HALO®

TECHNICAL REPORT: AMT-TR062003

TITLE: **ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS IN GRILLED MEAT BY UHPLC/MS/MS**

MARKET SEGMENT: FOOD/BEVERAGE

AUTHOR:
Andrew Harron Ph.D., Application Scientist

ABSTRACT

A LCMS method was developed for the analysis of polycyclic aromatic hydrocarbons in meat samples following QuEChERS extraction. The HALO® PAH column demonstrated its performance and reliability by resolving 6 sets of isomeric PAHs in under 8 minutes. In addition, charbroiled meat samples (chicken and beef) were extracted and analyzed for PAH contamination, revealing quantitatible levels of PAHs in cooked steak.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs), formed from the incomplete combustion of fossil fuels, have been detected in foodstuffs throughout the world. In food, PAHs may be formed during industrial processing and domestic food preparation, such as barbecuing, smoking, drying, roasting, baking, frying or grilling. They can also enter food supply chains through contaminated air and water, and accumulate in various animals of food chains, such as livestock and fisheries. Listed as a confirmed human carcinogen by the US EPA, extensive exposure to PAHs by humans and animals has resulted in the development of cancer. PAHs, and various alkylated derivatives have a number of isomers and traditionally are analyzed by GC or GC-MS. However, because these isomers have to be chromatographically separated, the instrument run time is very long.

The analysis of PAHs by LCMS is challenging due to their low polarity and low solubility in aqueous environments, often requiring special post column derivation before introduction into the mass spectrometer. However, LCMS offers the ability for faster run times, provided that the isomeric species can be adequately separated. Fused-Core® technology is an ideal candidate to be used for PAH analysis, due to their high resolution and high throughput capabilities. Here we present an LCMS method using Fused-Core® technology to separate 18 PAHs in under 8 minutes and in addition the screening and quantitation of PAH compounds in cooked meat samples.

KEY WORDS:

PAHs, LCMS, Meat extraction, food safety, HALO® PAH column, superficially porous particles, Fused-Core® technology.

EXPERIMENTAL

PAH Standards, LCMS grade ACN, and QuEChERS kits were obtained from Millipore Sigma. Formic acid was obtained from Thermo Fisher. Charbroiled steak and chicken samples were prepared and extracted via the QuEChERS method. The column used was a HALO 90 Å PAH, 2.7 µm, 2.1 x 100 mm column (Advanced Materials Technology, Wilmington DE). Samples were run on a Shimadzu Nexera (Shimadzu Scientific Instruments, USA) coupled to an LTQ Velos Pro Orbitrap (Thermo Fisher Scientific, Bremen, Germany).

TEST CONDITIONS

Column: HALO 90 Å PAH, 2.7 µm, 2.1 x 100 mm
Part Number: 92842-612
Mobile Phase A: Water/0.1% formic acid
B: Acetonitrile/0.1% formic acid
Flow Rate: 0.4 mL/min
Gradient:

Time	%B
0.0	40
5.0	100
8.0	100
8.01	40

Pressure: 289 bar
Column Temperature: 30 °C
Injection Volume: 1 µL
Sample Solvent: Methanol
LC System: Shimadzu Nexera

MASS SPECTROMETRY CONDITIONS

Source Conditions	
ESI voltage	5.5 kV
Heater Temp	400 °C
Sheath gas (arbitrary units)	35
Aux gas (arbitrary units)	8
Tube lens voltage	40 V

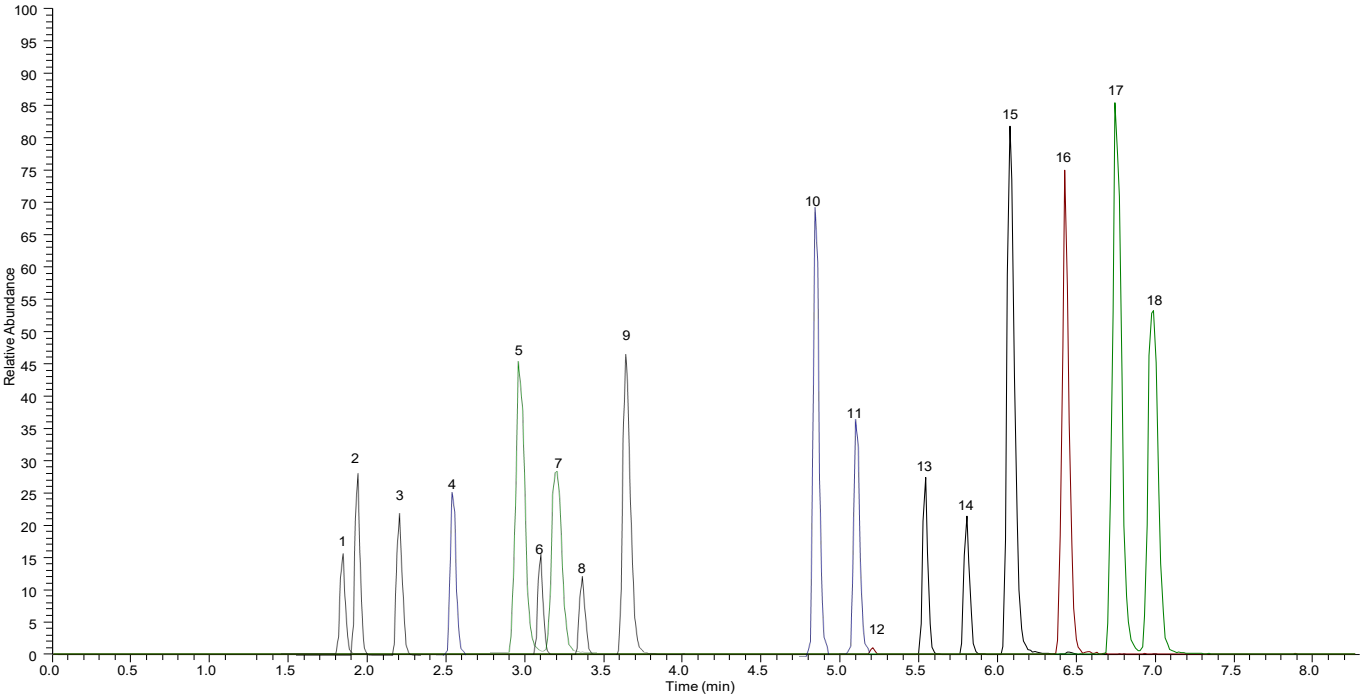


Figure 1. TIC of a high resolution separation of 18 PAHs in under 7.5 minutes

RESULTS

Using the HALO® PAH column 18 PAHs were able to be separated in under 7.5 minutes. Notice the clear separation of 6 sets of isomeric compounds (**Figure 1**). Table 1 shows the identities and elution order of the 18 compound mixture. Included in this table is a color code to show the isomeric compounds that were easily resolved by the HALO® PAH column, showing both high resolution and high throughput, which is a standard of HALO® performance.

Table 1. Peak identities and elution order

Compound	Precursor ion	Fragment 1	Fragment 2	Peak elution number
Naphthalene	128	78	102	1
Acenaphthylene	152	126	151	2
1-Methylnaphthalene	142	89	115	3
2-Methylnaphthalene	142	115	141	4
Acenaphthene	154	126	153	5
Fluorene	166	115	165	6
Phenanthrene	178	151	176	7
Anthracene	178	152	176	8
Fluoranthene	202	150	200	9
Pyrene	202	150	200	10
Benzo[a]anthracene	228	150	226	11
Chrysene	228	200	226	12
Benzo[b]fluoranthene	252	224	250	13
Benzo[k]fluoranthene	252	224	250	14
Benzo[a]pyrene	252	224	250	15
Dibenzo[a,h]anthracene	278	248	276	16
Benzo[ghi]perylene	276	248	274	17
Indeno[1,2,3-cd]pyrene	276	246	274	18

In order to test the performance and reliability of the HALO® PAH column, a screening and quantitation experiment was performed in which meat samples were investigated for PAH contamination following cooking. Charbroiled steak and chicken samples were obtained and then subjected to QuEChERS extraction. The extracted samples were then dried down, reconstituted in methanol and injected. No PAHs were detected in the chicken sample, in both the raw sample (data not shown) and in the cooked sample (**Figure 2**).

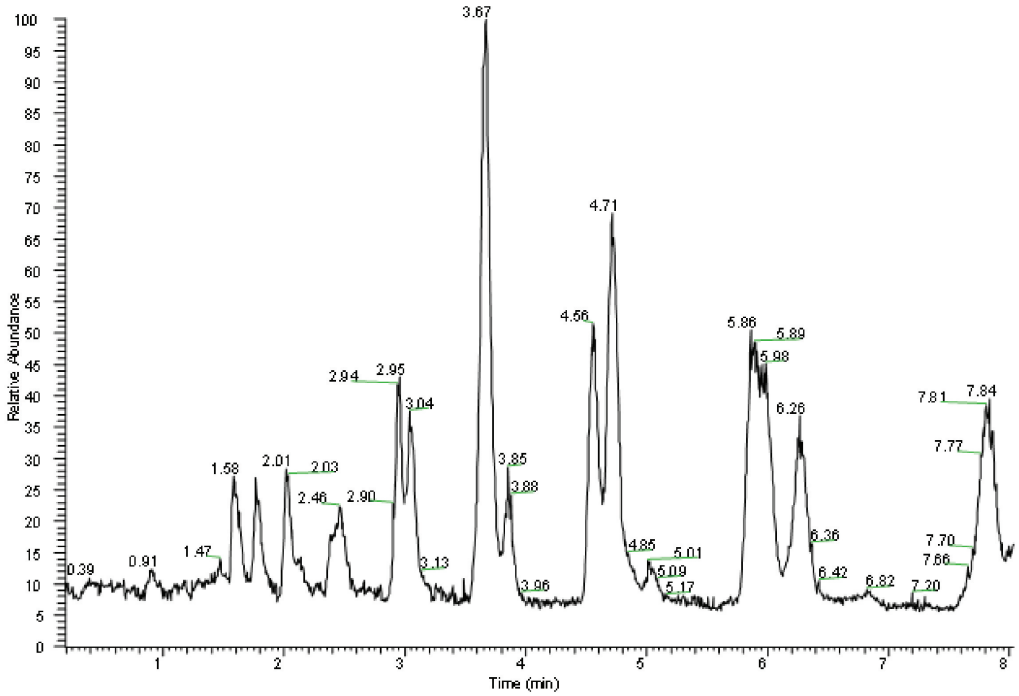


Figure 2. TIC of complex meat matrix of charbroiled chicken showing no PAHs detected

The steak sample, however, did indeed show evidence of PAH contamination (**Figure 3**), with chrysene and benzo[a]pyrene, found at detectable levels in the cooked steak sample.

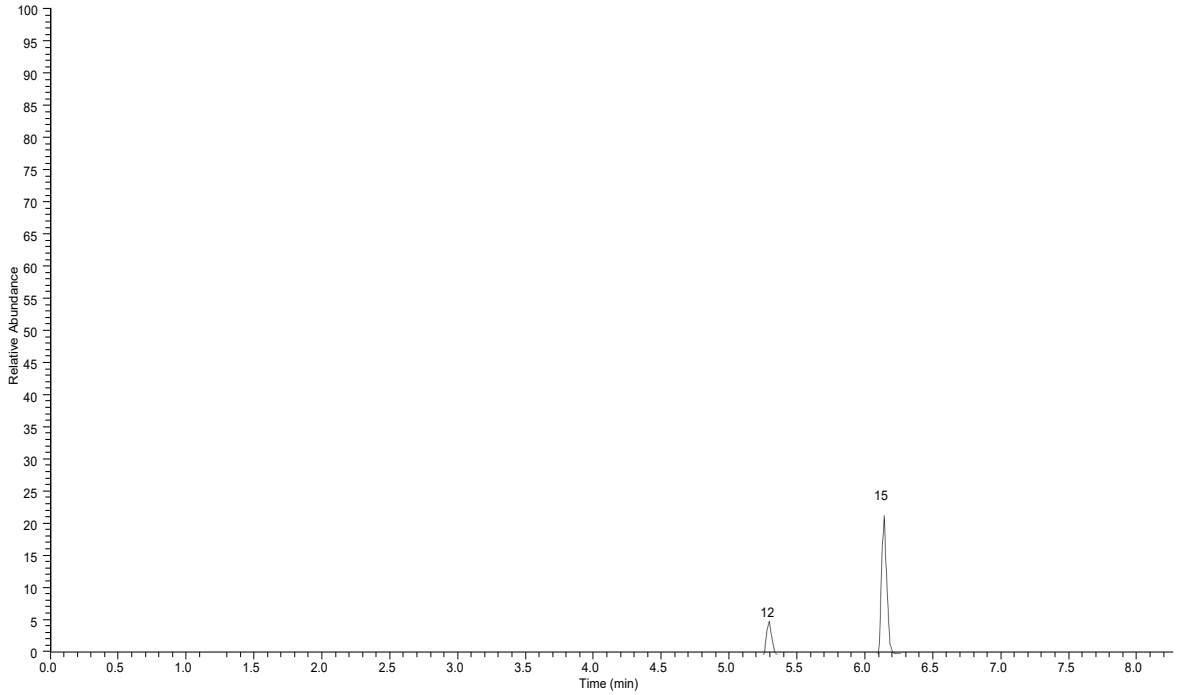


Figure 3. Extracted ion chromatogram of the extracted steak sample showing PAH contamination of chrysene and benzo[a]pyrene.

In order to determine the level of PAH contamination in the steak, a calibration curve was prepared at concentrations from 1 ppb-100 ppb, and the level of PAHs was quantified in the sample, with 1 ppb being the lower limit of detection.

Figure 4. Calibration curve of benzo [a] pyrene

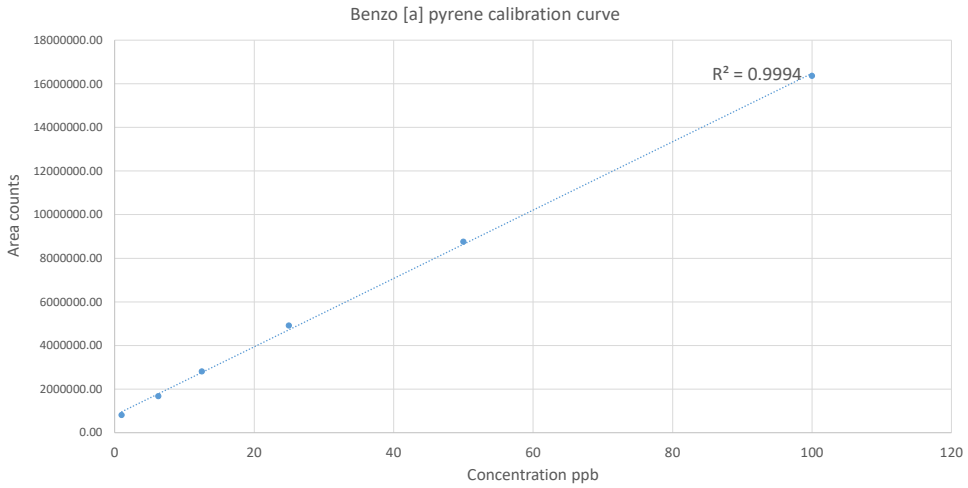
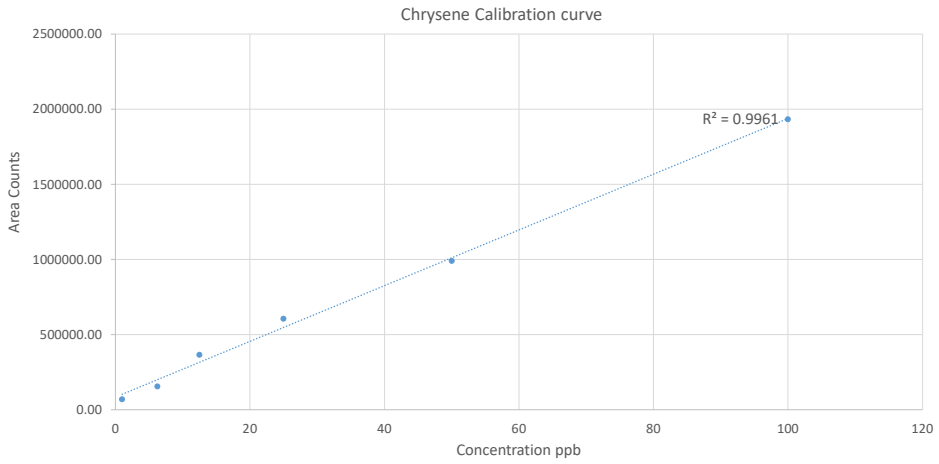


Figure 5. Calibration curve of chrysene



The concentration of benzo[a]pyrene was found to be 1.98 ppb and 2.55 ppb for chrysene (**Figures 4,5**). Smoke that is generated from fat that drips into the grill is the most likely explanation of the presence of these two PAHs. The EPA offers limited guidance on the maximum allowable limit of PAHs in food, however other entities such as the European Union have established limits. Maximum limits have been set by Commission Regulation (EC) No 1881/2006 for PAHs in key foodstuffs. The limit for cooked meat is 5 ppb for meat, and these results fall below those limits[1-3].

CONCLUSION

The HALO® PAH column continues in the tradition of HALO® products by offering high resolution separations, in high throughput time frames. 18 PAH compounds with 6 sets of isomeric compounds were able to be quickly and efficiently resolved in under 8 minutes. In addition, the high resolution separation of the HALO® PAH column, enabled chrysene and benzo [a] pyrene to be resolved from a complex meat matrix, enabling quantitation of PAH contamination present in barbequed steak. The concentration of PAHs in the sample, were below those established by the EU, and demonstrates that not only can the HALO® PAH column be used in the stringent regulatory testing of current established methods, but also be relied upon as future regulations dictate the establishment of new methods, requiring lower limits of detection. The HALO® PAH column offers a rugged and reproducible particle design meeting the needs of complex matrix testing. Fused-Core® technology is ideal for PAH analysis in particular, enabling customers to achieve analytical goals of speed, accuracy, and precision LC separations.

REFERENCES:

1. *Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs, as amended by Regulations 1126/2007 and 629/2008*
2. *Commission Regulation (EU) No 1327/2014 of 12 December 2014 amending Regulation (EC) No 1881/2006 as regards maximum levels of polycyclic aromatic hydrocarbons (PAHs) in traditionally smoked meat and meat products and traditionally smoked fish and fishery products*
3. *Regulation (EC) No 2065/2003 of the European Parliament and of the Council of 10 November 2003 on smoke flavourings used or intended for use in or on foods*