Application Note

Food and Beverage Testing

Extraction and Analysis of Polycyclic Aromatic Hydrocarbons in Infant Formula

Using Agilent Captiva EMR–Lipid cartridges by GC/MS with hydrogen carrier gas

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Abstract

This application note presents a sample preparation method for the extraction and cleanup of polycyclic aromatic hydrocarbons (PAHs) from infant formula. The use of Agilent Captiva Enhanced Matrix Removal–Lipid (EMR–Lipid) provides highly selective, efficient lipid removal from the infant formula with acceptable analyte recoveries. The solvent exchange to isooctane allows for a favorable GC/MS injection solvent. The application also showcases the use of hydrogen (H_2) carrier gas with the Agilent HydroInert source[1] on the Agilent 8890 GC coupled with the Agilent 5977C GC/MSD.

Introduction

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.

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. This selective interaction mechanism makes it ideal for multiclass, multiresidue analysis in fatty-food matrices.

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 dichlorination reactions in the MS source may occur, and thus make the application of H_2 for GC/MS and GC/MS/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]

This study investigates the analysis of PAHs in infant formula using Captiva EMR–Lipid pass-through cleanup for sample preparation, followed by GC/MS using the HydroInert source and H_2 carrier gas.

Experimental

Sample preparation

The sample preparation method development followed the previously published PAH method used in beef and salmon.[5] Prior to a solvent extraction, infant formula powder first needs to be dissolved in water. The crude extract can then further be cleaned using Captiva EMR–Lipid 3 mL cartridges. For the analysis of PAHs on the GC/MS, the cleaned extract was back-extracted with isooctane—a more GC-amenable solvent. An outline of the sample preparation procedure is shown in Figure 1. The entire sample preparation procedure introduced a 5× dilution of the infant formula powder sample.

Instrumental analysis

Regarding quantification, PAHs can be quantified using GC/MS. GC/MS allows accurate identification of the target analytes and their respective internal standards with high selectivity, thereby reducing analytical errors.[2] The PAH extraction from infant formula was performed using H_2 and the HydroInert source on the 8890 GC coupled with a 5977C GC/MSD (Figure 2).

Weigh 2 g of infant formula powder into a 50 mL tube (p/n 5610-2049).

For prespiking QCs, spike standards into infant formula powder carefully. Vortex for 10 seconds (internal standards p/n 5191-4509).

Add 2 mL of water, then vortex for 5 minutes.

Add 10 mL of 80/20 acetonitrile (ACN)/ethyl acetate (EtOAc), and vortex for 1 minute.

Add Agilent Bond Elut QuEChERS extraction salt (original; p/n 5982-6550) and add ceramic homogenizers (one or two).

Cap the tubes tightly and shake vigorously on Geno/Grinder at 1,500 rpm for 5 minutes.

Centrifuge the tubes at 5,000 rpm for 5 minutes.

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

Transfer 2.5 mL of the above mixture to an Agilent Captiva EMR–Lipid 3 mL cartridge (p/n 5190-1003) and use gravity elution (or with low level of pressure when needed). The flow rate should be 3 to 5 seconds per drop.

Add 0.625 mL of 72/18/10 ACN/EtOAc/water for additional gravity elution.

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

Transfer 2 mL of eluent to a new 15 mL tube (p/n 5610-2039); add 3.6 mL of water, mix 30 seconds.

Add 1.44 mL of isooctane to each sample tube. Cap tightly.

Vortex for 15 minutes, and centrifuge at 5,000 rpm for 5 minutes.

Transfer the upper layer for GC/MS.

Figure 1. Infant formula sample preparation procedure chart.

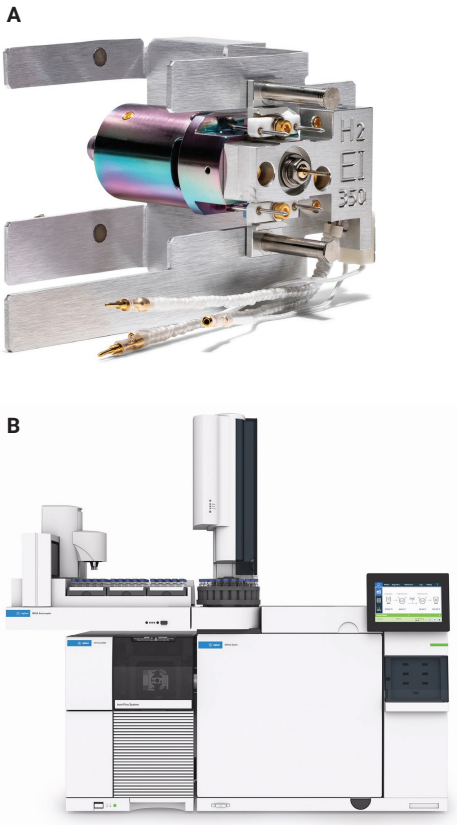

Figure 2. Agilent HydroInert source (A) and Agilent 8890 GC and 5977C GC/MSD system (B).

The 8890 GC system was configured with an Agilent J&W DB-EUPAH GC column (part number 121-9627) combined with an Agilent 5977C Inert Plus GC/MSD with a HydroInert source. Tables 1 and 2 summarize the GC/MS instrumentation and consumables used in this study.

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 internal standard sandwich injection)
L2 Air Gap	0.2 µL
Inlet Temperature	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 Temperature 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 and Flow Rate	H_2, 0.9 mL/min constant flow
Transfer Line Temperature	320 °C
Ion Source Temperature	320 °C
Quadrupole Temperature	150 °C
Data Acquisition	Selective ion monitoring (SIM)
Tune	etune.u
Gain Factor	5

Results and discussion

Sample preparation

Low regulatory limits and food matrices add layers of complexity to the analysis of PAHs. As a result, extensive, multistage sample preparation methods are usually necessary. Several factors can affect the quantification of PAHs, such as solubility, temperature, ionic strength, interactions with the matrix of origin, and so on. PAHs are highly hydrophobic compounds, especially heavy PAHs with three or four rings, which typically bear high log P values above 5. Therefore, they are easily accumulated in matrices with high lipid content or other nonpolar components.[6] Accordingly, each food matrix has a specific sample preparation according to its composition. Thus, in-depth knowledge of the matrix of interest is essential for determining the appropriate steps for PAH analysis.[7]

Infant formula is a relatively fatty food matrix, containing 5 to 20% fat. The dry powder must be dissolved in water before 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 solvent for extraction provides enough strength to extract hydrophobic PAHs from fatty matrices. The additional elution on Captiva EMR–Lipid assures the complete elution of targets from Captiva EMR–Lipid cartridges during pass-through cleanup. 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.

For infant formula powder, it is important to dissolve the dry powder first to achieve efficient solvent extraction. The addition of water to dissolve the infant formula powder was investigated by comparing a higher water volume of 10 mL to the lower volume of 2 mL. Figure 3 shows the target-recovery comparison using the two different water-addition volumes. The results clearly demonstrated that the lower water volume (2 mL) for powder dissolving played a significant role in

heavy-PAH recoveries. This is because the higher water volume (10 mL) can result in the reduced solubility of more hydrophobic PAHs and cause target losses during extraction. As a result, the 2 g of infant formula was dissolved into 2 mL of water for the following solvent extraction.

Analytical system

Due to recent helium supply shortages, required organizations have had to investigate the use of H_2 carrier gas. However, most GC/MS analyses have reduced sensitivity and hydrogenation or dechlorination in the source.

A GC/MS total ion chromatogram (TIC) scan of the infant formula blank is shown in Figure 4. The full scan of the blank matrix displays the sample matrix chromatographic background baseline. A matrix blank was used for postspiking standard samples.

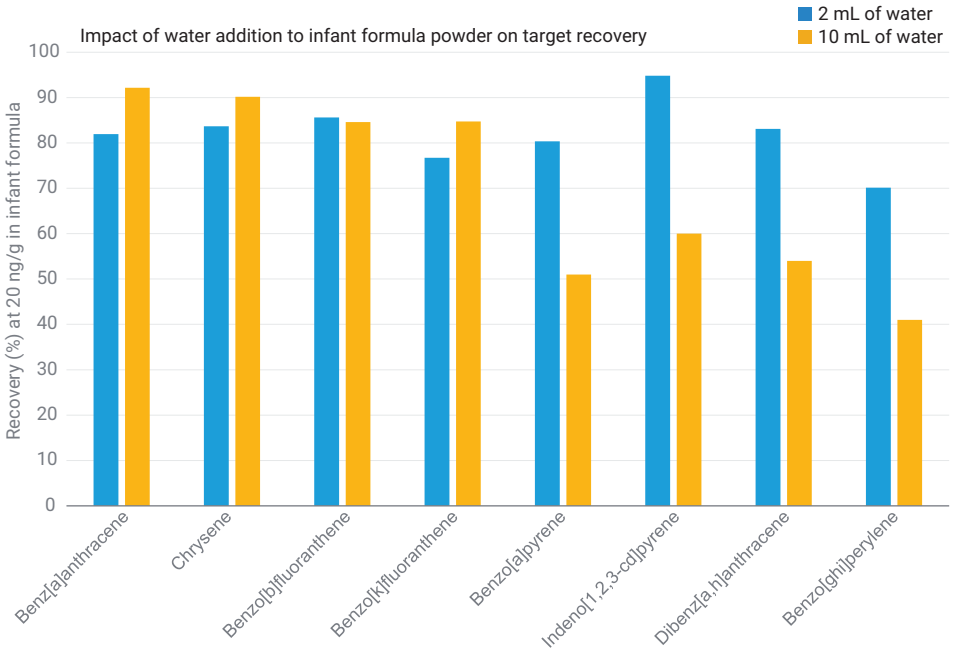

Figure 3. Comparison of PAH target recoveries for different water volumes used to dissolve infant formula before solvent extraction.

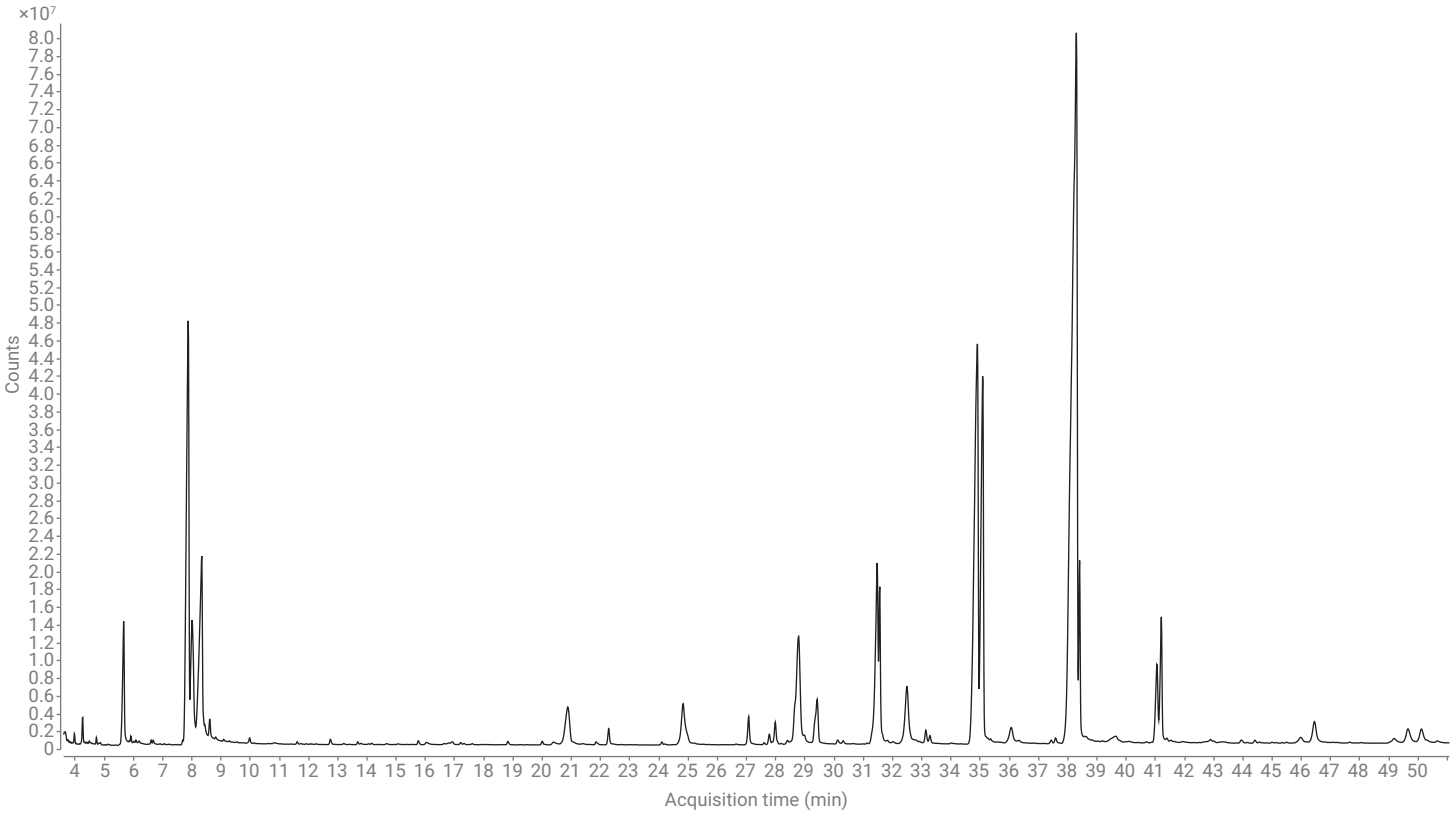

Figure 4. GC/MS TIC scan of infant formula matrix blank.

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 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.

Method recovery and reproducibility

The examination of prespiked samples allowed the evaluation of the developed quantitation method performance. A GC/MS SIM chromatogram of target PAHs in a postspiked infant formula sample (1 ng/g in the infant formula extract) is shown in Figure 5.

Table 3. Analysis data for target PAHs.

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

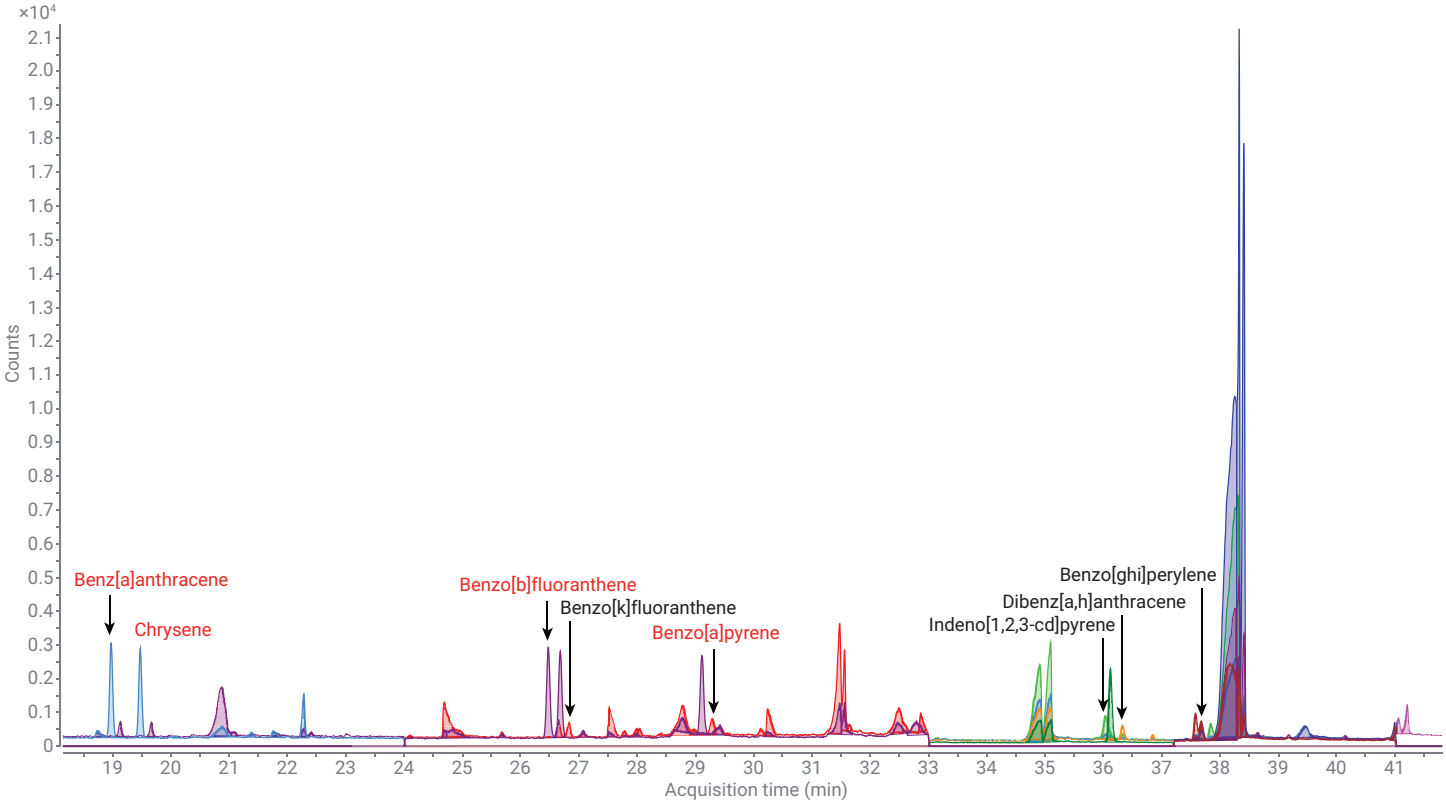

Figure 5. GC/MS SIM chromatogram of PAHs in a postspiked infant formula sample (1 ng/g in the infant formula extract).

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 6. The four critical PAH compounds—BaP, BaA, BbF, and Chr—are 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.

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.

Conclusion

This application note presents a sample preparation method using solvent extraction followed by Agilent Captiva EMR—Lipid pass-through cleanup for PAH analysis in infant formula. The study also showed that 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.

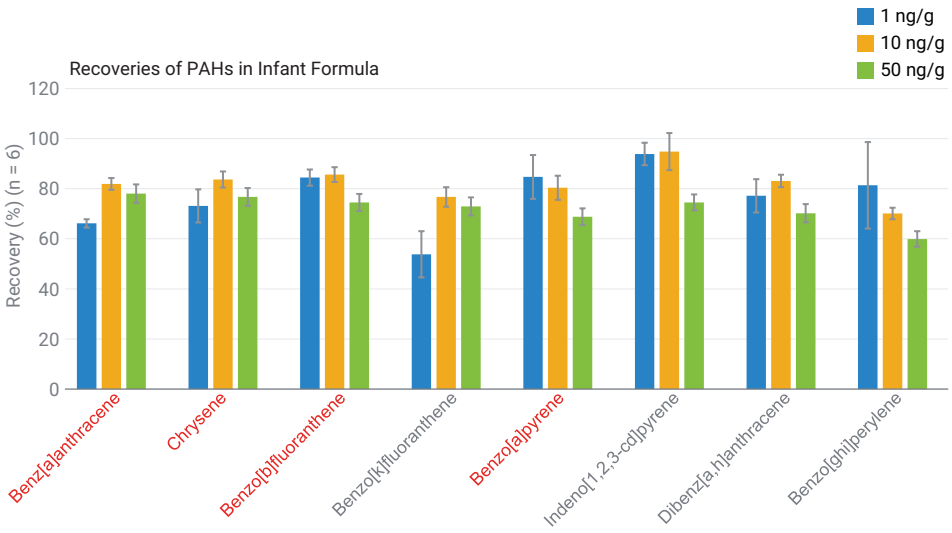

Figure 6. Method recoveries and reproducibility for targeted PAHs in infant formula.

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