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Simplified and Efficient PFAS Trace Extraction in Cereal-Based Infant Food Using Cleanert LipoNo Beads

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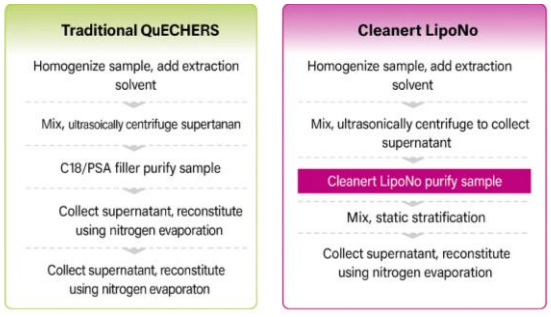
Introduction

Perfluoroalkyl substances (PFAS) are highly stable synthetic organic compounds widely used in industrial and commercial applications, including food packaging materials and non-stick cookware. Their hydrophobic, fully fluorinated alkyl chains and strong carbon–fluorine bonds confer exceptional chemical stability and environmental persistence [1]. As a result, PFAS can migrate into food products through environmental contamination or contact with food packaging materials, making dietary intake an important exposure pathway, particularly for vulnerable populations such as infants. Cereal-based infant foods constitute a complex solid matrix with high contents of carbohydrates, proteins, and lipids, which can complicate the extraction and analysis of PFAS. Although numerous analytical methods have been developed for PFAS determination in aqueous samples, considerably fewer validated procedures are available for solid food matrices[2], especially infant foods. Existing methods commonly employ QuEChERS or solid liquid extraction followed by dispersive solid-phase extraction (dSPE) clean-up. Although effective, these clean-up steps can be time-consuming, and costly, and may not sufficiently remove co-extracted lipids and matrix interferences. Variants such as solid liquid extaction combined with dSPE are frequently applied to complex food matrices; however, the efficiency of dSPE clean-up may be limited when dealing with lipid-rich or highly processed foods such as cereal-based infant food products.

In this study, a novel clean-up technique was evaluated as an alternative to the conventional dSPE method for the determination of PFAS in cereal-based infant food which contains approximately 10% fat along with dietary fibers, carbohydrates, and proteins. Agela's Cleanert LipoNo (**MS-LN0415**) – a newly developed fat-removal sorbent – features a surface modified with multiple long carbon chains that selectively adsorb fats in a high-lipid samples (e.g., meat, eggs, milk). Cleanert LipoNo incorporates an innovative filler manufacturing process with large particles that enable static stratification upon standing, thereby simplifying operation and reducing processing time (**Fig. 1**).

Figure 1. Purification workflow comparison: Quechers vs LipoNo



The procedure is simple and suitable for processing large batches of samples. The aim of this work was to assess the performance of the Cleanert LipoNo clean-up in terms of extract cleanliness, analytical efficiency, and method robustness, as well as its potential to reduce solvent consumption, sample preparation time, and overall analytical costs. The results of this study provide insight into the applicability of Cleanert LipoNo as a more efficient and cost-effective clean-up strategy for routine PFAS analysis in complex infant food matrices.

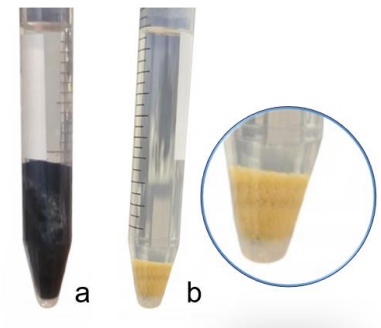
Sample Preparation

The extraction of cereal-based infant food samples was performed following the solid-liquid extraction protocol described in EURL-POPs guidance [3]. The resulting supernatant was then cleaned using both Cleanert LipoNo beads **(a)** and a dSPE purification salt replicating the same composition reported in the method **(b)**.

Table 1. Sample preparation procedure

Step	Description		
1*	Sample (1 g) + STD + ILIS in 15mL centrifuge tube and add 4 mL of LC-MS water.		
2*	Add 5 mL ACN and shake.		
3*	Ultrasonic bath (15 min) and Centrifugation (4000 x g, RT, 10 min)		
Repeat the extraction twice before moving to steps 4a or 4b			
Step	**Description**	**Step**	**Description**
4.a	Transfer all supernatant into a dSPE Tube - 15mL	4.a	Transfer all supernatant in centrifuge tube with Cleanert Lipo (No MS-LN0415)
5.a	Vortex 3 min	5.a	Vortex 3 min and Let stand the centrifuge tube for 5 min and than collect supernatant
6.a	Centrifuge (4000 x g, RT, 10 min), and than collect supernatant	6.a	Solvent evaporation to dryness at 40 °C
7.a	Solvent evaporation to dryness at 40 °C	7.a	Dissolve residue in 300 µL MeOH/1% formic acid (2:1:v:v) in ultrasonic bath
8.a	Dissolve residue in 300 µL MeOH/1% formic acid (2:1:v:v) in ultrasonic bath	8.a	Transfer supernatant in PP vial
9.a	Transfer supernatant in PP vial	9.a	UHPLC-HRMS/MS
10.a	UHPLC-HRMS/MS		

Figure 2. Actual images of the clean-up step for approaches (**a**) and (**b**)



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UHPLC-HRMS/MS Conditions

Column: Luna Omega PS C18 1.6µm
Dimensions: 100 x 2.1mm
Delay Column: Luna Omega PS C18 3µm
Dimensions: 50 x 2.1mm
Part No.: 00D-4752-AN (delay 00B-4758-AN)
Mobile Phase: A: 2 mM NH_4AcO in H_2O; B: MeOH:ACN 1:1 (v/v)

Gradient:

Time (min)	% B
0	10
1	10
2	50
13	90
14	100
15	100
16	10
22	10

Flow Rate: 0.350mL/min
Injection Volume: 5µL
Temperature: 40°C
Detection: Electrospray Ionization QExactive+ Orbitrap® (ESI-HRMS/MS)

Ionization and PRM parameters

Acquisition method	PRM
Resolution	35000
AGC target	1x10E6
Maximum IT (ms)	150
Isolation window (m/z)	1.6
Ion Source:	H-ESI
Polarity:	Negative
Sheath gas (A.U.)	30
Auxiliary gas (A.U.)	4
Capillary spray voltage (kV)	-3.0
Capillary temperature (°C)	250
Auxiliary gas temperature (°C)	250
S-lens (%)	40

Figure 3. Chromatographic separation of target PFAS spiked at 0.500 µg/kg in cereal-based infant food

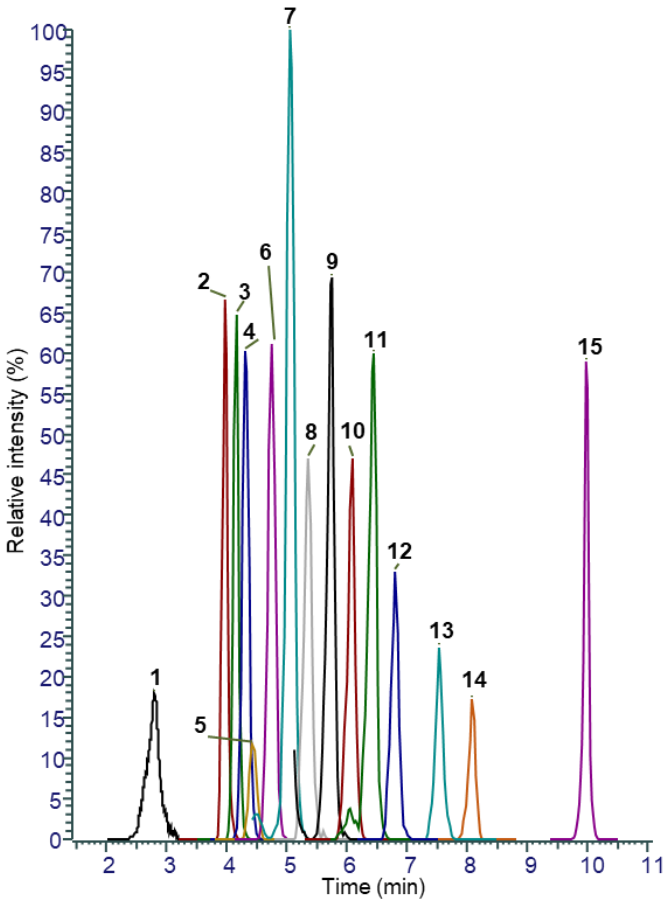

Table 2. PFAS peak's identification, respective retention time, internal standard, and PRM transitions

Peak No.	Retention Time	Analyte	Internal Standard	Precursor ion (m/z)	Fragment ion (m/z)	CE (eV)
1	2.79	**PFBA**	$^{13}C_3$-PFBA	212.9790	168.9898	10
2	3.96	**PFPeA**	$^{13}C_5$-PFPeA	262.9761	218.9858	10
3	4.14	**PFBS**	$^{13}C_4$-PFBS	298.9432	98.9559	50
4	4.29	**PFHxA**	$^{13}C_6$-PFHxA	312.9733	268.9836	10
5	4.41	**GenX**	$^{13}C_3$-GenX	284.9779	168.9898	10
6	4.74	**PFHpA**	$^{13}C_7$-PFHpA	362.9703	318.9799	10
7	5.04	**PFHxS**	$^{13}C_6$-PFHxS	398.9371	79.9575	50
8	5.35	**PFOA**	$^{13}C_8$-PFOA	412.9670	368.9772	10
9	5.74	**PFHpS**	$^{13}C_8$-PFOS	448.9341	79.9575	50
10	6.08	**PFNA**	$^{13}C_9$-PFNA	462.9642	418.9743	10
11	6.44	**PFOS**	$^{13}C_8$-PFOS	498.9311	79.9575	50
12	6.79	**PFDA**	$^{13}C_9$-PFDA	512.9606	468.9704	10
13	7.53	**FOSA**	$^{13}C_8$-FOSA	497.9468	77.9658	42
14	8.07	**PFDS**	$^{13}C_8$-PFOS	598.9246	79.9575	50
15	9.98	**PFUnDA**	$^{13}C_9$-PFUnDA	562.9578	518.9666	10

Quantification and apparent recoveries

An isotope dilution method was employed to quantify target PFAS in cereal-based infant food spiked at 0.500 µg/kg. Each sample was prepared with 4 replicates and tested in both cleanup procedures. Aiming to evaluate effectiveness of extraction and clean up steps the apparent recovery was calculated and shown in **Fig. 4 and Table 3**.

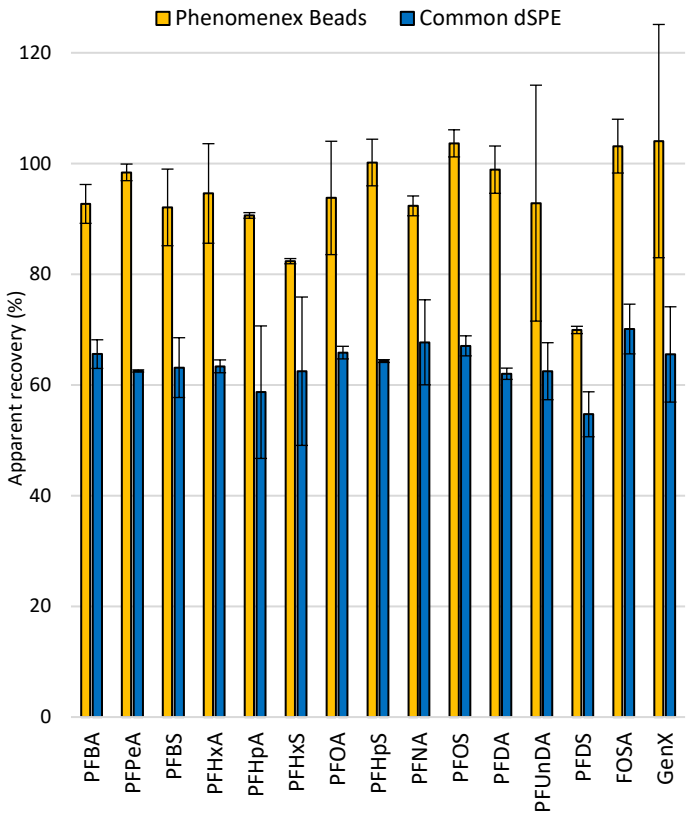

Figure 4. Flow chart representing Method Performance Data Comparison for cereal-based infant food Spiked at 0.500 µg/kg of the Target Analytes (n=4).

Table 3. Method Performance Data Comparison for cereal-based infant food Spiked at 0.500 µg/kg of the Target Analytes (n=4) with apparent recovery % and RSD%.

	Phenomenex Beads (a)		Common dSPE (b)	
Analyte	Apparent recovery (%)	RSD (%)	Apparent recovery (%)	RSD (%)
PFBA	92.70	3.51	65.57	2.59
PFPeA	98.40	1.50	62.52	0.20
PFBS	92.07	6.92	63.13	5.40
PFHxA	94.59	8.99	63.36	1.16
PFHpA	82.36	0.47	62.48	13.40
PFHxS	93.78	10.24	65.84	1.14
PFOA	100.18	4.21	64.31	0.25
PFHpS	92.35	1.79	67.70	7.68
PFNA	103.64	2.45	67.06	1.81
PFOS	98.89	4.27	62.02	1.02
PFDA	92.84	21.31	62.48	5.15
PFUnDA	104.05	21.07	65.51	8.61
PFDS	103.14	4.86	70.10	4.49
FOSA	69.93	0.66	54.70	4.06
GenX	90.62	0.51	58.69	11.97

Results and Discussion

Accuracy and precision were assessed using four replicates of cereal-based infant food spiked with the PFAS target analytes. Apparent recovery (**Table 4** and **Fig. 3**) was markedly improved compared to the classical dSPE purification when operating after identical extraction conditions, showing an increase of nearly 30%, from 64% obtained with dSPE clean-up to 94% achieved using the beads, while maintaining good repeatability. In addition, absolute recoveries of the internal standards were calculated, yielding average values of 85% for the bead-based clean-up and 97% for the dSPE procedure.

From a practical point of view, the proposed approach resulted in a faster clean-up procedure that is intuitive and simple to perform. The advantages of the method are clearly illustrated in **Fig. 1** and **Fig. 2**, which highlights the differences compared to the conventional purification. The use of Cleanert LipoNo also enabled the recovery of a larger volume of supernatant while minimizing the risk of co-extracting residues from the aqueous and salt phases; this aspect can contribute to improved method sensitivity. In addition, the simplified handling and reduced number of operational steps associated with the **bead-based** clean-up led to lower analysis time and overall costs, making the approach economically advantageous for routine applications.

Conclusion

The proposed bead-based clean-up approach provides a fast, effective, and user-friendly alternative to conventional dSPE purification. The method simplifies the clean-up workflow and significantly improves apparent recovery, showing an increase of nearly 30% compared to dSPE, while maintaining good repeatability.

Overall, the results demonstrate that the bead-based method offers enhanced analytical performance and represents a promising option for PFAS routine applications in infant food.

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Ordering Information

Luna Omega

1.6 µm Minibore Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
PS C18	00A-4752-AN	00B-4752-AN	00D-4752-AN	00F-4752-AN	AJ0-9508

for ID: 2.1mm

‡SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

5 µm Minibore Columns (mm)					SecurityGuard Cartridges‡
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	4 x 2.0* (10/pk)
PS C18	00A-4753-AN	00B-4753-AN	00D-4753-AN	00F-4753-AN	AJ0-7605

for ID: 2.0-3.0mm

5 µm MidBore Columns (mm)				SecurityGuard Cartridges (mm)
Phases	50 x 3.0	100 x 3.0	150 x 3.0	4 x 2.0* (10/pk)
PS C18	00B-4753-Y0	00D-4753-Y0	00F-4753-Y0	AJ0-7605

for ID: 2.0-3.0mm

5 µm Analytical Columns (mm)					SecurityGuard Cartridges (mm)
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0* (10/pk)
PS C18	00B-4753-E0	00D-4753-E0	00F-4753-E0	00G-4753-E0	AJ0-7606

*SecurityGuard Analytical cartridges require holder, Part No.: KJ0-4282

for ID: 3.1-8.0mm

5 µm Analytical Columns (mm)					SecurityGuard Cartridges (mm)
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0* (10/pk)
PS C18	00B-4753-E0	00D-4753-E0	00F-4753-E0	00G-4753-E0	AJ0-7606

Cleanert LipoNo

LipoNo Tube	
Code	Description
MS-LN0415	Cleanert LipoNo,15mL, 50/Pk

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