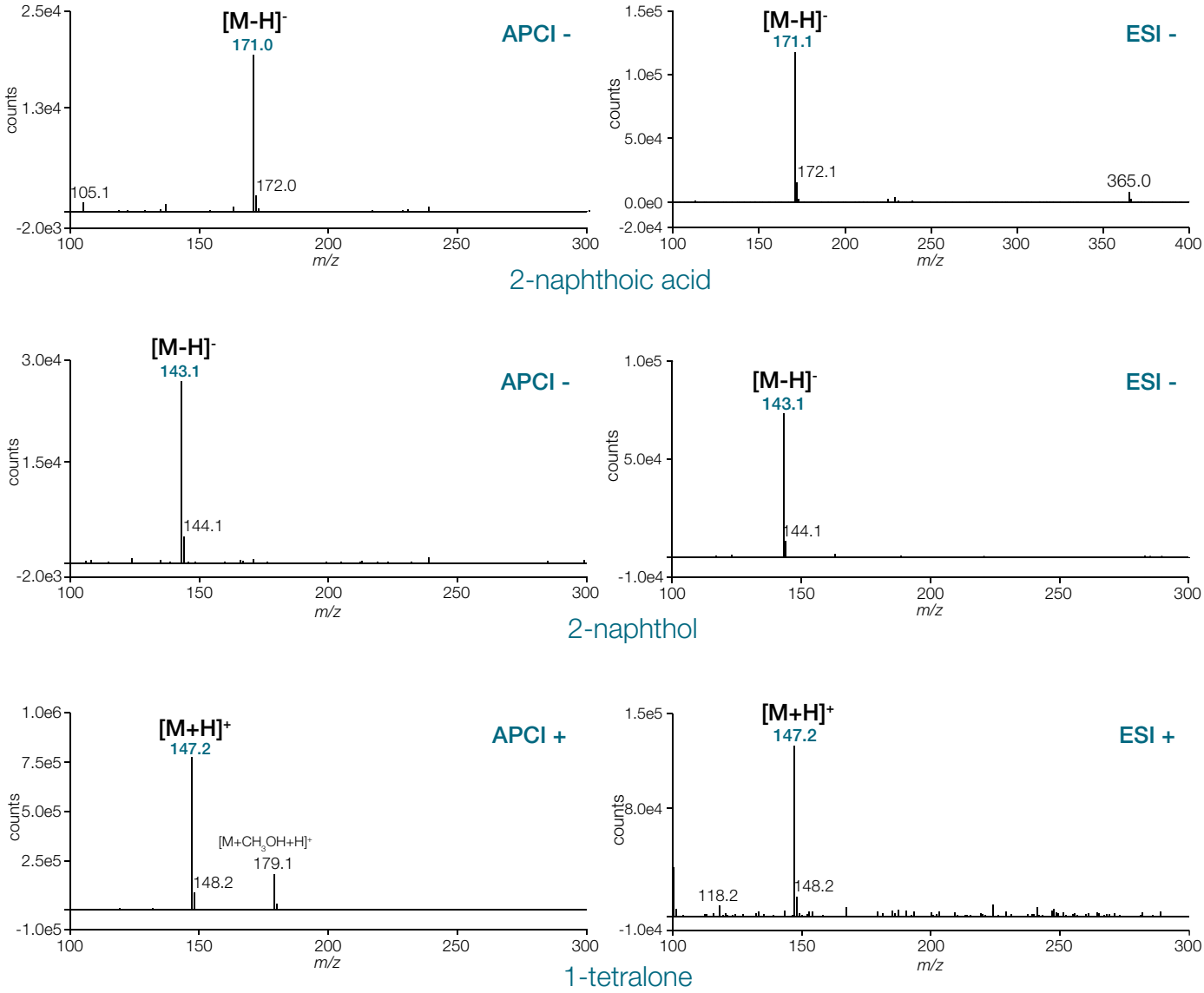


Figure 3. Background subtracted mass spectra for three analyzed oxy-PAH compounds in positive (+) and negative (-) APCI and ESI mode; most abundant *m/z* ions are marked; background spectra were obtained from blank areas next to the corresponding peaks.

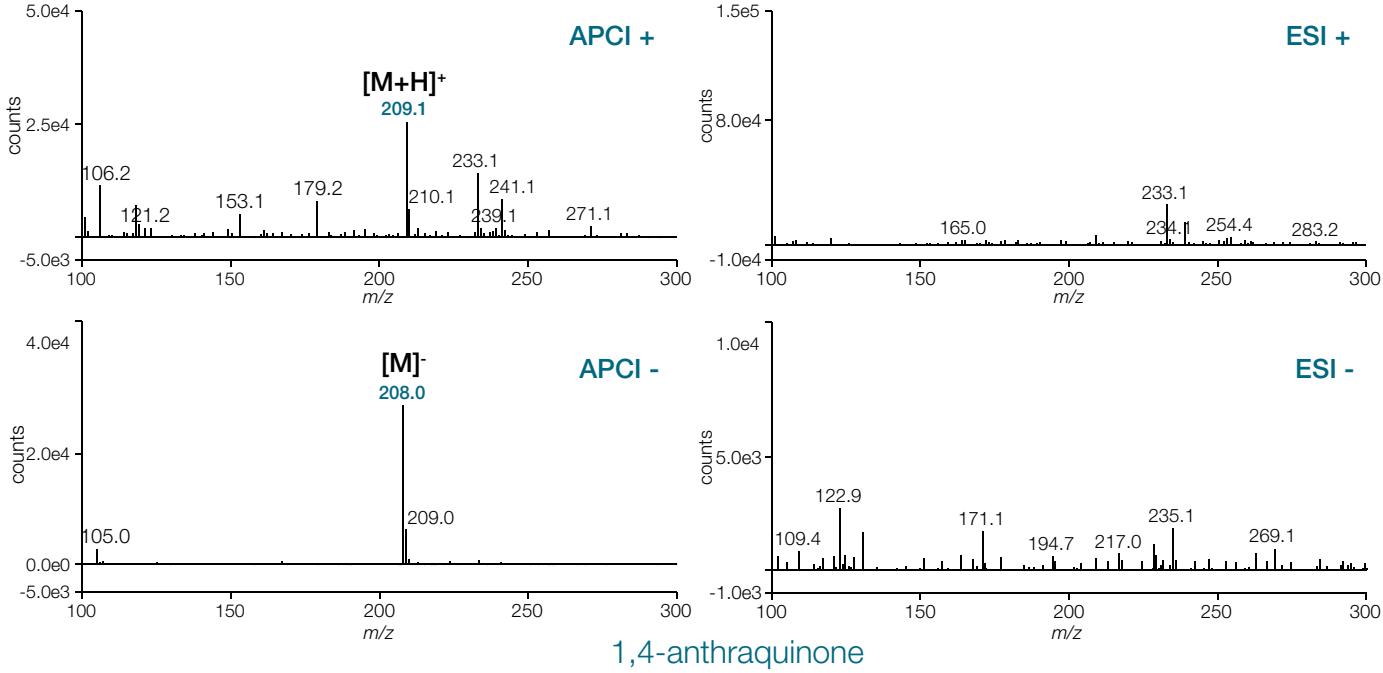


Figure 4. Background subtracted mass spectra for 1,4-anthraquinone in positive (+) and negative (-) APCI and ESI mode; most abundant *m/z* ions are marked; background spectra were obtained from blank areas next to the corresponding peaks.

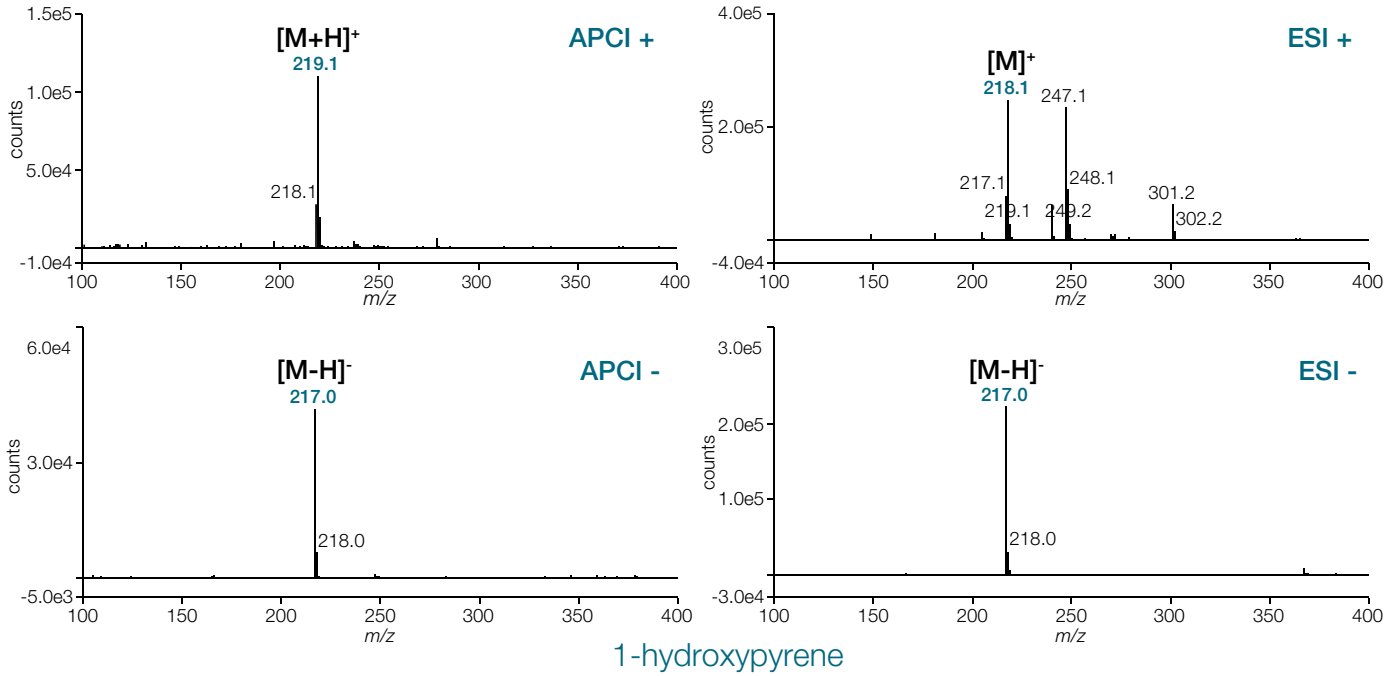


Figure 5. Background subtracted mass spectra for 1-hydroxypyrene in positive (+) and negative (-) APCI and ESI mode; most abundant *m/z* ions are marked; background spectra were obtained from blank areas next to the corresponding peaks.

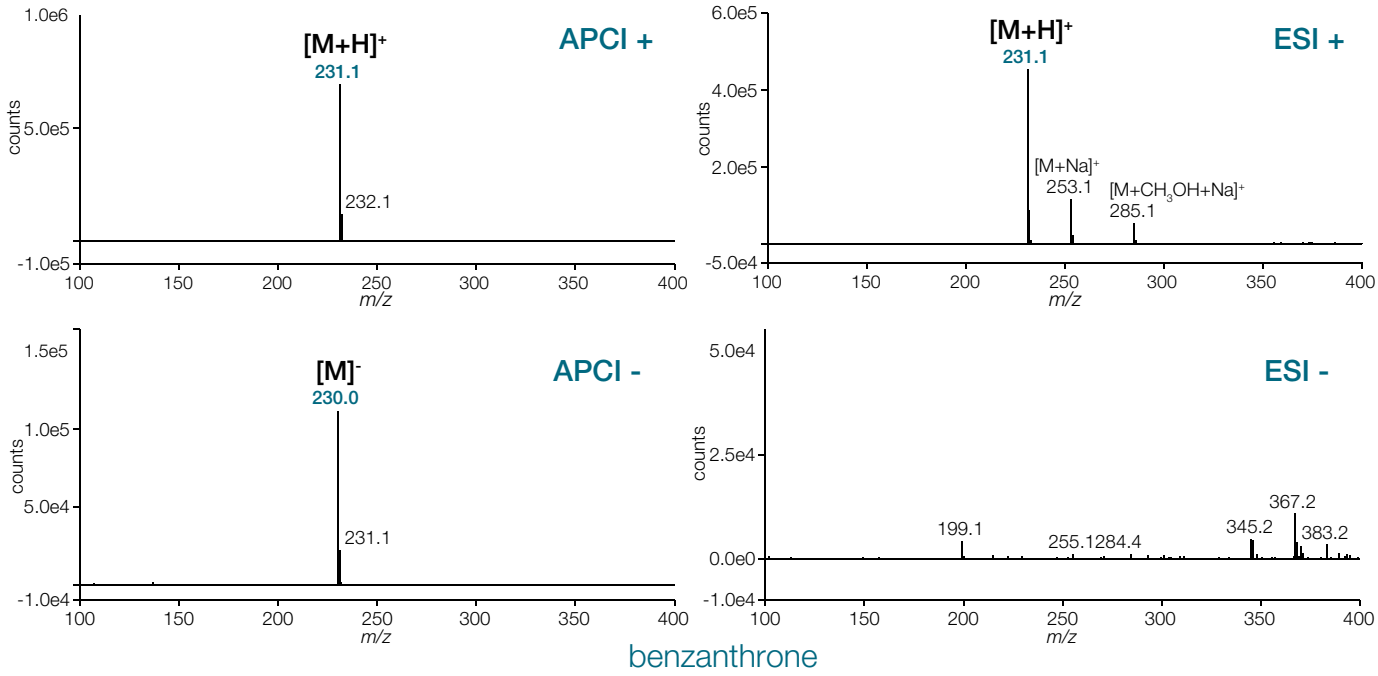


Figure 6. Background subtracted mass spectra for benzanthrone in positive (+) and negative (-) APCI and ESI mode; most abundant *m/z* ions are marked; background spectra were obtained from blank areas next to the corresponding peaks.

A comparison of MS peak areas and signal-to-noise ratios (S/N) of the most abundant *m/z* is given in Figure 7. From part C and D of Figure 7 it becomes clear that ESI in negative polarity exhibits some benefits over APCI in terms of sensitivity. Peak areas for the three compounds detected in ESI negative mode are higher than in APCI negative mode. However, S/N is only higher for two of these three components, and with APCI two more analytes were detected in negative mode. Parts A and B of the same figure showcase a strong advantage of APCI in positive mode. Signal responses are similar to or higher than with ESI but S/N is better for all detected compounds in APCI. In summary, APCI should be preferred over the standard ESI mode for these types of analyses.

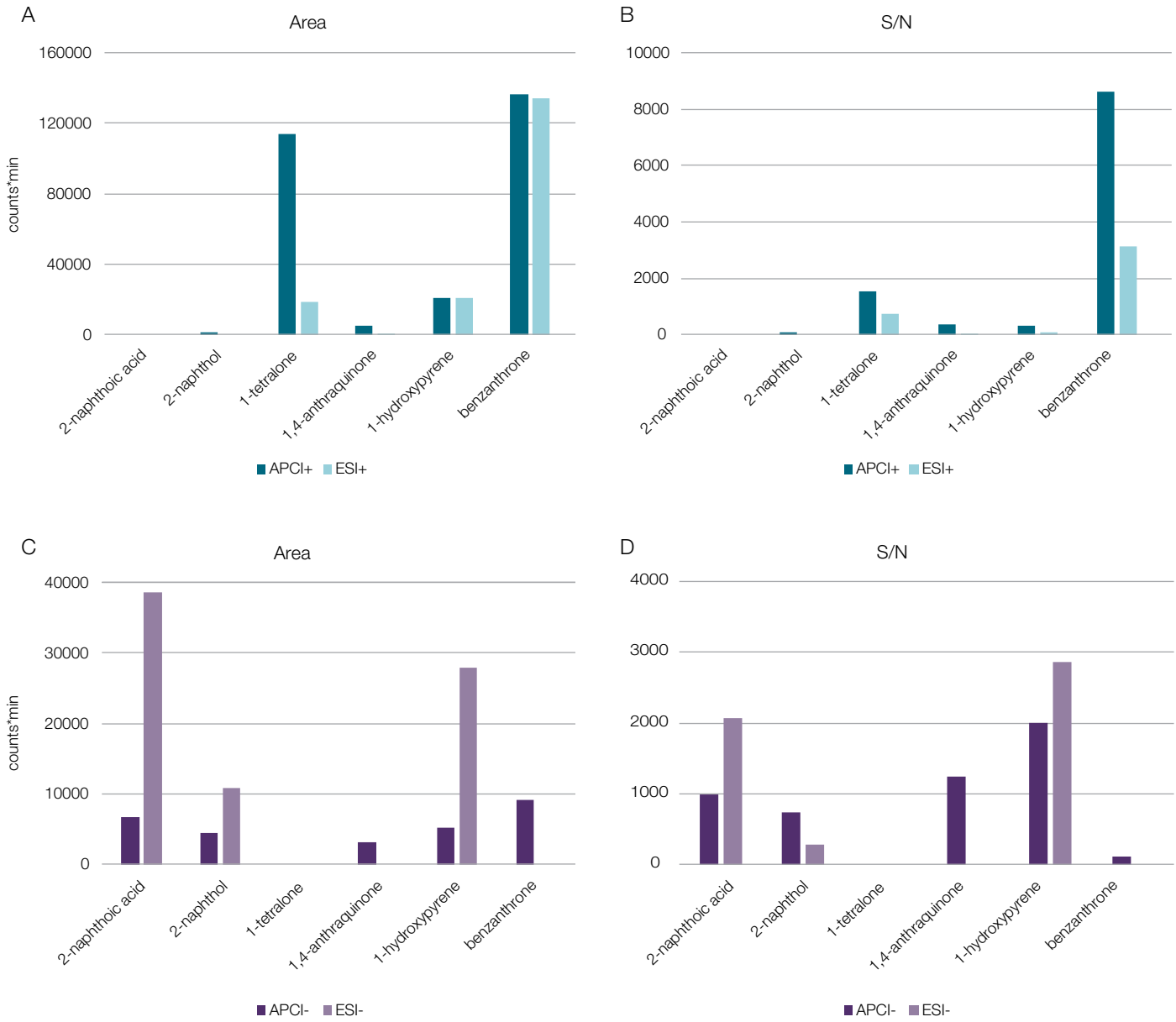


Figure 7. Comparison of MS peak areas and S/N in positive (+) and negative (-) APCI and ESI mode for the six oxy-PAHs

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Conclusions

Although standard ESI mode showed higher detection sensitivity for some analytes in negative polarity, APCI mode was the preferred MS mode in the current application because of the following:

- All six oxy-PAHs were easily detected in APCI mode, while in ESI mode one compound was barely detectable with a S/N≈12.
- Molecules with a lack of acidic hydrogen, which were not ionizable by negative ESI, could be ionized by electron capture in negative APCI.
- S/N values were better with APCI for most analytes.
- Fewer adducts were formed in APCI mode.

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