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Extraction and Analysis of Polycyclic Aromatic Hydrocarbons in Infant Formula

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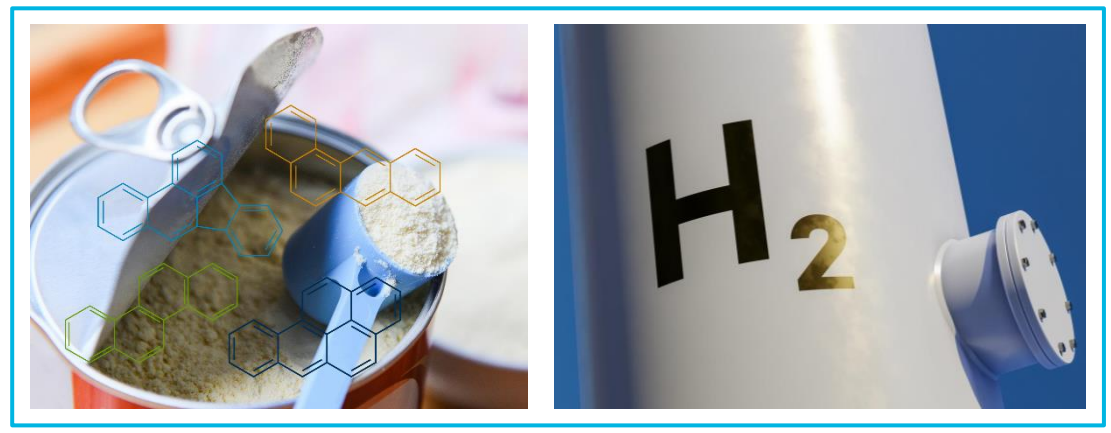
Introduction

PAH Exposure

One of the common ways for humans to encounter PAH exposure is through food consumption. Several countries have drafted legislation to establish tolerable limits for PAHs in foods, food products, and beverages, as well as to enforce monitoring strategies for the most relevant compounds.[2] Furthermore, regulatory agencies such as the World Health Organization (WHO) and the European Commission (EC) have launched regulations to decrease the concentration of PAHs in food, especially through strategies to control the processes that induce their formation.[2]

There is particular concern about the levels of PAHs in infant formula. The EC defines infants as "children under the age of 12 months," and infant formula as "food used by infants during the first months of life and satisfying by themselves the nutritional requirements of such infants until the introduction of appropriate complementary feeding".[3] The current European legislation provides specific PAH parameters for processed cereal-based food and baby food for infants and young children; infant formulae; and follow-on formulae.[4] According to Commission Regulation (EU) number 835/2011, the content of benzo[a]pyrene (BaP) and PAH4 (the sum of BaP, benz[a]anthracene (BaA), benzo[b]fluoranthene (BbF), and chrysene (Chr)) in processed cereal-based food and baby food for infants and young children should not exceed 1 µg/kg.



H_2 Carrier

GC/MS is the most commonly used technique for analysis of PAHs allowing for trace analysis of PAHs in foods with selectivity and sensitivity. With the increased global helium (He) crisis in the market, laboratories are looking for a more sustainable alternative to helium and exploring the option of H_2 carrier gas. The economic benefits of H_2 carrier gas for GC are widely known but resulting hydrogenation and dechlorination reactions in the MS source may occur, and thus make the application of H_2 for GC/MS challenging. The Agilent HydroInert source is a newly designed extractor source for GC/MSD that addresses these issues and improves performance with H_2 carrier gas in GC/MS.[1]

Agilent Captiva EMR–Lipid Pass-Through Cleanup

Low regulatory limits and food matrices add layers of complexity to the analysis of PAHs. Several factors can affect the quantification of PAHs, such as solubility, temperature, ionic strength, interactions with the matrix of origin, and so on. As a result, an extensive, multistage sample preparation method is necessary:

- Infant formula is a relatively fatty food matrix, containing 5 to 20% fat. It's required to dissolve the dry powder first, to achieve the efficient solvent extraction.
  - The water addition to dissolve the infant formula powder was investigated by comparing the typical water volume of 10 mL to the much less volume of 2 mL. Figure 1 shows the targets recovery comparison using the two different water addition volume. The results clearly demonstrated that the less water volume (2 mL) for powder dissolving played an important role for heavy PAHs recoveries. This is because the more water volume (10 mL) can result in the reduced solubility of more hydrophobic PAHs and cause these targets loss during extraction. As a result, the 2 g of infant formula was dissolved into 2 mL of water for the following solvent extraction.

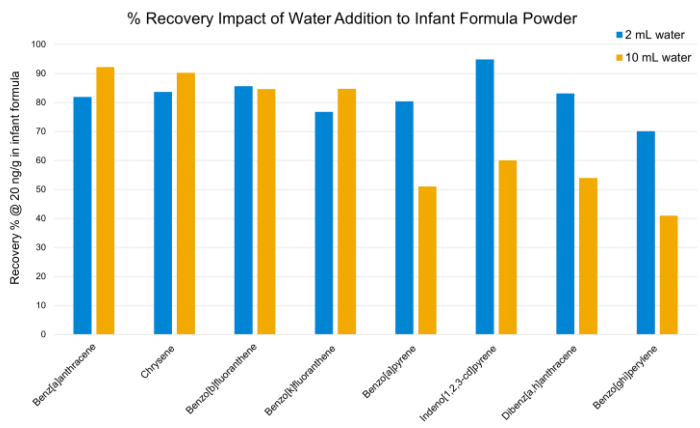


Figure 1. Target Recovery Comparison using different water addition volumes

Weigh 2 g of infant formula powder into a 50 mL tube (PN 5610-2049)

For pre-spiking QCs, spike standards into infant formula powder carefully
Vortex 10 sec (ISTD PN 5191-4509)

Add 2 mL of water, and then vortex for 5 mins — The dry powder needs to be dissolved with water prior to the solvent extraction

- After solvent extraction from the infant formula matrix, a cleanup/purification step is essential to isolate the analytes of interest and to remove potential interferences, especially fatty co-extractives such as triglycerides and fatty acids, where Captiva EMR–Lipid can provide an efficient matrix cleanup.[2]
  - The use of 20/80 EtOAc/ACN for the extraction provides enough strength to extract hydrophobic PAHs from fatty matrices.

Add 10 mL of 80:20 Acetonitrile (ACN)/Ethyl Acetate (EtOAc) & vortex for 1 min — Improves the solvent strength to extract hydrophobic PAHs

Add QuEChERS extraction salt (original; PN 5982-6550) and add ceramic homogenizers (1-2)

Cap the tubes tightly and shake vigorously on Gino Grinder @ 1500 rpm for 5 mins

Centrifuge the tubes @ 5000 rpm for 5 mins

- The Captiva EMR–Lipid pass-through cleanup has gained considerable attention since its introduction. The EMR–Lipid sorbent selectively interacts with the unbranched hydrocarbon chains of lipids, leaving "bulky" target analytes in solution for subsequent analysis.
  - The additional elution on Captiva EMR–Lipid assures the complete elution of targets from Captiva EMR–Lipid cartridges during pass-through cleanup.

Transfer 2.7 mL of supernatant and mix with 0.3 mL of water

Transfer 2.5 mL of above mixture to Captiva EMR-Lipid 3 mL cartridge (PN 5190-1003) and use gravity elution

Add 0.625 mL of 72:18:10 ACN/EtOAc/Water (H_2O) for additional gravity elution

Once dripping stops, apply 6-9 psi pressure to completely dry the EMR-Lipid cartridges

- The isooctane back extraction after cleanup makes it easier to switch from the extraction solvent to a more GC-amenable solvent and provides partial sample concentrating.
  - The entire sample preparation procedure introduces a 5× dilution of the infant formula powder sample.

Transfer 2 mL of eluent to a new 15 mL tube (PN 5610-2039), add 3.6 mL of water, mix 30 sec

Add 1.44 mL of Isooctane to each sample tube. Cap tightly — GCMS amenable solvent

Vortex 15 minutes, and centrifuge @ 5000 rpm for 5 minutes

Transfer the upper layer for GC/MS

Infant formula matrix chromatograms

Figure 2. A GC/MS total ion chromatogram (TIC) scan of the infant formula.

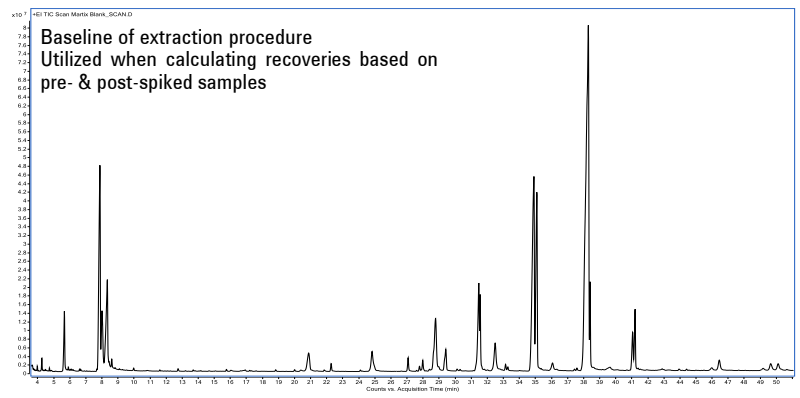
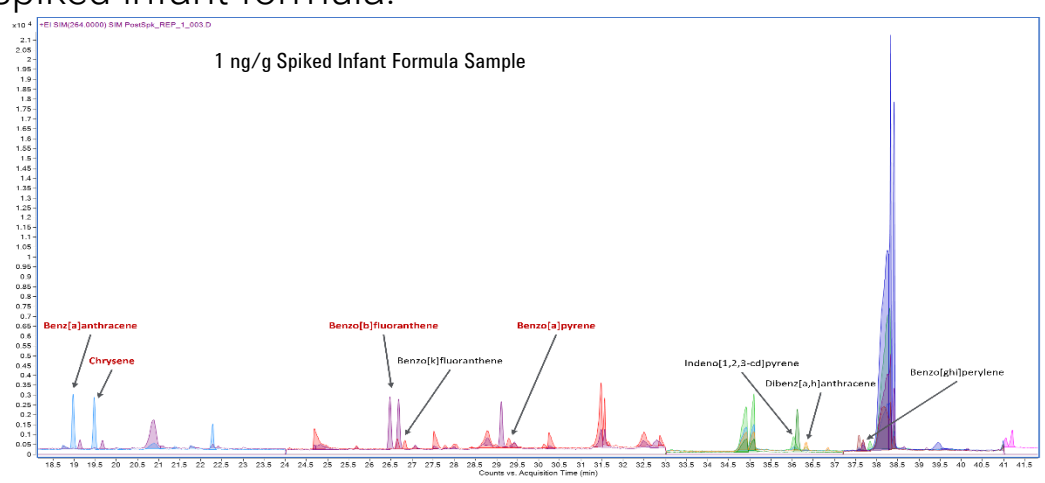


Figure 3. A GC/MS SIM chromatogram of PAHs in a post spiked infant formula.



Instrument

GC/MS is the preferable mode of detection as it confirms the identity of the analyte with high selectivity and for using stable isotope labeled PAHs as internal standards, thereby reducing analytical errors.[1] Tables 1 and 2 provide information on the instrumentation, consumables, and parameters, respectively.

Agilent 8890/5977C GC/MS

Table 1. GC and MSD instrumentation and consumables.

Part	Description
GC	Agilent 8890 GC system
MS	Agilent 5977C Inert Plus GC/MSD
Source	Agilent HydroInert source with 9 mm HydroInert extraction lens
Syringe	Agilent Blue Line autosampler syringe, 10 µL, PTFE-tip plunger (p/n G4513-80203)
Column	Agilent J&W DB-EUPAH GC column, 20 m, 0.18 mm, 0.14 µm, 7-inch cage (p/n 121-9627)
Inlet Liner	Agilent inlet liner, Ultra Inert, split, low pressure drop, glass wool (p/n 5190-2295)

Table 2. GC and MSD instrument conditions.

Parameter	Value
Injection Volume (L1)	2 µL
Injection Type	Two-layer sandwich (L1, L2)
L1 Air Gap	0.2 µL
L2 Volume	0.5 µL (used for ISTD sandwich injection)
L2 Air Gap	0.2 µL
Inlet Temp	320 °C
Inlet Mode	Pulsed splitless
Septum Purge Flow	3 mL/min
Septum Purge Flow Mode	Switched
Injection Pulse Pressure	40 psi until 0.75 min
Purge Flow to Split Vent	50 mL/min at 0.7 min
Column Temp Program	60 °C (1 min hold); 60 °C/min to 180 °C (hold 0 min); 3 °C/min to 335 °C (hold 15 min)
Carrier Gas & Flow Rate	H_2, 0.9 mL/min constant flow
Transfer Line Temp	320 °C
Ion Source Temp	320 °C
Quadrupole Temp	150 °C
Data Acquisition	Selective ion monitoring (SIM)
Tune	etune.u
Gain Factor	5

Due to recent pressures on the helium (He) supply, required organizations have had to actively investigate the use of hydrogen (H_2) carrier gas. However, most GC/MS analyses have reduced sensitivity and hydrogenation or dechlorination in the source. The PAHs extracted from infant formula was performed utilizing H_2 and the Agilent HydroInert source on the Agilent 8890/5977C GC/MS.

Agilent HydroInert source

- Allows for the use of Hydrogen Carrier Gas with better supply and reduced cost
- Faster, shorter Separations
- Reduces loss of sensitivity and spectral anomalies
- Reduced source cleanings and maintenance

Results and Discussion

Method Recovery and Reproducibility

Target analyte recoveries for eight PAHs were calculated based on the direct peak-area comparison of the prespiked and postspiked infant formula samples, and the results are shown in Figure 4. The four critical PAH compounds—BaP, BaA, BbF, and Chr—are circled in red. Three levels of spiked samples were used for method recovery and reproducibility validation, which included 1, 10, and 50 ng/g in infant formula with six replicates at each level.

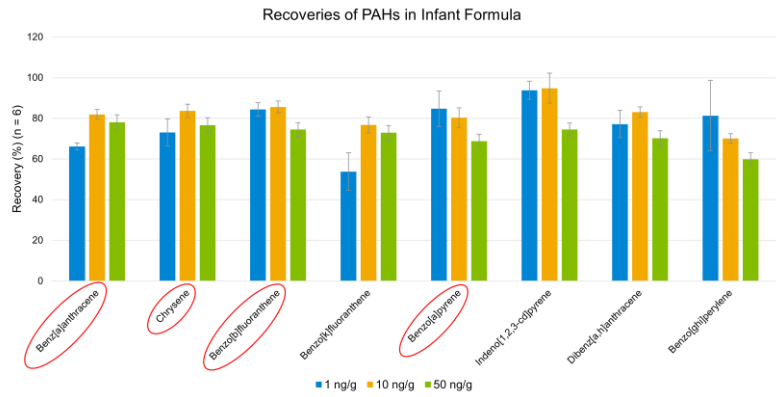


Figure 4. Method recoveries and reproducibility for targeted PAHs in infant formula.

The results confirmed that the method delivered acceptable >60% recoveries (60 to 95%) with <20% RSD, except for benzo[k]fluoranthene at 1 ng/g level (54% recovery), and benzo[ghi]perylene (34.6% RSD). The two outliers are mostly due to the low sensitivity of the instrument detection method and more matrix impact at the 1 ng/g level. The instrument method sensitivity and matrix impact to low-level spiked samples also resulted in higher RSDs at the 1 ng/g level.

Analytical System

For quantitation of PAHs in infant formula, a matrix-matched calibration was used with seven calibration levels from 0.1 to 20 ppb in vial (0.5 to 100 µg/kg in infant formula). Target analyte retention times (RTs) and linearity values are displayed in Figure 5 and Table 3.

Acquiring a quantitation level below 1 µg/kg for BaP and PAH4 allows accurate quantitation for the Commission Regulation (EU) number 835/2011.

Table 3. Analysis data for target PAHs.

Compound	RT (min)	Linearity	Quantifier Ion (*m/z*)	Qualifier Ion 1 (*m/z*)	Qualifier Ion 2 (*m/z*)
Benzo(a)anthracene-d12	19.00	0.999	240.1	236.1	
Benz[a]anthracene (BaA)	19.15		228	226	229
Chrysene-d12	19.50	0.997	240	236	
Chrysene (Chr)	19.69		228.1	226.1	229
Benzo[b]fluoranthene-d12	26.50	0.998	264	260	
Benzo[b]fluoranthene (BaF)	26.67		252	250	253
Benzo[k]fluoranthene-d12	26.70	0.994	264.1	260.1	
Benzo[k]fluoranthene	26.85		252	250	253
Benzo[a]pyrene-d12	29.14	0.995	264.1	260.1	
Benzo[a]pyrene (BaP)	29.31		252.1	250.1	248
Indeno(1,2,3-cd)pyrene-d12	35.91	0.998	288	284	
Indeno[1,2,3-cd]pyrene	36.05		276	274	277
Dibenzo(ah)anthracene-d14	36.14	0.998	292	288	
Dibenz[a,h]anthracene	36.35		278.1	276.1	279.1
Benzo(ghi)perylene-d12	37.71	0.997	288	287	
Benzo[ghi]perylene	37.86		276.1	274.1	277
Dibenzo[a,i]pyrene-d14	46.45		316	317	

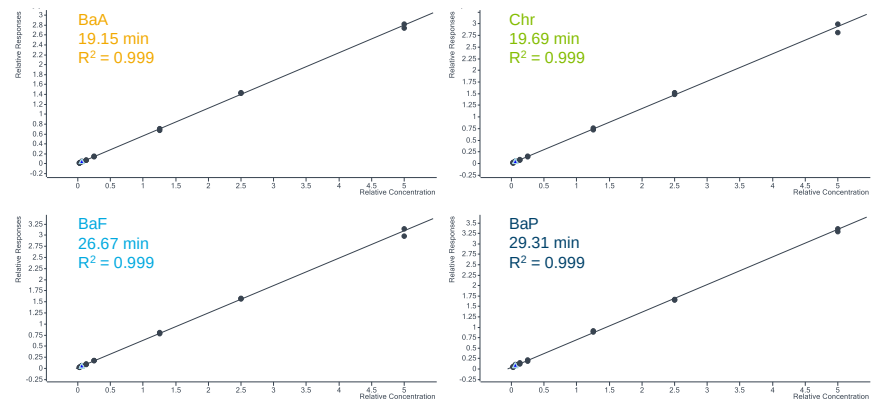


Figure 5. Matrix Matched Calibration for PAH4 seven calibration over 0.1 – 20 ppb in vial (0.5 – 100 µg/kg in infant formula).

Conclusions

- An Agilent Captiva EMR—Lipid pass-through cleanup for PAH analysis in infant formula and the use of the Agilent HydroInert source with H_2 carrier gas on the Agilent 8890 GC and 5977C GC/MSD system can be used for the determination of PAHs at low concentrations.
- The method delivered acceptable recovery, reproducibility, and quantitation results that meet the EU regulation for PAH analysis in food.

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