Analysis of PAH Compounds Using LC/Single Quadrupole MS with a Field Free APCI Source

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1 Overview

Purpose – A study was conducted to compare sample throughput and detection limits between conventional reverse phase HPLC/MS and UHPLC/MS using a novel "field free" APCI source. The study was performed on a 16 component mixture of PAHs.

Methods – A recently introduced PerkinElmer "field free" APCI source was interfaced to a PerkinElmer Flexar™ FX-10 UHPLC system coupled to a Flexar SQ 300 MS detector.

Results – Going from a 5 µm to a 1.9 µm particle size improved the detection limits by an average factor of 6.5 for the 16 component PAH mixture and decreased the total run time by a factor of 3. The detection limits varied from 1.5 ng to 6.0 pg.

2 Introduction

Methods for LCMS analysis of polycyclic aromatic hydrocarbons (PAHs) were developed using conventional reverse phase UHPLC coupled to a single quadrupole mass spectrometer featuring a proprietary "field free" Atmospheric Pressure Chemical Ionization (APCI) source. PAHs are a group of semi-volatile organic compounds that consist of one or more aromatic rings. They are common byproducts of fossil fuel combustion which lead to atmospheric pollutants. These non-polar compounds are usually not ionizable by ESI and are of interest since they have been identified as carcinogenic, mutagenic and teratogenic. In this work, a comparison between conventional reverse phase HPLC/MS to UHPLC/MS was performed to determine the sample throughput and detection limits for a 16 component mixture of PAHs.

3 Methods

A field free APCI source was interfaced to a PerkinElmer Flexar FX-10 UHPLC system and coupled to a Flexar SQ 300 MS detector. A PerkinElmer Brownlee Analytical DB PAH Column: 3.2 X 150 mm, 5 µm, was used for the conventional reverse phase LC. A sixteen component mixture of various PAHs was injected on-column at a solvent flow rate of 1.0 ml/min. A gradient was run from 50% to 60% ACN in water for 6 minutes, from 60% to 100% ACN over 6 minutes and then 100% ACN for an additional 6 minutes. For the UHPLC analysis, a PerkinElmer Brownlee Analytical DB PAH Column: 2.1 X 50 mm, 1.9 µm, was run at a solvent flow rate of 0.6 ml/min. The same PAH mixture was injected and a gradient was run from 50% to 100% ACN over 5 minutes then held at 100% ACN for 1 minute. The optimized field free APCI source parameters used were held constant for both the conventional reverse phase LC and the UHPLC separations. The corona current was set to 5 µA at the APCI vaporizer temperature was set to 350°C.

Atmospheric Pressure Chemical Ionization Source

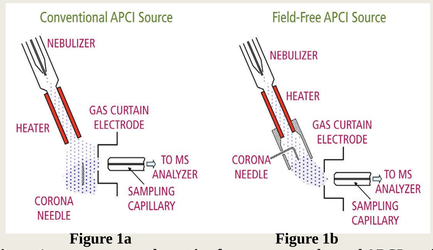

Figure 1a Figure 1b

Figure 1 : Figure 1a represents a schematic of most commonly used APCI configuration. Note that the heated gas exiting the vaporizer assembly experiences rapid expansion minimizing the exposure of the analyte to the corona discharge needle. Figure 1b illustrates the Field Free APCI configuration where the corona discharge needle is contained within the grounded vaporizer assembly shielding the needle electric field. This configuration allows the high electric field in the corona discharge region to be separated from the ion focusing region into vacuum.

Chemical Structures of the 16 Polyaromatic Hydrocarbons

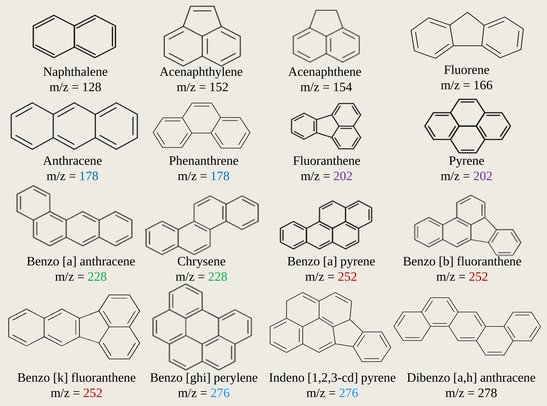

Figure 2 : The chemical structures of the 16 different PAH's are shown that were separated by conventional reverse phase HPLC and UHPLC before analysis by a field free APCI source coupled to a Flexar SQ 300 MS detector.

4 Results

Conventional LC/MS Acquisition Parameters

Analyte	Ion Type	Ion (m/z)	Capillary Exit Voltage/V	Dwell Time/ms	Retention Window/min	Retention Time/min
Naphthalene	M+	128	120	100	0-8.2	4.83
Acenaphthylene	M+	152	200	100	0-8.2	5.80
Acenaphthene	M+	154	110	100	0-8.2	7.40
Fluorene	M+	166	110	100	0-8.2	7.75
Anthracene	M+	178	170	100	8.2-16.5	8.61
Phenanthrene	M+	178	170	100	8.2-16.5	9.32
Fluoranthene	M+	202	180	100	8.2-16.5	10.02
Pyrene	M+	202	180	100	8.2-16.5	10.49
Benzo[a] anthracene	M+	228	180	100	8.2-16.5	11.87
Chrysene	M+	228	180	100	8.2-16.5	12.15
Benzo [a] pyrene	M+	252	200	100	8.2-16.5	13.09
Benzo [b] fluoranthene	M+	252	200	100	8.2-16.5	13.51
Benzo [k] fluoranthene	M+	252	200	100	8.2-16.5	13.98
Benzo [ghi] perylene	M+	276.1	200	100	8.2-16.5	15.32
Indeno [1,2,3-cd] pyrene	M+	276.1	200	100	8.2-16.5	15.74
Dibenzo [a,h] anthracene	M+	278.1	180	100	8.2-16.5	14.68

Figure 3 : Acquisition parameters used for the conventional HPLC/MS separation. In order to find the retention times of the 16 PAH compounds the mass spectrometer was operated in scan mode.

Conventional LC/MS Chromatogram of a 16 Component PAH Mixture

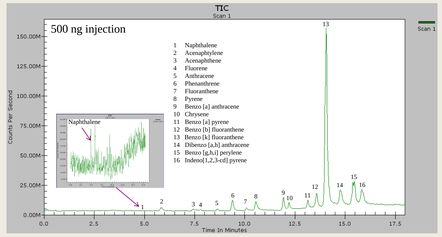

Figure 4 : The total ion chromatogram for the conventional HPLC/MS separation is shown. In this experiment 500 ng was injected on column. A single analysis required 18 minutes of run time.

UHPLC/MS Acquisition Parameters

Analyte	Ion Type	Ion (m/z)	Capillary Exit Voltage/V	Dwell Time/ms	Retention Window/min	Retention Time/min
Naphthalene	M+	128	120	50	0-3.2	1.23
Acenaphthylene	M+	152	200	50	0-3.2	1.48
Acenaphthene	M+	154	110	50	0-3.2	1.85
Fluorene	M+	166	110	50	0-3.2	1.95
Anthracene	M+	178	170	50	0-3.2	2.21
Phenanthrene	M+	178	170	50	0-3.2	2.43
Fluoranthene	M+	202	180	50	0-3.2	2.73
Pyrene	M+	202	180	50	0-3.2	2.90
Benzo[a] anthracene	M+	228	180	75	3.2-6.0	3.57
Chrysene	M+	228	180	75	3.2-6.0	3.66
Benzo [a] pyrene	M+	252	200	75	3.2-6.0	4.26
Benzo [b] fluoranthene	M+	252	200	75	3.2-6.0	4.47
Benzo [k] fluoranthene	M+	252	200	75	3.2-6.0	4.66
Benzo [ghi] perylene	M+	276.1	200	75	3.2-6.0	5.30
Indeno [1,2,3-cd] pyrene	M+	276.1	200	75	3.2-6.0	5.43
Dibenzo [a,h] anthracene	M+	278.1	180	75	3.2-6.0	5.10

Figure 5 : The acquisition parameters used for the UHPLC/MS separation. The retention times of the 16 PAH compounds were determined using scan mode. Note that the retention times for all 16 components are considerably shorter when compared to the separation using conventional HPLC. In addition the pulse counting dwell times were shortened to increase the sampling rate necessary for narrower eluting peaks do to the use of the 1.9 µm particle size column.

UHPLC/MS Chromatogram of a 16 Component PAH Mixture

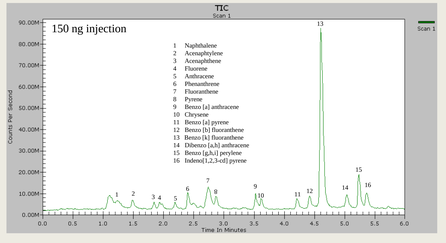

Figure 6 : The total ion chromatogram for the UHPLC/MS separation. Note that the peak widths are narrower when compared to the conventional HPLC/MS separation. This allowed a lower injection volume of 150 ng as compared to 500 ng due to the increase in sensitivity with the UHPLC method. In addition, the total run time to achieve the separation was decreased by a factor of 3 increasing the sample throughput time.

Mass Spectrum of Benzo[k]fluoranthene

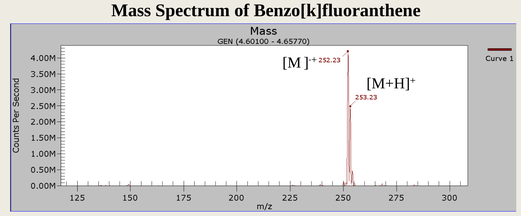

Figure 7 : Mass spectrum for benzo[k] fluoranthene. Note that two different species were detected, one being the molecular radical cation and the other being the protonated species. For all PAH's analyzed, the radical cation was the most intense ion detected. Consequently, the radical cation was used to acquire all SIM data in order to determine the detection limits and linearity response of the PAH's.

Linearity Response for Benzo[k]fluoranthene (0.05 ng-500 ng)

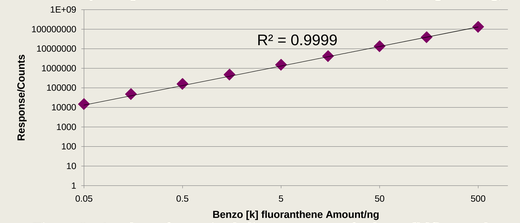

Figure 8 : 4 orders of magnitude linear response for benzo[k]fluoranthene is demonstrated with the SQ 300 MS operating in SIM mode.

On-Column Detection Limits

Analyte	HPLC SIM Detection Limit (ng)	UHPLC SIM Detection Limit (ng)	Decrease in Detection Limit
Naphthalene	15.015	0.459	32.7 X
Acenaphthylene	2.492	0.390	6.4 X
Acenaphthene	4.144	0.285	14.5 X
Fluorene	4.288	1.502	2.9 X
Anthracene	1.502	0.258	5.8 X
Phenanthrene	0.291	0.111	2.6 X
Fluoranthene	0.601	0.099	6.1 X
Pyrene	0.180	0.060	3.0 X
Benzo[a] anthracene	0.366	0.075	4.9 X
Chrysene	0.655	0.105	6.2 X
Benzo [a] pyrene	0.661	0.150	4.4 X
Benzo [b] fluoranthene	0.372	0.123	3.0 X
Benzo [k] fluoranthene	0.021	0.006	3.5 X
Benzo [ghi] perylene	0.051	0.021	2.4 X
Indeno [1,2,3-cd] pyrene	0.111	0.036	3.1 X
Dibenzo [a,h] anthracene	0.114	0.048	2.4 X

Figure 9 : The SIM detection limits for HPLC/MS and UHPLC/MS analyses are presented. Note that increased sensitivity was achieved with increasing molecular weight.

5 Conclusions

For the conventional HPLC/MS analysis, all sixteen PAHs were separated with detection limits ranging between 15 ng to 21 pg. To complete this experiment a total run time of 18 minutes was needed. For the UHPLC/MS analysis the on-column detection limits achieved were between 1.5 ng to 6 pg. In order to complete one UHPLC/MS run, a total run time of 6 minutes was needed. From these experiments the results showed that going from a 5 µM particle size to a 1.9 µm particle size can lower detection limits substantially with a significant increase in throughput. The detection limits were decreased by an average factor of 6.5 for the 16 components, and the total run time for the analysis was decreased by a factor of 3.