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Single-run gas chromatography-mass spectrometry method for the analysis of phthalates, polycyclic aromatic hydrocarbons, and pesticide residues in infant formula based on dispersive microextraction techniques

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ABSTRACT

For the first time, three important contaminant groups (phthalates, polycyclic aromatic hydrocarbons, and pesticides), traditionally monitored by separated methods, were simultaneously analyzed in complex infant formula matrices. An accurate and sensitive analytical method based on gas chromatography-mass spectrometry (GC–MS) was developed for 45 food contaminants. Dispersive solid-phase extraction and low-density solvent-based dispersive liquid–liquid microextraction techniques were combined for clean-up and analyte enrichment purposes. Distinct GC–MS injection conditions were studied with the highest analytical responses obtained at high temperatures in pulsed splitless mode at high pulse pressures. Low matrix effects were observed for the majority of the analytes, indicating a possible relation of these effects with the physicochemical parameters of the analytes. Adequate method performance characteristics were achieved, covering limits of detection and quantification sufficiently low to monitor the regulated compounds at the maximum limits fixed for infant formula. Furthermore, the greenness and practicality of the proposed method were evaluated through the AGREE and BAGI metric tools, respectively. In summary, the application of the proposed method to commercial infant formulas, with the identification and quantification of benzo[*a*]pyrene, benz[*a*]anthracene, dibutyl phthalate, di(2-ethylhexyl) phthalate, dimethyl phthalate, and diisobutyl phthalate at levels between 1.4 and 47.1 μg kg^{-1} in a total of 35.0 % samples, demonstrates their suitability for routine analysis as well as contributes to the first data on the co-occurrence of PAHs and phthalates in infant formula.

1. Introduction

Dietary intake has been recognized as an important pathway of exposure to multiple chemicals of distinct nature. Many of them are harmless to humans depending on both dose and exposure frequency, whereas others represent a threat to health even at low levels. Evidence suggests that the risks associated with exposure to "chemical cocktails" depend on the effects resulting from antagonism, synergism, potentiation, or additivity [1]. In this way, the identification and quantification of the main mixtures of contaminants that may occur in diets comprise one of the great challenges nowadays [2]. In parallel, the availability of novel analytical methods, able to simultaneously determine contaminants of different classes at low concentration levels in matrices of complex composition, is decisive in risk assessment of concurrent exposure to food toxicants, especially, approaches in line with the Green Chemistry concept.

Prevention constitutes one of the twelve principles of Green Chemistry, which is based on the idea that it is better to prevent waste than to treat it later [3]. Accordingly, the application of microextraction techniques has shown to be a key strategy for sample preparation towards environmental friendliness [3,4]. The expressive reduction in both sample size and quantity of organic solvents and other hazardous reagents, associated with a low generation of waste, comprise greener features of these techniques [5,6]. Moreover, the simple and easy operation and good adaptability to several matrices facilitate the combination of microextraction techniques with each other and with other conventional sample treatments, mainly, for target analyte isolation, clean-up, and preconcentration purposes [6]. In this context, dispersive solid-phase extraction (d-SPE) and dispersive liquid–liquid microextraction (DLLME) techniques are highlighted.

DLLME is a liquid-phase microextraction based on a ternary component solvent system formed by the dispersion of microliters of a

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non-polar water-immiscible extraction solvent (acceptor phase) throughout the aqueous sample solution in the presence of a few milliliters of a disperser solvent (acetone, acetonitrile, ethanol, and others) miscible in both aqueous and acceptor phases [4,5,7]. The extensive contact area attained between the two phases benefits the mass transfer of analytes into the microdroplets of the extractant, whose extraction equilibrium is achieved instantaneously, corroborating the high enrichment factor [4,5,7]. Halogenated solvents, including chloroform, carbon tetrachloride, dichloromethane, and others, are common extractants used in DLLME [4,7]. Notwithstanding the few microliters applied, the replacement of these chlorinated solvents with less harmful non-halogenated solvents, including those with lower density than water, contributes to an eco-friendly DLLME procedure, the so-called low-density solvent-based DLLME (LDS-DLLME) [4,7]. Conversely, no additional organic solvents are required in the d-SPE procedure, in which micrograms of a sorbent (or a mixture of them) are directly added to the sample solution, followed by fast agitation and centrifugation [5,7,8]. The dispersion of the sorbent as fine particles throughout the liquid phase favors their interaction with the analytes (target isolation) or interfering matrix components (clean-up), whose technique efficiency is associated with the adsorption capacity, surface area, and dispersibility of sorbent materials such as graphitised carbon black (GCB), octadecylsilane (C18), primary-secondary amine (PSA), among others [5,7,8].

For food control, special attention has been given to phthalates, polycyclic aromatic hydrocarbons (PAHs), and residues of pesticides concerning their genotoxicity, carcinogenicity, and endocrine-disrupting potential [9–12]. Phthalates comprise esters of 1,2-benzene dicarboxylic acid with extensive industrial applications as plasticisers in the manufacture of polymers like polyvinyl chloride, as detergents in personal care products, and in the production of epoxy resins, adhesives, and others [9,10]. Since these compounds are not covalently bound to their native polymer, they can be leached from materials for the environment leading to the contamination of food [9,10]. PAHs are also widely distributed in the environment. These compounds are constituted by two or more fused aromatic rings resulting from incomplete combustion or pyrolysis of organic matter at high temperatures, including wood and fossil fuel combustion, motor vehicle emission, and other sources [11,13]. In addition to environmental pollution, the presence of these chemicals in foods is associated also with thermal processing such as grilling, roasting, drying, and smoking with combustion gases [11,13]. A maximum level of 1.0 μg kg^{-1} of benzo[*a*]pyrene, either individually or in combination with benz[*a*]anthracene, benzo[*b*]fluoranthene, and chrysene, was fixed for infant formula and follow-on formula [14]. Additionally, restrictions on the use and/or prohibition of pesticides in agricultural commodities destined for the production of infant foods do not assure that the final product is free from these chemicals [15]. Many of them present high stability in the environment, with bioaccumulation and biomagnification capacity in trophic chains [12,15]; besides, processing treatments might not be efficient in the elimination of pesticide residues in foods [12]. Thus, a default maximum residue level (MRL) of 10.0 μg kg^{-1} has been prioritized in infant formula and follow-on formula, except for cadusafos, demeton-S-methyl, ethoprophos, fipronil, and propined with MRLs varying between 4.0 and 8.0 μg kg^{-1} [15,16].

Gas chromatography (GC) and high-performance liquid chromatography (HPLC), both coupled to mass spectrometry (MS), have been the techniques of choice for the analysis of PAHs, phthalates, and pesticide residues in food, based on the distinct selectivity and sensitivity obtained in selected ion monitoring (SIM) or selected reaction monitoring (SRM) data acquisition modes [17–19]. Additionally, HPLC with fluorescence detection has also been widely used for PAHs [13]. Since the HPLC-based methods require organic solvents for the separation process, GC has often been considered a greener technique [3]. It estimates that between 1.0 and 1.5 L of liquid waste can be daily generated by a single HPLC system; furthermore, typical mobile phases involve harmful organic solvents such as acetonitrile and methanol [3].

Recent literature overview showed the prevalence of conventional sample preparation approaches in the analysis of PAHs, phthalates, and pesticide residues in infant formulas, including liquid–liquid extraction (LLE), solid–liquid extraction (SLE), pressurized liquid extraction, solid-phase extraction (SPE), and QuEChERS (Tables S1, S2, and S3 of Supplementary Material). However, significant amounts of chemicals, energy- and time-consuming steps, high manipulation of extracts, and critical production of waste, including disposable plastic SPE cartridges, are the main shortcomings of these approaches [4,5,8]. Although QuEChERS and SPE require smaller volumes of organic solvents than LLE and SLE, a considerable quantity of salts and organic solvents are consumed in the extraction and liquid–liquid phase separation steps and elution and conditioning of SPE cartridges.

Furthermore, as far as we know, the simultaneous determination of PAHs, phthalates, and pesticide residues in infant formula has never previously been reported. In this way, a simplified and high-throughput sample preparation approach, based on the d-SPE and LDS-DLLME techniques, was developed for the determination of 45 contaminants by GC–MS (Table 1). The study also included (i) the evaluation of the influence of distinct GC–MS injection conditions on the analytical response, (ii) the relation of the matrix effects with the physicochemical parameters of the analytes, (iii) the evaluation of the greenness and practicality of the method through metric tools, (iv) the establishment of analytical performance characteristics, and (v) the method application to commercial infant formula samples.

2. Material and methods

2.1. Chemicals and standard solutions

Analytical standards of phthalate diesters (≥98 % purity) were acquired from Sigma-Aldrich Co. (St. Louis, Mo, USA). Individual stock solutions were prepared in acetonitrile at concentrations between 2094.6 μg mL^{-1} (di-n-octyl phthalate) and 2871.4 μg mL^{-1} (benzyl butyl phthalate), and then an intermediate multi-phthalates solution at 100.0 μg mL^{-1} was prepared in acetonitrile from the individual stock solutions, which were all maintained at –20 °C in amber glass flasks.

PAHs analytical standards (97.9–99.9 % purity) were purchased from Sigma-Aldrich Co. (St. Louis, Mo, USA), Supelco (Bellefonte, PA, USA), and RMM BCR-08IR (Geel, Belgium). Individual stock solutions were prepared in toluene at concentrations between 360.7 μg mL^{-1} (dibenzo[*a,h*]pyrene) and 1053.6 μg mL^{-1} (benz[*a*]anthracene). An intermediate multi-PAHs solution at 50.0 μg mL^{-1} was prepared in toluene from the individual stock solutions and all solutions were stored in amber glass flasks at –20 °C.

Analytical standards of pesticides (92.8–99.9 % purity) were supplied by Sigma-Aldrich (Saint Louis, MO, USA), Fluka (Neu-Ulm, Germany), Dr. Ehrenstorfer GmbH (Augsburg, Germany), and Riedel-de-Haën (Seelze, Germany). Triphenyl phosphate (TPP; 99.3 % purity), used as an internal standard (IS), was acquired from Sigma-Aldrich (Saint Louis, MO, USA). Individual stock solutions for most pesticides were prepared in acetonitrile at concentrations between 955.8 μg mL^{-1} (*p,p'*-DDT) and 1785.6 μg mL^{-1} (heptachlor). Particularly for simazine, a stock solution at 563.7 μg mL^{-1} was prepared in methanol; whereas, individual stock solutions of dieldrin (831.6 μg mL^{-1}) and hexachlorobenzene (862.3 μg mL^{-1}) were prepared in a mixture of acetonitrile: toluene (1:1, v/v). From the individual stock solutions, an intermediate multi-pesticides solution at 100.0 μg mL^{-1} was prepared in acetonitrile and kept at –20 °C in amber glass flasks.

A multi-contaminants solution, containing all 45 analytes, at 10.0 and 5.0 μg mL^{-1} was prepared in acetonitrile from the intermediate standard solutions, and then working standard solutions, at 1.0, 0.5, and 0.025 μg mL^{-1}, were weekly obtained by appropriate dilution before use. Specifically for the TPP, individual intermediate (100.0 and 10.0 μg mL^{-1}) and working (1.0 and 0.5 μg mL^{-1}) solutions were prepared in

Table 1
Retention time (RT, min), molecular weight (MW, g moL^{-1}), *n*-octanol–water partition coefficient (log K_{ow}), and GC–MS parameters of the target analytes.

RT	Analyte	MW *	log K_{ow} *	Selected ion monitoring (SIM, *m/z*)		Start time of window, min (data acquisition rate, scan/s)
				Quantifier	Qualifier	
7.80	Dichlorvos	220.97	1.43	185	79; 109	7.00 (8.88)
8.76	Diuron	233.09	2.68	187	124; 159	8.50 (8.92)
10.18	Dimethyl phthalate	194.18	1.60	163	77; 194	10.00 (8.88)
11.66	Diethyl phthalate	222.24	2.47	149	150; 177	11.40 (8.94)
12.61	Trifluralin	335.28	5.34	306	264; 290	12.30 (8.93)
12.96	Dimethoate	229.30	0.78	87	93; 125	12.80 (3.75)
13.03	Simazine	201.66	2.18	201	173; 186	
13.15	Atrazine	215.68	2.61	200	173; 215	
13.40	Hexachlorobenzene	284.80	5.73	284	214; 249	13.25 (2.78)
13.60	Lindane (γ-HCH)	290.80	3.72	181	147; 219	
13.70	Diazinon	304.35	3.81	137	179; 304	
14.30	Diisobutyl phthalate	278.34	4.11	149	104; 223	14.20 (8.88)
14.64	Acetochlor	269.77	4.14	146	162; 223	14.50 (4.88)
14.82	Alachlor	269.77	3.52	160	188; 237	
15.10	Dibutyl phthalate	278.34	4.50	149	150; 223	15.00 (2.37)
15.12	Malathion	330.40	2.36	125	93; 173	
15.15	Heptachlor	373.30	6.10	272	237; 337	
15.48	Chlorpyrifos	350.60	4.96	197	258; 314	15.35 (4.85)
15.56	Triadimefon	293.75	2.77	208	57; 181	
15.82	Aldrin	364.90	6.50	263	293; 329	15.70 (8.91)
16.10	Thiabendazole	201.25	2.47	201	129; 174	15.95 (8.91)
16.33	Procymidone	284.13	3.00	283	96; 255	16.21 (8.81)
17.00	α-Endosulfan	406.90	3.80	241	195; 239	16.90 (8.93)
17.42	Dieldrin	380.90	5.40	79	263; 277	17.20 (8.82)
17.70	*p,p'*-DDD	320.00	6.02	237	165; 235	17.65 (3.36)
17.75	Endrin	380.90	5.20	263	245; 317	
17.80	β-Endosulfan	406.90	3.80	241	195; 339	
18.12	Benzyl butyl phthalate	312.40	4.73	149	91; 104	18.00 (8.92)
18.40	Endosulfan sulfate	422.90	3.66	272	387; 422	18.25 (2.55)
18.46	*p,p'*-DDT	354.50	6.91	237	165; 235	
18.60	Tebuconazole	307.82	3.70	250	125; 163	
19.35	Benz[*a*]anthracene	228.30	5.76	228	226; 229	19.10 (4.87)
19.42	Chrysene	228.30	5.73	228	226; 229	
19.50	Di(2-ethylhexyl) phthalate	390.60	7.60	149	167; 279	
19.81	λ-Cyhalothrin	449.80	7.00	181	141; 197	19.68 (8.92)
20.32	5-Methylchrysene	242.30	6.00	242	239; 241	20.10 (8.96)
20.66	Di-n-octyl phthalate	390.60	8.10	149	150; 279	20.48 (8.87)
20.85	Prochloraz	376.70	4.60	180	266; 308	20.76 (8.87)
21.56	Benzo[*b*]fluoranthene	252.30	5.78	252	250; 253	21.45 (8.96)
22.26	Benzo[*a*]pyrene	252.30	6.13	252	250; 253	22.17 (8.96)
25.35	Indeno[1,2,3-*cd*]pyrene	276.30	6.58	276	274; 277	25.20 (5.77)
25.40	Dibenz[*a,h*]anthracene	278.30	6.50	278	276; 279	
30.83	Dibenzo[*a,l*]pyrene	302.40	7.71	302	300; 303	30.60 (8.85)
33.33	Dibenzo[*a,i*]pyrene	302.40	7.30	302	300; 303	
33.70	Dibenzo[*a,h*]pyrene	302.40	7.00	302	300; 303	

* Source: https://pubchem.ncbi.nlm.nih.gov.

acetonitrile. All solutions were stored in amber glass flasks at –20 °C.

Acetonitrile and methanol, for HPLC analysis, were acquired from J. T. Baker® (Avantor Performance Materials, Inc. C.V. Xalostoc, Mexico). Toluene (Chromasolv® for HPLC, 99.9 %) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Primary-secondary amine (PSA) sorbent (40.0 μm) was supplied by Supelco (Bellefonte, PA, USA). A Milli-Q purifier system (Direct 8, Millipore, Bedford, MO, USA) was used to obtain the deionized water used in the sample preparation step.

2.2. Commercial infant formula samples

Samples of infant formula intended for babies from 0 to 12 months, including starter ($n = 10$) and follow-up ($n = 10$) infant formulas, were acquired in the city of Campinas, SP, located in the southeastern region of Brazil. A total of twenty different brands of infant formula were randomly collected from drugstores and supermarkets, comprising the most popular commercial brands available in the retail market at the time of sampling. All samples were maintained in their original packaging and stored at 20 °C until the analysis.

2.3. Simultaneous determination of PAHs, phthalates, and pesticide residues in infant formula by GC–MS

2.3.1. Sample preparation

Dispersive microextraction techniques were combined for clean-up and target-enrichment purposes as follows:

(i) Acetonitrile-based extraction with dispersive solid-phase extraction (d-SPE) clean-up: 2.0 g of powdered infant formula was weighed into a 15 mL glass centrifuge tube and 40.0 μL of a TPP solution at 1.0 μg mL^{-1} (IS) was added. The sample was extracted with 5.0 mL of acetonitrile under vigorous vortex agitation for 1 min, followed by centrifugation at 3700 xg for 15 min at 20 °C (Centrifuge 5804R, Eppendorf, Hamburg, Germany). In sequence, an aliquot of 2.5 mL of the extract was transferred to another 15 mL glass centrifuge tube containing 25.0 mg of PSA sorbent, followed by a fast vortex agitation and centrifugation at 3700 xg for 5 min at 20 °C. Then, the acetonitrile extracted was collected.

(ii) Analytes enrichment by low-density solvent-based dispersive liquid–liquid microextraction (LDS-DLLME): 2.0 mL of

acetonitrile extract (as a disperser solvent) and 200.0 μL of toluene (extraction solvent) were mixed and rapidly injected into 6.0 mL of deionized water, an extraction medium placed into a 15 mL glass centrifuge tube. After fast vortex agitation, a cloudy solution was formed in which the toluene was dispersed as tiny droplets throughout the all-water-acetonitrile phase. The mixture was centrifuged at 3700 xg for 5 min at 20 °C and the phase separation was achieved. Then, an aliquot of 100.0 μL of the upper layer (toluene) was collected and transferred to a glass insert placed into a glass vial for subsequent GC–MS analysis.

2.3.2. Gas chromatography-mass spectrometry analysis

An Agilent 7890A gas chromatography system equipped with a 7693 autosampler and interfaced to a 5975C inert MSD single quadrupole with an electron ionization (EI) source at 70 eV was used. The system was operated under the ChemStation platform and the data acquisition was in selected ion monitoring (SIM) mode. The chromatographic analyses were established on an HP-1MS ultra-inert capillary column (30 m x 0.25 mm x 1.0 μm; Agilent Technologies, Santa Clara, CA, USA). The injector was kept at 300 °C and 2.0 μL of toluene extract was injected in pulsed splitless mode as follows: injection pulse pressure at 50 psi until 0.5 min and purge flow to split vent of 100.0 mL min^{-1} at 1 min. The initial oven temperature was set at 85 °C, which was increased to 330 °C at a rate of 12 °C min^{-1} and then held for 15 min, resulting in a total run time of 35.4 min. The solvent delay was 7 min. Ultra-high purity helium (99.999 %) was used as carrier gas at a constant flow of 1.2 mL min^{-1}. The temperature of the transfer line, EI source, and quadrupole mass analyser was set at 320 °C, 300 °C, and 180 °C, respectively.

2.3.3. Identification and quantification criteria

Three ions were monitored for each analyte being the most abundant and/ or characteristic as quantifier ion and the other two ions as qualifiers, all organized into 28 ion groups in SIM mode as detailed in Table 1. The identification of the compounds in the samples was based on the retention time (±0.1 min) and ion ratio (±30 %) equal to those observed in the spiked samples [20]. Linear and non-forced-through-zero matrix-matched calibration curves, containing at least 5 concentration levels each, were used for the quantification of the analytes by plotting the analyte peak area/ IS peak area ratio versus concentration level (μg kg^{-1}). All the samples were analysed in triplicate and obtained results were not corrected for recovery.

2.4. Matrix effect

The matrix effect was estimated using the post-extraction addition method [21], in which a blank extract was spiked at 20.0 μg kg^{-1} of standard equivalent in the sample. In parallel, a multi-analyte standard solution at the same concentration level was prepared in 1.0 mL of toluene (0.08 μg mL^{-1}). No internal standard was used in the trials since it could also be subject to the effect. The matrix effect (ME) was calculated as indicated in Eq. (1):

$$ME\ \% = [(area\ in\ spiked\ extract - area\ in\ solvent)/area\ in\ solvent] \times 100 \quad (1)$$

where 0 % denotes no effect, values above 0 % indicate signal enhancement, and values below 0 % denote signal suppression [21]. The ME was categorized as high (ME < –50 % or ME > 50 %), medium (–50 % < ME < –20 % or 20 % > ME > 50 %), or low (–20 % < ME < 20 %) [22].

2.5. Quality assurance

Before the analysis, all materials used in the sample preparation step such as disposable pipette tips, glass centrifuge tubes, and stainless-steel spatulas, were rinsed successively with methanol, acetone, and hexane, all HPLC-grade. A procedural blank containing only reagents and solvents, and then processed as a sample, was routinely prepared to monitor possible contamination pertaining to laboratory materials and chemicals [23]. Phthalates, specifically di(2-ethylhexyl) phthalate, dibutyl phthalate, and diisobutyl phthalate, were detected in the procedural blanks, whose mean absolute values were subtracted from the contents found in the infant formula samples. Therefore, only the samples with phthalate signals at least twice greater than those detected in procedural blanks were reported as positives for these compounds. Toluene, as a solvent blank, was injected before and after each set of samples and the carryover phenomenon was not observed for any of the target analytes.

3. Results and discussion

3.1. Development of the analytical method

3.1.1. Sample preparation approach

The ubiquity of certain phthalates in the environment comprises one of the main challenges for the development of multi-residue methods involving this contaminant group. Particularly, concerning the several phthalate sources in the laboratory (sampling containers, plastic materials, organic solvents, chemicals, and some parts of the chromatographic system) that contribute to GC–MS background levels and potential contamination of samples [23]. Therefore, a straightforward sample preparation with a reduced number of steps and minimal extract manipulation was the focus of this study.

Powdered infant formula samples were directly extracted with acetonitrile under vigorous vortex agitation. A consistent homogenization between the matrix and the solvent, with suitable recoveries and reproducibility (Table 2), was achieved at a ratio of 1: 2.5 (sample to extraction solvent, w/v), without the need for matrix hydration, thus avoiding the demand for salt-induced liquid–liquid phase separation. The use of acetonitrile as an extractant ensures the extraction of analytes with a wide range of polarity; moreover, smaller co-extractive content in the extract could be expected due to the low solubility of both highly polar proteins and highly non-polar lipids in the solvent [24,25].

In sequence, dispersive solid-phase extraction (d-SPE) and low-density solvent-based dispersive liquid–liquid microextraction (LDS-DLLME) techniques were successively applied to the acetonitrile extract to minimize the matrix interferences and obtain a high enrichment factor, respectively. d-SPE with primary-secondary amine (PSA) sorbent (10.0 mg per mL extract) provided a cost- and time-effective clean-up, supported by low matrix effects for most compounds, without compromising the recovery rates (Table 2). Specifically, PSA acts as both a weak anionic exchanger and polar phase sorbent retaining mainly free fatty acids, sugars, organic acids, and certain pigments [26].

Afterward, the cleaned-up acetonitrile extract (2.0 mL) was employed as a disperser solvent in the LDS-DLLME technique, in which a micro-volume of toluene (200.0 μL) was used for extraction and enrichment purposes. Toluene was preferentially chosen based on our previous study with suitable recoveries obtained for 4-, 5-, and 6-ring PAHs from fruit baby foods [27]. In addition, to the best of our knowledge, this organic solvent had never been applied in LDS-DLLME for the extraction of phthalates and pesticides, corroborating the novelty of the proposed method. The very fine droplets of toluene dispersed in the aqueous acetonitrile phase create an infinitely large interface area between the two immiscible liquids, so rapid extraction equilibrium occurs with an effective mass transfer between the phases [4]. The use of toluene, an aromatic hydrocarbon (C_7H_8) of lower density ($d_{20\,°C} = 0.87$ g mL^{-1}) and higher boiling point (110.6 °C) than those chlorinated solvents commonly used as an extraction solvent in DLLME, favors the recovery of compounds containing aromatic rings in their chemical structure such as phthalates and PAHs; facilitates its collection at the top of the aqueous phase after centrifugation; and avoids possible alterations in the micro-volume during the extract manipulation. Finally, the

Table 2
Method performance characteristics for simultaneous analysis of phthalates, polycyclic aromatic hydrocarbons, and pesticide residues in infant formula matrix [a].

Analytes	LOD μg kg^{-1}	LOQ μg kg^{-1}	Linear range (R^2)	Recovery [b] (repeatability; reproducibility [c]) %			ME (%)
				2.5 μg kg^{-1}	20.0 μg kg^{-1}	100.0 μg kg^{-1}	
Pesticides							
Acetochlor	0.5	1.0	1.0–100.0 (0.9981)	96.6 (9.3; 12.6)	104.3 (1.4; 2.3)	103.5 (1.6; 2.7)	4.7
Alachlor	0.5	1.0	1.0–100.0 (0.9985)	107.1 (4.8; 14.2)	107.0 (4.2; 6.4)	105.8 (1.5; 2.4)	6.2
Aldrin	1.0	2.5	2.5–100.0 (0.9979)	96.4 (6.3; 7.9)	99.9 (1.6; 2.8)	99.4 (1.0; 1.9)	9.9
Atrazine	0.5	1.0	1.0–100.0 (0.9978)	107.2 (4.4; 6.3)	103.3 (2.5; 4.1)	99.0 (1.8; 3.0)	6.2
Chlorpyrifos	1.0	2.5	2.5–100.0 (0.9977)	104.5 (5.3; 8.2)	102.5 (1.3; 2.1)	100.9 (1.3; 2.0)	8.9
λ-Cyhalothrin	1.0	2.5	2.5–100.0 (0.9979)	103.7 (6.5; 11.0)	92.8 (3.4; 5.0)	92.3 (4.2; 6.7)	4.2
p,p'-DDD	0.5	1.0	1.0–100.0 (0.9972)	85.7 (2.9; 4.7)	99.0 (1.6; 2.6)	95.9 (0.8; 1.4)	11.0
p,p'-DDT	0.5	1.0	1.0–100.0 (0.9964)	98.7 (0.5; 10.0)	99.8 (4.0; 5.8)	110.4 (1.9; 3.0)	4.4
Diazinon	1.0	2.5	2.5–100.0 (0.9981)	110.9 (2.2; 7.8)	103.9 (2.2; 3.2)	102.0 (1.9; 2.8)	5.2
Dichlorvos	5.0	10.0	10.0–100.0 (0.9926)	n.a.	107.3 (5.5; 8.0)	101.3 (2.5; 4.3)	0.5
Dieldrin	5.0	10.0	10.0–100.0 (0.9951)	n.a.	97.7 (4.8; 6.5)	102.0 (0.9; 1.4)	7.1
Dimethoate	5.0	10.0	10.0–100.0 (0.9915)	n.a.	111.8 (5.9; 9.8)	112.5 (6.6; 10.6)	–0.7
Diuron	0.5	1.0	1.0–100.0 (0.9969)	88.0 (10.3; 17.8)	96.0 (2.4; 4.0)	100.3 (2.8; 4.6)	5.0
α-Endosulfan	5.0	10.0	10.0–100.0 (0.9989)	n.a.	108.0 (10.3; 12.2)	115.0 (2.2; 3.3)	6.8
β-Endosulfan	5.0	10.0	10.0–100.0 (0.9962)	n.a.	87.9 (3.0; 8.9)	114.6 (1.9; 3.0)	4.3
Endosulfan sulfate	0.5	1.0	1.0–100.0 (0.9986)	98.9 (4.7; 10.2)	108.1 (1.7; 2.4)	106.9 (1.0; 1.6)	4.5
Endrin	2.5	5.0	5.0–100.0 (0.9984)	n.a.	104.9 (3; 5.4)	104.7 (1.0; 2.2)	15.8
Heptachlor	0.5	1.0	1.0–100.0 (0.9978)	106.0 (4.4; 7.3)	98.6 (3.5; 5.0)	102.4 (2.3; 3.7)	4.9
Hexachlorobenzene	1.0	2.5	2.5–100.0 (0.9977)	95.0 (3.4; 5.7)	94.6 (1.3; 2.0)	93.0 (1.0; 1.6)	6.9
Lindane (γ-HCH)	2.5	5.0	5.0–100.0 (0.9982)	n.a.	101.9 (1.7; 2.5)	100.6 (1.2; 2.0)	7.9
Malathion	1.0	2.5	2.5–100.0 (0.9975)	94.3 (3.2; 8.2)	100.4 (2.6; 3.8)	99.8 (3.3; 5.1)	13.0
Prochloraz	5.0	10.0	10.0–100.0 (0.9942)	n.a.	115.0 (2.4; 6.1)	108.4 (3.1; 4.7)	1.2
Procymidone	5.0	10.0	10.0–100.0 (0.9975)	n.a	105.5 (2.5; 3.7)	104.0 (1.4; 2.3)	6.0
Simazine	1.0	2.5	2.5–100.0 (0.9975)	118.5 (0.6; 5.5)	103.9 (2.8; 4.3)	100.6 (3.3; 5.1)	3.6
Tebuconazole	1.0	2.5	2.5–100.0 (0.9975)	111.4 (6.4; 9.9)	107.8 (3.5; 5.8)	105.1 (1.7; 2.5)	17.3
Thiabendazole	5.0	10.0	10.0–100.0 (0.9916)	n.a.	100.3 (6.9; 11.0)	104.3 (3.4; 5.3)	18.9
Triadimefon	2.5	5.0	5.0–100.0 (0.9981)	n.a.	99.5 (2.9; 4.5)	100.8 (0.9; 1.5)	15.9
Trifluralin	0.5	1.0	1.0–100.0 (0.9985)	102.2 (2.5; 4.3)	99.2 (1.7; 2.6)	99.2 (2.1; 2.9)	2.6
Phthalates							
Benzyl butyl phthalate	1.0	2.5	2.5–100.0 (0.9974)	87.3 (3.3; 5.5)	101.4 (1.6; 2.5)	99.8 (1.0; 1.7)	14.3
Di(2-ethylhexyl) phthalate	5.0	10.0	10.0–100.0 (0.9965)	n.a.	116.4 (1.4; 7.0)	104.2 (9.8; 14.5)	79.9
Dibutyl phthalate	5.0	10.0	10.0–100.0 (0.9948)	n.a.	98.9 (1.3; 4.6)	102.3 (6.3; 9.1)	7.9
Diethyl phthalate	2.5	5.0	5.0–100.0 (0.9975)	n.a.	101.9 (1.7; 2.9)	98.9 (1.7; 2.6)	4.4
Diisobutyl phthalate	5.0	10.0	10.0–100.0 (0.9941)	n.a.	98.7 (1.0; 9.9)	103.8 (6.5; 10.0)	13.4
Dimethyl phthalate	1.0	2.5	2.5–100.0 (0.9975)	114.5 (0.8; 3.3)	103.0 (2.3; 3.8)	99.0 (1.6; 2.5)	2.7
Di-n-octyl phthalate	0.5	1.0	1.0–100.0 (0.9979)	92.9 (4.7; 7.3)	100.3 (1.2; 2.1)	94.1 (3.6; 5.6)	39.7
Polycyclic aromatic hydrocarbons							
Benz[*a*]anthracene	0.5	1.0	1.0–100.0 (0.9951)	81.6 (2.7; 3.8)	99.1 (1.0; 1.8)	97.5 (4.2; 6.1)	14.0
Benzo[*a*]pyrene	0.5	1.0	1.0–100.0 (0.9968)	95.0 (6.3; 9.4)	88.9 (2.3; 2.7)	87.3 (9.0; 12.9)	7.5
Benzo[*b*]fluoranthene	0.5	1.0	1.0–100.0 (0.9965)	110.2 (6.4; 9.5)	97.8 (3.1; 5.3)	92.4 (7.4; 10.2)	4.3
Chrysene	0.5	1.0	1.0–100.0 (0.9942)	78.5 (3.8; 6.2)	100.6 (1.1; 1.9)	97.7 (4.2; 5.7)	15.3
Dibenz[*a,h*]anthracene	1.0	2.5	2.5–100.0 (0.9949)	101.1 (8.1; 11.8)	86.5 (5.3; 9.8)	80.1 (4.3; 14.0)	–29.6
Dibenzo[*a,h*]pyrene	5.0	10.0	10.0–100.0 (0.9967)	n.a.	84.4 (4.0; 5.7)	74.9 (4.1; 13.6)	–71.7
Dibenzo[*a,i*]pyrene	5.0	10.0	10.0–100.0 (0.9987)	n.a.	77.9 (4.2; 11.5)	79.7 (1.0; 12.9)	–75.4
Dibenzo[*a,l*]pyrene	5.0	10.0	10.0–100.0 (0.9947)	n.a.	87.0 (5.7; 10.7)	81.3 (4.3; 13.7)	–55.9
Indeno(1,2,3-*cd*)pyrene	5.0	10.0	10.0–100.0 (0.9961)	n.a.	80.7 (5.3; 8.6)	85.8 (5.3; 12.7)	–28.1
5-Methylchrysene	1.0	2.5	2.5–100.0 (0.9951)	80.2 (4.7; 8.2)	99.9 (0.5; 1.0)	96.7 (4.6; 6.7)	10.1

LOD: limit of detection; LOQ: limit of quantification; R^2: coefficient of determination; ME: matrix effect; n.a.: not applicable because the spiked level is lower than the LOQ established for the analyte. [a] Commercial sample of powdered infant formula based on skimmed milk, whey, and vegetable oils, among other ingredients, containing 9 % proteins, 28 % fats, and 55 % carbohydrates, intended for infants between 0 and 6 months of age. [b] $n = 5$. [c] Precision in terms of relative standard deviation (RSD) under repeatability ($n = 5$) and within-laboratory reproducibility ($n = 10$) conditions.

application of LDS-DLLME provides an additional clean-up based on the liquid–liquid partitioning of matrix co-extractives, suggesting a higher concentration of polar co-extractives in the acetonitrile–water phase than in the toluene layer.

In comparison with other sample treatments used for determining PAHs, phthalates, and pesticide residues in infant formulas by GC–MS, the proposed sample preparation stands out by low consumption of non-halogenated organic solvents (5.2 mL per sample) and other chemicals (25.0 mg of PSA sorbent per sample), as well as it does not require salts mixture, disposable plastic SPE cartridges, and preconcentration step through solvent evaporation, thus saving costs, energy and time (Tables S1, S2, and S3 of the Supplementary Material).

3.1.2. GC–MS analysis

To improve the method detectability, several GC–MS injection conditions were explored. A comparison between pulsed splitless and conventional splitless injection techniques is shown in Fig. 1. Particularly for pulsed splitless, the better conditions associated with the injection pulse pressure (20, 30, 40, or 50 psi) and pulse duration (0.25, 0.5, or 1 min) were studied (Figs. 1 and 2). In addition, the influence of injector temperature (250, 275, or 300 °C) and injection volume (1.0 or 2.0 μL) on the analytical signals were also evaluated.

As can be seen in Fig. 1, the pulsed splitless injection provided the highest analytical response for all analytes when compared to the conventional splitless technique. Although too little has been explored, similar behaviour was reported in the analyses of organophosphorus pesticides in green beans [28] and water samples [29], as well as in the determination of impurity profiling of methamphetamine [30]. In pulsed splitless mode, the increase of pressure on the top of the column, for a short time during the sample injection, leads to a higher flow rate of the carrier gas through the injector, and hence the sample vapours are quickly transported from the inlet into the column. As a consequence,

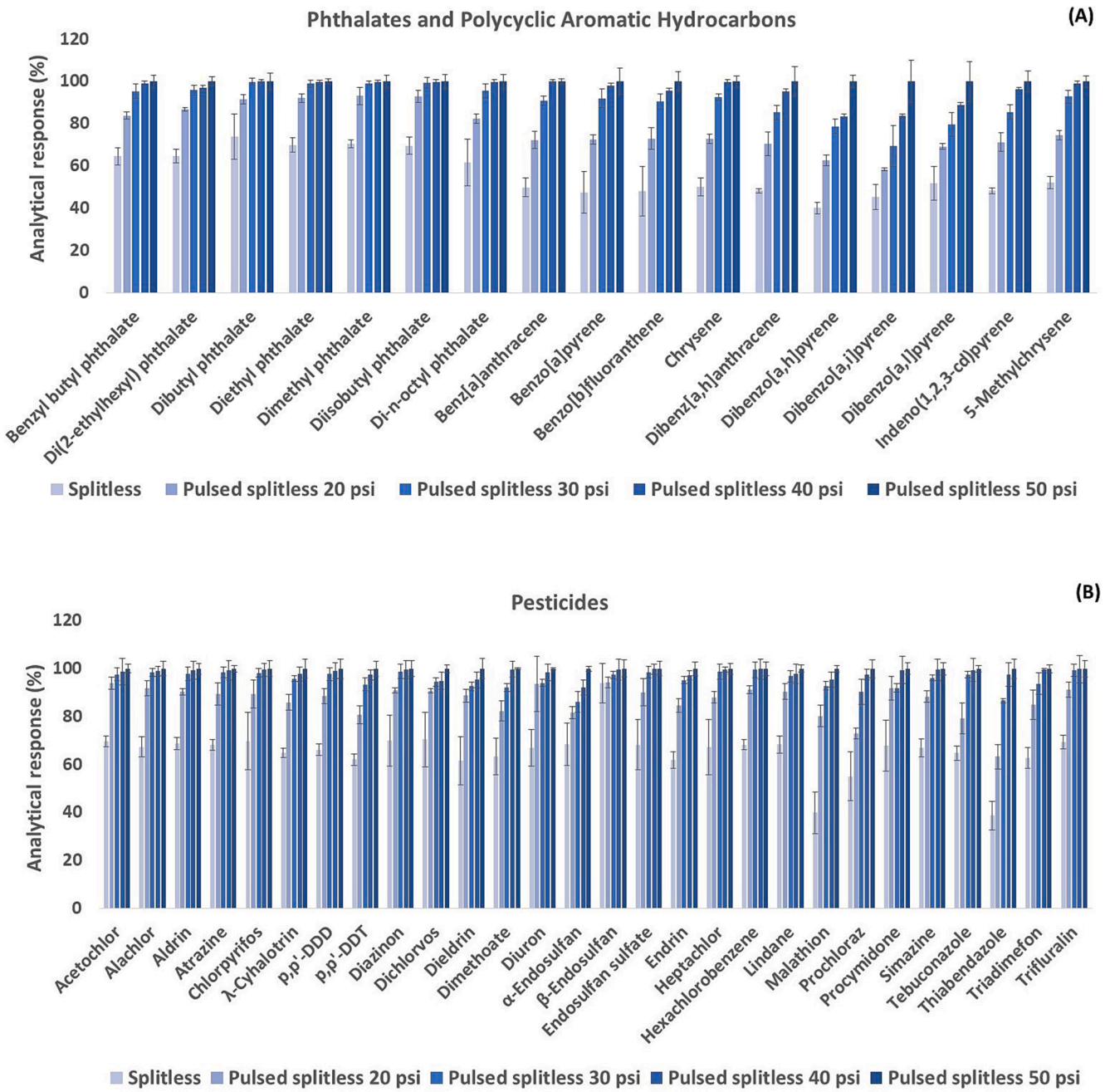

Fig. 1. Effect of the injection technique (splitless or pulsed splitless at different pulse pressures) on the analytical response (normalized to peak area) of phthalates and PAHs (A) and pesticides (B), using an infant formula extract spiked at 20.0 μg kg^{-1}. GC–MS injection conditions: sample volume of 2.0 μL; injector at 300 °C; and pulse duration of 0.5 min in pulsed splitless mode. (Mean ± standard deviation, $n = 3$).

the residence time of the analytes in the hot vaporizing chamber and their interaction with the active inlet surfaces is minimized, resulting in an enhanced analytical response [31].

The great impact of the injection pulse pressure (20–50 psi) in the analytical response was verified (Fig. 1). High pressures such as 40 psi (carrier gas flow at 4.8 mL min^{-1}) or 50 psi (6.8 mL min^{-1}) resulted in higher signal for the vast majority of the analytes when compared with 20 psi (2.0 mL min^{-1}), for example. Particularly, for the last eluting compounds (dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, and dibenzo[*a,l*] pyrene), a distinguished response was achieved by applying a pulse pressure at 50 psi, suggesting the positive effect of high pressures at the injection port in the detectability of less volatile compounds (Fig. 1A). Regarding the pulse duration, 0.25 and 0.5 min resulted in better analytical signal for the most of compounds under study, when compared with 1 min at 50 psi (Fig. 2). In particular, the majority of PAHs presented the highest responses at 0.5 min; however, for some pesticides, slight better responses were observed at 0.25 min (Fig. 2). Our results support previous studies in which high pulse pressures (60–70 psi), for a short time (1 min), achieve greater analytical signals [32]. In contrast, pressures above 60 psi with pulse duration exceeding 1 min can result in lower responses and losses of early eluting volatile analytes which have been attributed to the partial evaporation of compounds from the solvent layer caused by the large column flow rate [32].

A comparison of peak intensities obtained from different injection temperatures (250, 275, or 300 °C), in pulsed splitless mode at 50 psi for 0.5 min, is presented in Fig. 3. A strong influence of injector temperature on the analytical response was observed, mainly, for those less volatile compounds that appeared after 21 min run (benzo[*b*]fluoranthene,

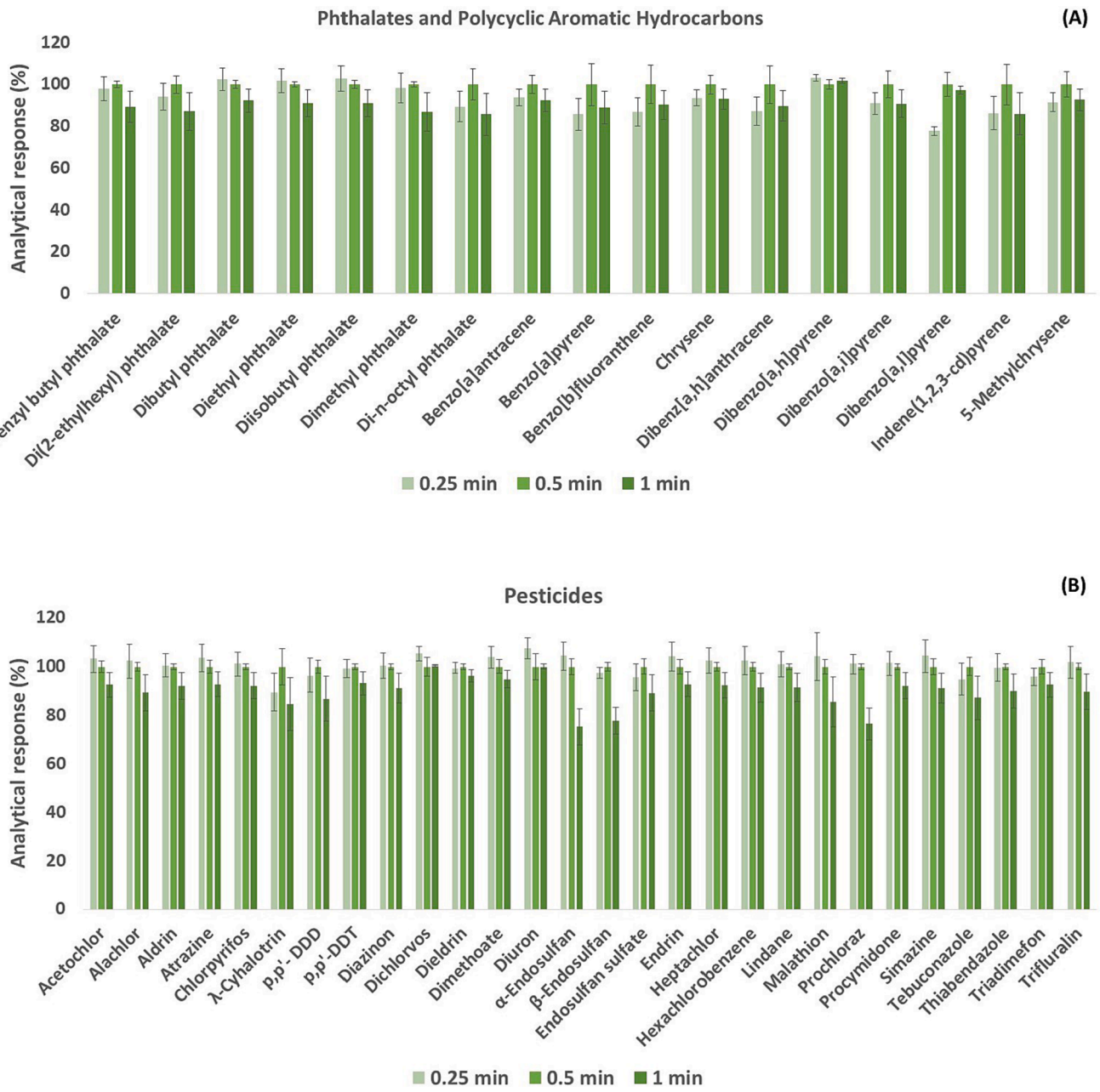

Fig. 2. Effect of the pulse duration (0.25, 0.5, or 1 min) in pulsed splitless injection technique on the analytical response (normalized to peak area) of phthalates and PAHs (A) and pesticides (B), using an infant formula extract spiked at 20.0 μg kg^{-1}. GC–MS injection conditions: sample volume of 2.0 μL; injector at 300 °C; and pulse pressure at 50 psi. (Mean ± standard deviation, $n = 3$).

benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, dibenz[*a,h*]anthracene, dibenzo[*a,l*]pyrene, dibenzo[*a,i*]pyrene, and dibenzo[*a,h*]pyrene), with higher signals observed at 300 °C. For the other analytes, a slight increment in the response was also observed at high temperatures (Fig. 3). Additionally, the large difference between the injector (300 °C) and the initial oven (85 °C) temperatures contributes to obtaining narrow peak shapes. Since the column temperature is at least 25 °C lower than the boiling point of the sample solvent (toluene, B.P. = 110.6 °C), a liquid film is formed at the top of the column by the condensation of the solvent; thus, the evaporation of this film occurs during the temperature program and then the compounds are concentrated in a continuous smaller liquid film resulting in a narrow band of enriched sample components [33].

Finally, distinct injection volumes (1.0 or 2 μL) were evaluated. As expected, a larger injection volume (2.0 μL) provided the highest analytical responses. Interestingly, no chromatographic peak broadening was observed for 2.0 μL, maintaining the symmetry of peaks when compared with 1.0 μL (Fig. S1 of Supplementary Material). A high pressure applied to the column head during the pulsed splitless injection enables the introduction of considerable amounts of sample; in this way, the active sites in the inlet are "swamped" by the large volume injected facilitating the transference of a wide portion of "unharmed analytes" to the column [28,32]. In summary, the better sample injection conditions were fixed as 2.0 μL of sample volume in pulsed splitless mode at 50 psi for 0.5 min at 300 °C.

3.1.3. Matrix effects

Ideally, low (–20.0 % < ME < 20.0 %) or medium (–50.0 % < ME < –20.0 % or 20.0 % > ME > 50.0 %) matrix effects (ME) were observed for the majority of the analytes (91.1 %) under study. Whereas, high

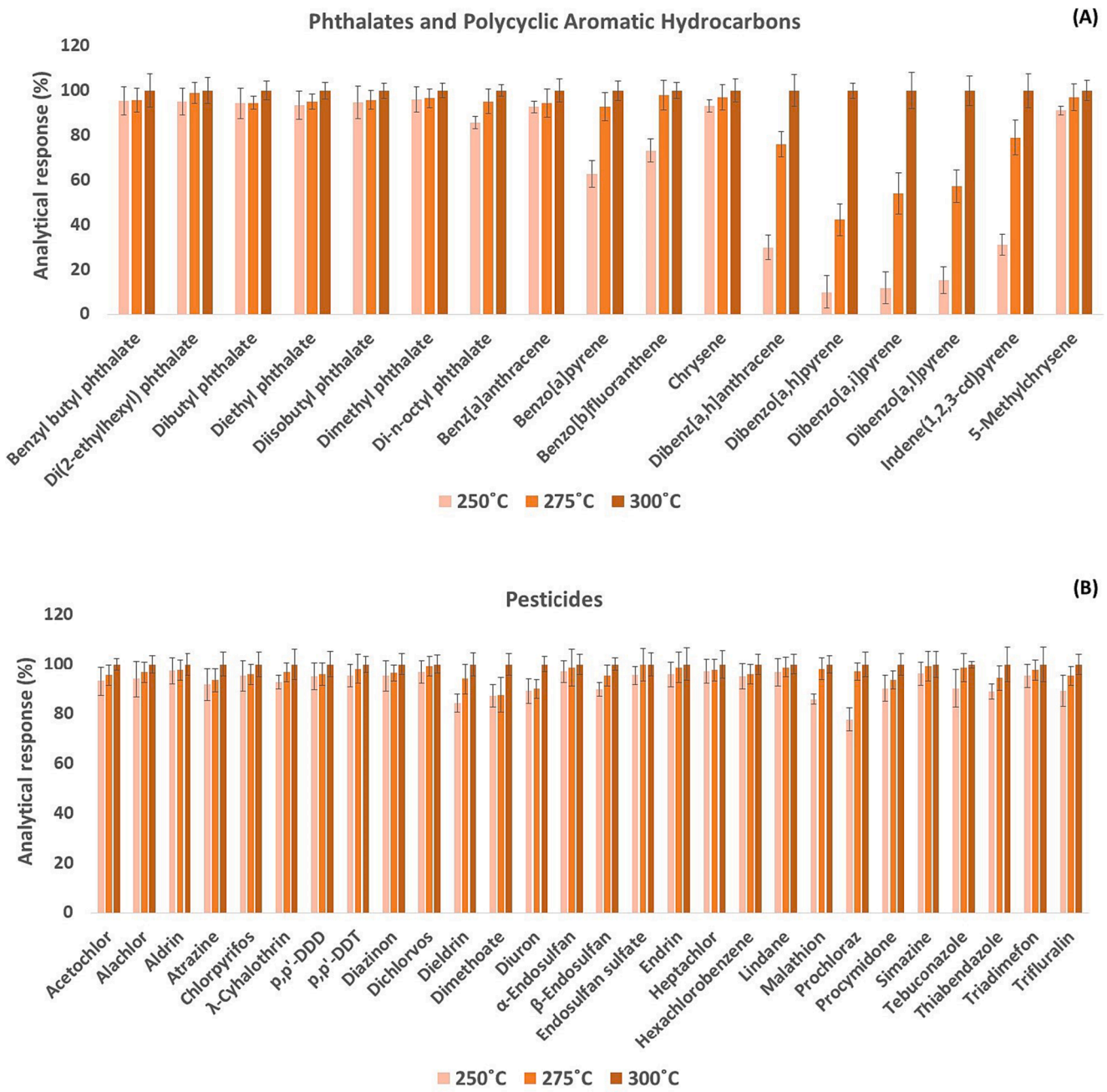

Fig. 3. Effect of the injector temperature (250, 275, or 300 °C) on the analytical response (normalized to peak area) of phthalates and PAHs (A) and pesticides (B), using an infant formula extract spiked at 20.0 μg kg^{-1}. GC–MS injection conditions: sample volume of 2.0 μL; pulsed splitless injection at 50 psi for 0.5 min. (Mean ± standard deviation, $n = 3$).

matrix effects (ME < –50.0 % or ME > 50.0 %) were verified for only four compounds, namely, di(2-ethylhexyl) phthalate, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, and dibenzo[*a,l*]pyrene (Table 2). These findings indicate the efficiency of the proposed sample preparation regarding the low extraction and/ or removal of matrix co-extractives. Moreover, the pulsed splitless injection has been associated with lower matrix effects when compared with the conventional splitless technique [32,34].

Matrix-induced signal enhancement was the main phenomenon observed affecting 86.7 % of the analytes; while, signal suppression was verified particularly for the pesticide dimethoate and the PAHs dibenz[*a,h*]anthracene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene, and indeno[1,2,3-*cd*]pyrene (Table 2). In GC–MS analysis, signal enhancement has been associated with the deposition of matrix co-extractives in the injector port and chromatographic column which blocks active sites resulting in a more efficient analyte transfer and consequently a greater analytical signal in matrix extracts than solvent-only solutions [31,35]. In parallel, the gradual accumulation of non-volatile matrix components in the GC inlet and front part of the column provides new active sites which may result in analytical signal suppression for certain analytes [31,35].

To establish, tentatively, a relation between the effects observed and the analyte features, the matrix effect was plotted against the retention time and log K_{ow} values (Fig. 4). Interestingly, it was observed a uniform distribution of the matrix effects over the retention time and log K_{ow} values for the majority of analytes, with some outliers (Fig. 4). The largest matrix effects were verified mainly for the analytes with the highest log K_{ow} values (≥ 6.5) as well as those less volatile with higher

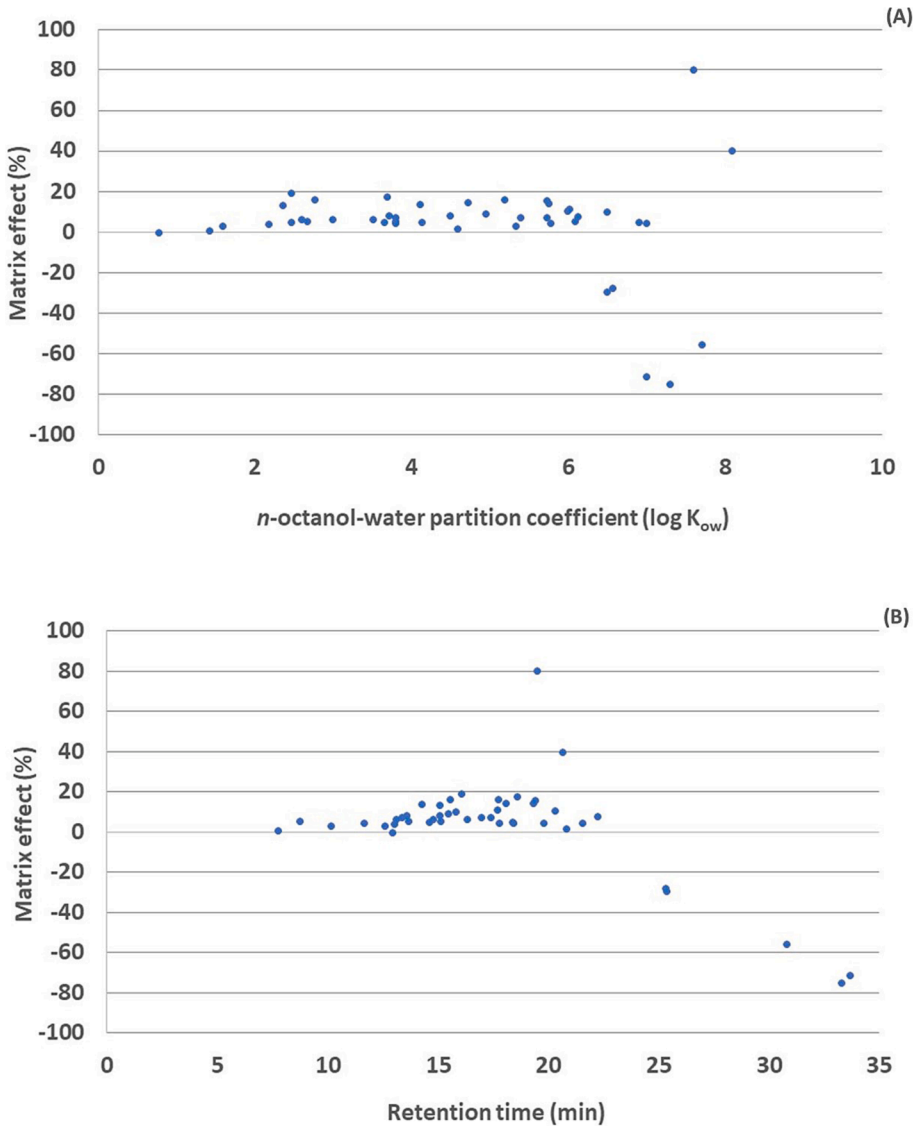

Fig. 4. Matrix effect plotted against log K_{ow} (A) and retention time in GC–MS (B) of the 45 compounds.

retention time (≥ 19.5 min), including di(2-ethylhexyl) phthalate, di-n-octyl phthalate, dibenz[*a,h*]anthracene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene, and indeno[1,2,3-*cd*]pyrene (Fig. 4). Similar behavior was reported in the analysis of pesticide residues in olive oil, in which the highest matrix effects were observed for those compounds with relevant retention time [36].

In addition to the physicochemical proprieties of the analyte, the magnitude of the matrix effect may be also influenced by other factors

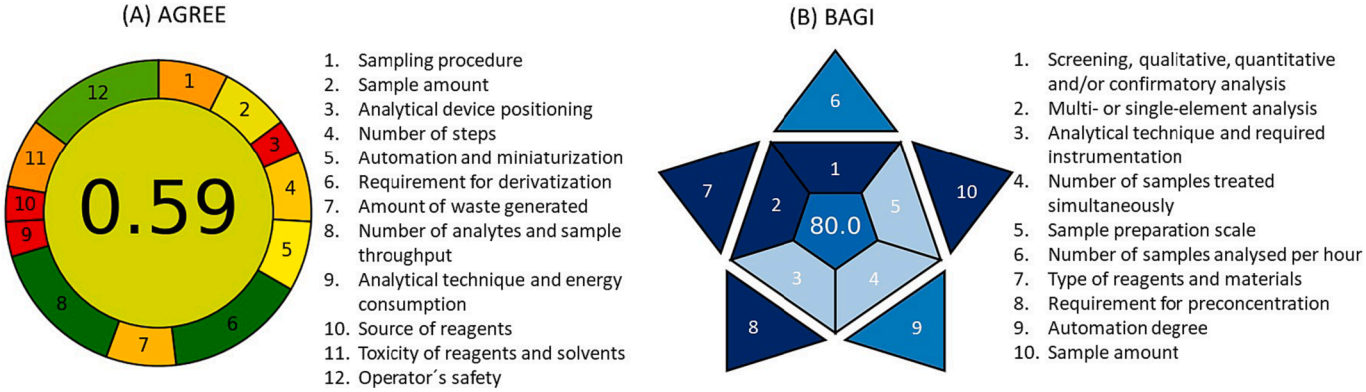

Fig. 5. Assessment of greenness and practicality of the proposed method through the Analytical Greenness – AGREE (A) and Blue Applicability Grade Index – BAGI (B) metric tools, respectively. The numbers 1 to 10 were added to the BAGI asteroid pictogram (Fig. 5B) to indicate the attributes evaluated in the metric tool. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

such as the nature of the matrix, analyte-to-matrix ratio, and GC–MS system conditions [31,35,37]. Since the matrix effects can severely affect the accuracy, detectability, and reproducibility of the analytical method, and hence compromise the quantitative analysis [37], matrix-matched calibration curves, prepared in extracts of a representative infant formula sample, were used to compensate for the matrix effects [20].

3.1.4. Evaluation of the analytical method through metric tools

The greenness and practicality of the proposed method were evaluated through the Analytical Greenness (AGREE) [38] and Blue Applicability Grade Index (BAGI) [39] metric tools, respectively. The AGREE is focused on the principles of Green Analytical Chemistry and it evaluates twelve criteria as detailed in Fig. 5A. Based on a unified scale between 0 (red) and 1 (dark green), an overall score of 0.59 was obtained demonstrating a certain greenness of the entire analytical procedure (Fig. 5A). The greener aspects were associated with the absence of derivatization (criterium 6), multi-analytes in a single run and the number of samples analysed per hour (criterium 8), and operator's safety (criterium 12), for which the highest weight was attributed. Whereas, the non-green features were mainly related to the GC–MS technique, namely, the analytical device positioning with off-line measurement (criterium 3), energy-intensive instrumentation (criterium 9), and the use of acetonitrile and toluene, both compatible with the technique but, solvents of non-renewable sources (criterium 10). Since chromatography systems coupled to mass spectrometry are decisive in the determination of contaminants in food, the lowest weight was assigned for these criteria (Fig. 5A). For all others, an average weight was attributed assuming that all of them are equally important.

The BAGI has been considered complementary to green metric tools and it is based on the practical aspects of White Analytical Chemistry [39]. The analytical method presented an overall score of 80.0 (Fig. 5B), on a scale from 25.0 (white) to 100.0 (dark blue), demonstrating excellent performance in terms of practicality and applicability. Among the ten main attributes assessed, the proposed method stands out for its quantitative and confirmatory analysis (attribute 1), multi-analytes determination (attribute 2), type of reagents and materials used (attribute 7), non-requirement for additional preconcentration step (attribute 8), and the amount of sample (attribute 10) (Fig. 5B).

3.2. In-house validation

Under the optimized GC–MS conditions, no interfering compounds were observed at the retention time of the analytes by comparing the extracted ion chromatograms obtained from the analyses of standard solution, blank matrix, and spiked infant formula samples. Thus, the analytical selectivity was confirmed based on the ability of the method to accurately determine the target analytes without interference from matrix co-extractives of similar behaviour [40].

Reliable limits of detection (LOD) and quantification (LOQ) were established using blank matrix extracts spiked at concentration levels that were decreased until achieving signal: noise ratio of 3:1 and 10:1, respectively. Since at least three ions are required for the identification employing a single quadrupole mass analyser in SIM mode [20], the LOD and LOQ values were dependent on the presence of the diagnostic ion of lower intensity in the sample extract. LODs ranged from 0.5 to 5.0 μg kg^{-1}, with values ≤ 2.5 μg kg^{-1} for most analytes; whereas, the LOQs were within the range of 1.0–10.0 μg kg^{-1}, in which the great majority of the compounds presented values ≤ 5.0 μg kg^{-1} (Table 2). Compared with previous GC–MS methods, the obtained LODs were lower or equal to those reported for certain phthalates and pesticides; particularly for the PAHs, the LODs were higher than the values described in the literature (Tables S1, S2, and S3 of Supplementary Material). In general, the LOD and LOQ obtained were sufficiently low for monitoring and quantifying the regulated analytes in infant formula at the maximum limits of 10.0 μg kg^{-1} [15,16] and 1.0 μg kg^{-1} [14] fixed for pesticide residues and PAHs (benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, and chrysene), respectively.

Individual matrix-matched calibration curves were prepared by adding appropriate aliquots of standard working solution to blank matrix extracts providing 1.0, 2.5, 5.0, 10.0, 20.0, 40.0, 60.0, 80.0, and 100.0 μg kg^{-1} of standard equivalent in the sample. Since the LOQ was fixed as the first concentration level of the calibration curves, different linear ranges varying from 1.0 to 100.0 μg kg^{-1} were obtained, including at least five concentration levels each (Table 2). Linearity was evaluated by the ordinary least squares method, whose regression equations included slope values between 0.0016 (linuron) and 0.1643 (di-n-octyl phthalate), intercept values from –0.0653 (dibenzo[*a,l*]pyrene) to 0.1952 (diethyl phthalate), and coefficients of determination (R^2) ≥ 0.9915. In addition, the homoscedasticity of the residuals of regression was evaluated by Levene *t* statistics, which were not significant ($p > 0.05$) indicating that the variance of the residuals over the studied concentration range was constant [41].

The accuracy of the method was demonstrated through recovery and precision experiments using a representative matrix spiked at levels 2.5, 20.0, and 100.0 μg kg^{-1}. Adequate mean recoveries, between 74.9 % (dibenzo[*a,h*]pyrene at the 100.0 μg kg^{-1} level) and 118.5 % (simazine at the 2.5 μg kg^{-1} level), were observed for all analytes (Table 2). According to Document N° SANTE 11312/2021, recovery values within the range of 70.0–120.0 % are required for analytical methods for pesticide residues in food and feed [20]. Particularly for PAHs, recoveries from 50.0 to 120.0 % are acceptable for methods of analysis of benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, and chrysene in food matrices [42]. The precision, evaluated in terms of relative standard deviation (RSD, %), was ≤ 10.3 % under repeatability conditions, and ≤ 17.8 % under within-laboratory reproducibility conditions (Table 2). In general, the RSD values shall be as low as possible, with values ≤ 20 % required in the pesticide analysis [20].

3.3. Application to real samples

The feasibility of the proposed method for routine analysis was confirmed by their application to infant formulas marketed in Brazil, with subsequent identification and quantification of certain PAHs and phthalates in some of them (35.0 %). Ideally, no residues of pesticides were observed in any sample.

Benzo[*a*]pyrene and benz[*a*]anthracene was observed separately in two samples at a mean concentration of 5.0 ± 0.2 μg kg^{-1} and 47.1 μg kg^{-1} ± 0.9 μg kg^{-1}, respectively; whose levels largely exceeded the maximum limit of 1.0 μg kg^{-1} set for PAHs in infant formulas by the European Commission [14]. Specifically, these two positive samples contained vegetable oils in their composition, such as soybean oil, sunflower oil, coconut oil, and safflower oil, which are recognised sources of PAHs in the diet. For instance, maximum levels of benzo[*a*]pyrene and benz[*a*]anthracene, within the ranges of 3.3–7.5 μg kg^{-1} and 3.4–6.7 μg kg^{-1}, respectively, were reported in vegetable oils from Brazil [43]. Regarding the reports on these PAHs in infant formulas, maximum concentrations of benzo[*a*]pyrene between 0.16 and 1.7 μg kg^{-1}, and benz[*a*]anthracene from 0.2 to 2.4 μg kg^{-1}, were found in samples from China, Iran, Italy, and Nigeria [44–47].

Dibutyl phthalate (5.0 ± 1.2 μg kg^{-1}), di(2-ethylhexyl) phthalate (38.6 ± 0.9 μg kg^{-1}), dimethyl phthalate (1.4 ± 0.1 μg kg^{-1}), and diisobutyl phthalate (21.7 ± 0.3 μg kg^{-1}) were detected separately in four infant formulas. Furthermore, the simultaneous occurrence of di(2-ethylhexyl) phthalate and dibutyl phthalate was verified in another sample at concentrations of 40.9 ± 0.2 μg kg^{-1} and 11.2 ± 0.4 μg kg^{-1}, respectively. The results obtained are in line with previous studies about phthalates in infant formulas. Contents between 6.2 and 11.0 μg kg^{-1} (dibutyl phthalate), 18.0 and 25.0 μg kg^{-1} (diisobutyl phthalate), and from 18.0 to 75.0 μg kg^{-1} (di(2-ethylhexyl) phthalate) were reported in samples acquired in Italy [48]. In goat milk-based infant formulas, average concentrations of 11.2 μg kg^{-1} (dimethyl phthalate), 29.5 μg

kg^{-1} (diisobutyl phthalate), 32.2 μg kg^{-1} (di(2-ethylhexyl) phthalate), and 63.3 μg kg^{-1} (dibutyl phthalate) were found in samples marketed in China [49].

Particularly, the presence of benzo[*a*]pyrene (5.0 ± 0.2 μg kg^{-1}) and dimethyl phthalate (1.4 ± 0.1 μg kg^{-1}), whose levels were discussed previously, was observed in the same infant formula sample. To the best of our knowledge, this is the first report on the simultaneous quantification of PAH and phthalate in a product intended for infant consumption.

4. Conclusions

An original analytical method based on GC–MS was developed for accurate and high-throughput determination of three important contaminant groups (PAHs, phthalates, and pesticide residues) in infant formula, which have been typically studied in food matrices by separated methods. The low quantity of non-halogenated solvents and other chemicals required in the sample preparation, associated with a high enrichment factor and reduced generation of waste, comprise the main features of the proposed approach. The greenness and practicality of the analytical method were confirmed through the AGREE and BAGI metric tools, respectively. Furthermore, low matrix effects were observed for the majority of the analytes, suggesting a possible relation of these effects with the analyte's retention time and *n*-octanol-water partition coefficient (log k_{ow}). Suitable method performance characteristics were achieved for 45 food contaminants, including LOD and LOQ low enough to monitor the regulated compounds at the maximum limits established in infant formula. Exploring different GC–MS injection conditions, distinguished analytical responses were obtained at high temperatures in pulsed splitless mode at high pulse pressures. Overall, the co-occurrence of phthalate and PAH in a commercial sample reinforces the priority of establishing novel analytical methods for monitoring multi-class toxicants in foods intended for infants and young children to ensure the safety and quality of these products, as well as to evaluate the dietary exposure of this vulnerable consumer group to chemicals of distinct nature.

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CRediT authorship contribution statement

Mateus Henrique Petrarca: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft. **Eduardo Vicente:** Methodology, Resources, Founding acquisition, Writing – review & editing. **Silvia Amelia Verdiani Tfouni:** Resources, Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2023.109824.

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