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Targeted and non-targeted analysis of pesticides and aflatoxins in baby foods by liquid chromatography coupled to quadrupole Orbitrap mass spectrometry

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ABSTRACT

In this study, 21 pesticides and 4 aflatoxins were monitored in baby food marketed in Brazil, applying ultra-high-performance liquid chromatography coupled to quadrupole-Orbitrap mass spectrometry (UHPLC-Q-Orbitrap-MS). The quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction method combined with dispersive solid-phase extraction (d-SPE) clean-up was applied and primary secondary amine (PSA), octadecylsilane (C18) and C18 with silica coated with zirconium dioxide (Z-Sep+) were used during the clean-up stage. Suitable performance criteria, set by the SANTE/2020/12830 guidelines, were achieved, and therefore, all targeted analytes were successfully validated. The method was applied to the analysis of 50 baby food samples. Cypermethrin was detected at 10.3 μg kg^{-1} (above maximum residue level (MRL) established by the European Union (EU)). Furthermore, suspect screening analysis was performed for reliable identification of contaminants not included in this study such as other pesticides, mycotoxins, hormones, veterinary drugs and their metabolites. Finally 10 pesticides and one metabolite were detected, demonstrating the suitability of the proposed approach.

1. Introduction

Infants are considered a sensible and vulnerable population group, because they intake more food per kilogram of body weight than adults do, and their detoxification system and metabolic pathways are not fully developed (Nougadère et al., 2020). Currently, there are a rich variety of food products designed for babies composed of vegetables, meats, fruits, and cereals (Prata et al., 2021). These products may be contaminated with pesticides (frequently applied to control plant pests and to increase productivity) and mycotoxins resulting from natural fungal growth during agricultural crops or harvest storage (Eyring et al., 2021). Thus, exposure to pesticides and mycotoxins is inevitable due to this food consumption.

The term pesticide includes a variety of compounds such as insecticides, fungicides, and herbicides and due to their ubiquitous presence in common food, they are associated with potential health hazards. Evidence suggests that pesticides mainly act on the nervous system (Notardonato et al., 2019) increasing the risk of developing neurodegenerative diseases. Furthermore, it is associated with diseases such as cancer and dysfunctions in the endocrine and reproductive systems (Petrarca et al., 2016). Among all aflatoxins, aflatoxin B1 was recognized as the most toxic mycotoxin and the strongest natural carcinogen (Beltrán et al., 2011). Additionally, the International Agency for Research on Cancer (IARC) classified aflatoxins B1, B2, G1 and G2 in group 1 as human carcinogens, which is a global human health concern (Internacional Agency for Research on Cancer, 2021). To protect children from harmful substance intake, the European Union has established different regulations for baby and infant processed food. Since 2006, the Directive 2006/125/EC establishes MRLs for pesticides in processed baby food at 10 μg kg^{-1}, and lower MRLs were also set for specific pesticides, such as fipronil (4 μg kg^{-1}). In addition, pesticides that should not be used in food commodities intended for the production of

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baby foods have also been regulated (European Commission, 2006). Though there are no specific Brazilian MRLs established for pesticide residues in baby foods, 500 active ingredients have MRLs set for a wide range of food commodities in that country (ANVISA, 2021). Concerning aflatoxins, the Regulation EC 165/2010 sets MRLs for baby foods, and for aflatoxin B1 it was set at 0.1 μg kg^{-1} (European Commission, 2010). In Brazil, MRLs of aflatoxins B1, B2, G1, and G2 were established at 1 μg kg^{-1} for cereal-based baby foods (ANVISA, 2011).

Based on this, the occurrence of these compounds in different baby foods should be evaluated, as exposure of the infant population to these substances should be taken into account when risk assessment studies are being performed. However, there are scarce data concerning the presence of these analytes in food intended for children available on the Brazilian market.

In Brazil, liquid chromatography–tandem mass spectrometry (LC–MS/MS) was applied for the analysis of mycotoxins in infant formula and milk-based products for young children (Tonon et al., 2018), in fruit-, meat-, vegetable-, and pasta-based baby food (da Silva et al., 2020), and in commercial cereal-based porridge baby food (Sartori et al., 2017). Furthermore, pesticides were analyzed in fruit-based baby foods using gas chromatography–mass spectrometry (GC-MS) (Petrarca et al., 2016) and LC-MS/MS (Petrarca et al., 2017), and in soy-based infant formula by LC with fluorescence detection (de Souza et al., 2021; Rodrigues & de Souza, 2018).

In the last 10 years, LC-MS has been used to analyse pesticides in fruit-, vegetable-, and/or cereal-based baby foods worldwide (Díaz-Galiano et al., 2021; Gilbert-López et al., 2012; Mirabelli et al., 2016; Torović et al., 2021; Vuković et al., 2012). In addition, pesticide and veterinary drug residues were simultaneously determined in different baby foods, included meat-based baby food (Gómez-Pérez et al., 2015; Jia et al., 2014). For cereal-based baby food, a multiresidue method was developed for the simultaneous determination of pesticides, plant hormones, veterinary drugs and mycotoxins (Danezis et al., 2016). Two polar herbicides were also analyzed in baby foods composed of meat, fish, cheese, vegetable, and fruits (Panseri et al., 2020). In relation to sample treatment, nowadays, the QuEChERS (quick, easy, cheap, effective, rugged, and safe) method has been largely used to monitor pesticide in baby foods (Petrarca et al., 2016), although other extraction procedures as ultrasound-assisted dispersive liquid–liquid microextraction (UA-DLLME) also provided suitable results.

Bearing in mind the properties of QuEChERS method, a study was carried out for the simultaneous analysis of twenty-one pesticides, belonging to seven distinct chemical classes, and four aflatoxins in baby foods. The pesticides were selected based on those compounds that have been detected previously in the literature (Díaz-Galiano et al., 2021; Nougadère et al., 2020; Petrarca et al., 2016, 2017; Torović et al., 2021), where at the same time, they have been detected by the Program on Pesticide Residue Analysis in Food, coordinated by Brazilian Sanitary Surveillance Agency (ANVISA), and the National Residue and Contaminant Control Program, coordinated by the Ministry of Agriculture, Livestock and Food Supplies, and they are authorized for use in the country by ANVISA (ANVISA, 2019, 2021; BRAZIL, 2019).

For that purpose, LC-Q-Orbitrap-MS was used to perform the detection, and identification of compounds with different physico-chemical properties at low concentration levels, and targeted and non-targeted analyses were performed. Thus, a suspect screening analysis was carried out for a reliable identification of pesticides, mycotoxins, and other contaminants not included in this study. To the best of our knowledge, this is the first study focused on the multiclass analysis of pesticide residues and mycotoxins in Brazilian baby foods, based on current trends zoomed in the development of multiresidue and multiclass methods. Futhermore, this work provides valuable data related to the presence of pesticides in meat and vegetables-based baby foods.

2. Material and methods

2.1. Equipment, material and reagents

Analytical standards of pesticides (λ-cyhalothrin, atrazine, azoxystrobin, chlorpyrifos, cypermethrin, deltamethrin, dimethoate difenoconazole, etofenprox, imazalil, kresoxim methyl, malathion, methidation, phosalone, phosmet, pirimicarb, pirimiphos-methyl, pyraclostrobin, tebuconazole, tetraconazole, and trifloxystrobin) were obtained from Agilent (North Kingstown, RI, USA), whose purity ranged from 95.8 to 99.9%. Reference standards of aflatoxin B1, B2, G1, and G2 were obtained from Sigma-Aldrich (St. Louis, MO, USA). All compounds present a purity ≥99.7%. Stock standard solutions were prepared in acetone, acetonitrile, or methanol at 1000 μg mL^{-1} and were stored at ≤5 °C. Acetonitrile and methanol, LC-MS grade, were acquired from Honeywell, (Morriston, NJ, USA) and acetone from Fluka (St. Louis, MO, USA). Water (LC-MS grade) was provided by Supelco (Darmstadt, Germany). The filters (0.2 μm nylon syringe) were acquired from Agilent Technologies (Santa Clara, CA, USA).

GCB, PSA, and florisil (magnesium silicate) sorbents were purchased from Scharlab (Barcelona, Spain). C18 sorbent was purchased from Agilent Technologies. Sodium chloride, anhydrous magnesium sulfate, and ammonium formate were provided by Sigma-Aldrich (St. Louis, MO, USA). Z-Sep + sorbent was purchased from Supelco (Bellefonte, PA, USA).

To calibrate HRMS analysers, a mixture of acetic acid, caffeine, Met-Arg-Phe-Ala-acetate salt and Ultramark 1621 (ProteoMass LTQ/FT-hybrid ESI positive), obtained from Thermo-Fisher (Waltham, MA, USA), was employed for UHPLC-Q-Orbitrap-MS calibration.

An analytical balance Pioneer PX124 (Ohaus, Nänikon, Switzerland), a vortex mixer WX (Velp Scientifica, Usmate, Italy), a Consul 21 high-volume centrifuge (Olto Alresa, Madrid, Spain), and a Reax 2 overhead shaker (Heidolph, Schwabach, Germany) were used for the extraction procedure.

2.2. UHPLC-Q-Orbitrap-MS analysis

For the separation of the compounds, a Vanquish Flex Quaternary LC (Thermo Scientific Transcend™, Thermo Fisher Scientific, San Jose, CA, USA) was used. A Zorbax Eclipse plus C18 (100 mm × 2.1 mm x 1.8 μm particle size) from Agilent Technologies (Santa Clara, CA, USA) was chosen. For a suitable elution of the compounds, an aqueous solution of ammonium formate (4 mM) and formic acid (0.1%) was selected as eluent A, whereas methanol was used as eluent B. A gradient profile was utilized, starting at 5% of eluent B (0–1 min); from 1 to 4 min, it was increased to 100% of eluent B and after that, this composition was kept for 6 min, before returning to the initial conditions in 0.5 min. Finally a re-equilibration time of 3.5 min was set, achieving a total running time of 14 min. The column temperature was set at 30 °C and the flow rate was set at 0.2 mL min^{-1}. Aliquots of 10 μL were injected.

A hybrid mass spectrometer, Q-Exactive Orbitrap (Exactive™, Thermo Fisher Scientific, Bremen, Germany) was coupled to the chromatographic system. A heated electrospray interface (ESI) (HESI-II, Thermo Fisher Scientific, San Jose, CA, USA), working in positive (ESI+) and negative ionization mode (ESI−) was used. ESI parameters were: spray voltage, 4 kV; sheath gas (N_2, 95%), 35 (arbitrary units); auxiliary gas (N_2, 95%), 10 (arbitrary units); S-lens RF level, 50 (arbitrary units); capillary temperature, 300 °C; and heater temperature, 305 °C. Four acquisition functions were used to acquire MS spectra, based on previous studies (Hergueta-Castillo et al., 2022): (1) full MS, ESI+, without fragmentation (the collision cell (HCD) was switched off), mass resolving power = 70,000 Full Width at Half Maximum (FWHM); AGC target = 1e6; (2) data independent mass spectrometry fragmentation (DIA-MS/MS), ESI+ (HCD on, collision energy = 30 eV), mass resolving power = 35,000 FWHM; AGC target = 1e5, (3) full MS ESI- without fragmentation (the collision cell was switched off), mass resolving

Table 1
Exact mass database including chemical group/use type, log K_{ow}, retention time (RT), theoretical accurate masses, elemental compositions and fragments of the detected ions of target compounds determined by UHPLC-Q-Orbitrap-MS.

Compound	Log K_{OW} [a]	Chemical Group/Use type [a]	Precursor ions (quantifier ions)				Fragment ions (qualifier ions)			RT [c]
			Elemental composition	Monitored ion	Theoretical mass (m/z) [b]	Mass error (ppm)	Theoretical mass (m/z)	Mass error (ppm)	Elemental composition	
λ-Cyhalothrin	6.20	Pyrethroid/Insecticide	$C_{23}H_{19}ClF_3NO_3$	$[M + NH_4]^+$	467.13438	−2.10	225.02885	−0.56	$C_9H_9ClF_3O$	9.37
Atrazine	2.50	Triazine/Herbicide	$C_8H_{14}ClN_5$	$[M + H]^+$	216.10105	−1.61	174.05410	−0.45	$C_5H_9ClN_5$	7.67
							96.05562	−2.88	$C_4H_6N_3$	
Azoxystrobin	2.50	Strobilurin/Fungicide	$C_{22}H_{17}N_3O_5$	$[M + H]^+$	404.12410	−2.03	372.09788	−0.96	$C_{21}H_{14}N_3O_4$	7.71
							344.10297	−0.95	$C_{20}H_{14}N_3O_3$	
Chlorpyrifos	4.70	Organophosphorous/ Insecticide	$C_9H_{11}Cl_3NO_3PS$	$[M + H]^+$	349.93356	−1.92	197.92747	−0.66	$C_5H_3Cl_3NO$	9.36
							321.90226	4.75	$C_7H_8Cl_3NO_3PS$	
Cypermethrin	6.60	Pyrethroid/Insecticide	$C_{22}H_{19}Cl_2NO_3$	$[M + NH_4]^+$	433.10802	−2.21	191.00250	−0.67	$C_8H_9Cl_2O$	9.57
Deltamethrin	4.60	Pyrethroid/Insecticide	$C_{22}H_{19}Br_2NO_3$	$[M + NH_4]^+$	521.00699	−2.06	280.91712	−8.00	$C_8H_{11}Br_2O$	9.60
Difenoconazole	4.40	Triazole/Fungicide	$C_{19}H_{17}C_{l2}N_3O_3$	$[M + H]^+$	406.07197	−2.18	251.00250	−3.49	$C_{13}H_9Cl_2O$	8.67
							337.03928	−4.17	$C_{17}H_{15}Cl_2O_3$	
Dimethoate	0.70	Organophosphorous/ Insecticide	$C_5H_{12}NO_3PS_2$	$[M + H]^+$	230.00690	−1.82	198.96470	−2.05	$C_4H_8O_3PS_2$	6.72
							170.96978	−2.54	$C_3H_8O_2PS_2$	
Etofenprox	6.90	Pyrethroid/Insecticide	$C_{25}H_{28}O_3$	$[M + NH_4]^+$	394.23767	−2.24	177.12739	−4.53	$C_{12}H_{17}O$	10.51
							349.17982	7.39	$C_{23}H_{25}O_3$	
Imazalil	3.82	Imidazole/Fungicide	$C_{14}H_{14}Cl_2N_2O$	$[M + H]^+$	297.05560	−1.82	158.97628	−2.28	$C_7H_5Cl_2$	7.20
							200.98685	−2.87	$C_9H_7Cl_2O$	
Kresoxim-Methyl	3.40	Strobilurin/Fungicide	$C_{18}H_{19}NO_4$	$[M + H]^+$	314.13868	−2.00	222.09134	−2.39	$C_{15}H_{12}NO$	8.40
							282.11247	0.00	$C_{17}H_{16}NO_3$	
Malathion	2.75	Organophosphorous/ Insecticide	$C_{10}H_{19}O_6PS_2$	$[M + H]^+$	331.04334	−1.76	99.00767	−1.82	$C_4H_3O_3$	8.00
							257.00656	−3.74	$C_7H_{14}O_4PS_2$	
Methidathion	2.20	Organophosphorous/ Insecticide	$C_6H_{11}N_2O_4PS_3$	$[M + H]^+$	302.96913	−2.12	145.00662	−2.45	$C_4H_5N_2O_2S$	7.73
							85.03964	−0.82	$C_3H_5N_2O$	
Phosalone	4.01	Organophosphorous/ Insecticide	$C_{12}H_{15}ClNO_4PS_2$	$[M + H]^+$	367.99414	−2.01	182.00033	−2.65	$C_8H_5ClNO_2$	8.60
							138.01050	−2.05	C_7H_5ClN	
Phosmet	2.96	Organophosphorous/ Insecticide	$C_{11}H_{12}NO_4PS_2$	$[M + H]^+$	318.00181	−1.98	160.03930	−2.47	$C_9H_6NO_2$	7.76
Pirimicarb	1.70	Carbamate/Insecticide	$C_{11}H_{18}N_4O_2$	$[M + H]^+$	239.15025	−1.74	182.12879	−2.46	$C_9H_{16}N_3O$	7.21
							72.04439	2.35	C_3H_6NO	
Pirimiphos-methyl	4.20	Organophosphorous/ Insecticide	$C_{11}H_{20}N_3O_3PS$	$[M + H]^+$	306.10358	−1.70	164.11822	−1.67	$C_9H_{14}N_3$	8.69
							108.05562	−0.87	$C_5H_6N_3$	
Pyraclostrobin	3.99	Strobilurin/Fungicide	$C_{19}H_{18}ClN_3O_4$	$[M + H]^+$	388.10586	−1.90	163.06278	−1.60	$C_9H_9O_2N$	8.51
							149.04713	4.96	$C_8H_7NO_2$	
Tebuconazole	3.70	Triazole/Fungicide	$C_{16}H_{22}ClN_3O$	$[M + H]^+$	308.15242	−2.07	70.03997	−5.71	C_2H_4N3	8.48
							125.01525	−1.88	C_7H_6Cl	
Tetraconazole	3.56	Triazole/Fungicide	$C_{13}H_{11}Cl_2F_4N_3O$	$[M + H]^+$	372.02881	−0.63	158.97628	−2.15	$C_7H_5Cl_2$	8.09
							184.99193	−2.82	$C_9H_7Cl_2$	
Trifloxystrobin	4.50	Strobilurin/Fungicide	$C_{20}H_{19}F_3N_2O_4$	$[M + H]^+$	409.13697	−1.95	186.05251	−2.74	$C_9H_7F_3N$	8.67
							206.08117	−2.28	$C_{11}H_{12}O_3N$	
Aflatoxin B1	1.23 [d]	Mycotoxin	$C_{17}H_{12}O_6$	$[M + H]^+$	313.07066	−1.90	285.07575	−0.88	$C_{16}H_{13}O_5$	7.14
							270.05227	−2.93	$C_{15}H_{10}O_5$	
Aflatoxin B2	1.45 [d]	Mycotoxin	$C_{17}H_{14}O_6$	$[M + H]^+$	315.08631	−1.79	287.05501	−0.38	$C_{15}H_{11}O_6$	7.05
							259.06010	−1.05	$C_{14}H_{11}O_5$	
Aflatoxin G1	0.50 [d]	Mycotoxin	$C_{17}H_{12}O_7$	$[M + H]^+$	329.06558	−2.03	311.05501	−3.10	$C_{17}H_{11}O_6$	6.90
							243.06519	−1.30	$C_{14}H_{11}O_4$	
Aflatoxin G2	0.71 [d]	Mycotoxin	$C_{17}H_{14}O_7$	$[M + H]^+$	331.08123	−1.72	245.04445	1.43	$C_{13}H_9O_5$	6.84
							313.07066	−3.27	$C_{17}H_{13}O_6$	

[a] Extracted from the EURL pesticides database, except for aflatoxins (EURL, 2021).
[b] m/z: mass-to-charge ratio.
[c] RT: retention time (minutes).
[d] PubChem data base (PubChem, 2021).

power = 70,000 FWHM; AGC target = 1e6, (4) data independent mass spectrometry fragmentation (DIA-MS/MS), ESI- (HCD on, collision energy = 30 eV), mass resolving power = 35,000 FWHM; AGC target = 1e5. Mass range in the full scan experiments was set m/z 50–750.

2.3. Sampling

Fifty commercial baby food samples were arbitrarily purchased from five stores in Campinas, São Paulo, Brazil, between March and April of 2021. The samples were organized in two baby food groups: the first containing meat and/or vegetables and the second group containing fruit purées and other ingredients such as cereals. The baby food samples were maintained at room temperature until analysis in their original packaging, i. e. glass jars (between 115 g and 170 g each), or plastic bags (99 g each).

2.4. Sample extraction

2.4.1. QuEChERS-based method

For QuEChERS-based method (Petrarca et al., 2016), 5 g of homogenized baby food sample spiked with the working standard solution (200 μg kg^{-1}) was weighed into a 50 mL conical centrifuge tube, and 10 mL of acetonitrile was added. Then the mixture was vortexed (1 min). After that, 1 g of NaCl and 4 g of $MgSO_4$ were added and the mixture was

vortexed (1 min) and then it was centrifuged at 3061×*g* for 10 min. Then, 1.5 mL of supernatant was transferred to a 15 mL conical centrifuge tube that contained 0.03 g of PSA, 0.03 g of C18, and 0.03 g Z-Sep+. The mixture was vortexed for 1 min and then centrifuged at 3061×*g* for 10 min. Prior to analysis, the extracts were filtered using a 0.2 μm nylon syringe filter. Then, 1 mL of the obtained extract was injected directly in LC system.

2.4.2. WAHSPE (water, acetonitrile, and n-heptane as solvents in combination with solid-phase extraction)-based method

The recently developed "WAHSPE" method (Eyring et al., 2021) comprised the following steps: 5 g of homogenized baby food sample spiked with working standard solution (200 μg kg^{-1}) was weighed into a 50 mL conical centrifuge tube, and 10 mL of acetonitrile with 5% formic acid + 10 mL water LC-MS grade + 10 mL of *n*-heptane were added, and then mixed by a mechanical shaker for 1 h. For the separation, 5 g of ammonium formate was added and vortexed for 1 min followed by a centrifugation step at 3061×*g* for 10 min. For the water and acetonitrile phases, no clean-up procedures were performed. The extracts were filtered through a 0.2 μm nylon syringe filter and 1 mL of each phase was injected separately into the LC system. Bearing in mind the characteristics of the selected compounds, the upper phase (*n*-heptane) was discarded.

2.5. Validation procedure

The developed method was validated to assure the reliability of the results. Validation of the optimized method was assessed considering the requirements of the SANTE guidelines (SANTE, 2019, 2021). The method validation procedure included selectivity, limit of detection (LOD), limit of quantification (LOQ), linearity (studied in solvent and in matrix-matched calibration curves), recovery, precision, which was evaluated at intra and inter-day conditions, and matrix effect. The performance characteristics of the method were evaluated using two "blank" baby foods, which were purchased from a local store, without the presence of the targeted analytes. These blank samples (one of them was meat and vegetables baby food, and the other one included fish and vegetables baby food) were used as representative baby food matrices to perform recovery and precision experiments and to perform the quantitation of the target compounds in samples employing matrix-matched calibration curves. Both representative "blank" baby food samples were extracted according to the final optimized QuEChERS-based method.

For the matrix effect analysis, standards in acetonitrile and standards prepared in blank matrix extracts were used, and matrix effect was estimated following equation (1):

$$\text{Matrix Effect } (\%) = \left[\frac{(\text{Matrix response} - \text{Solvent response})}{\text{Solvent response}}\right] x\ 100 \qquad \text{(Eq. 1)}$$

Linearity was evaluated in solvent and matrix-matched calibration curves, and seven calibration levels, with concentrations ranging from 2 to 100 μg kg^{-1}, were used. Precision was studied performing repeatability (intra-day precision) and reproducibility (inter-day precision) studies, and it was expressed in terms of relative standard deviation (RSD).

Five independent replicates of spiked baby food samples at each level (2, 10 and 100 μg kg^{-1}) were analyzed under the same chromatographic conditions and the same day by the same analyst to evaluate the repeatability conditions of the method. In addition, to the analysis of reproducibility conditions of the method, ten independent replicates of spiked baby food samples at each level (2, 10 and 100 μg kg^{-1}) were analyzed by the same analyst (six different days) under the same chromatographic conditions.

Intra-day recovery (%) was evaluated by analysing five spiked blank baby food samples at three levels (2, 10 and 100 μg kg^{-1}), and extracted during the same day. Inter-day recovery (%) was studied performing ten replicates, extracted in six consecutive days, at each concentration level (2, 10 and 100 μg kg^{-1}). LODs were estimated by monitoring spiked blank samples at low concentration levels (0.02, 0.1, 0.2, 0.4, 1, 2, 4, 10, and 100 μg kg^{-1}). The criteria used to set LODs were the retention time (RT) and isotopic pattern of the characteristic ion. LOQs were set as the lowest concentration level that could be detected and quantified with acceptable precision (RSD ⩽ 20%) and recovery (70–120%).

For reliable identification of compounds, retention time (RT), isotopic pattern, precursor ion (mass error lower than 5 ppm), and one fragment (mass error lower than 10 ppm) criteria were used (Hergueta-Castillo et al., 2022).

3. Results and discussion

3.1. Chromatographic and MS conditions

With the aim of developing a database of targeted compounds, a previous characterization of the analytes was performed. For UHPLC-Q-Orbitrap-MS, the essential information included ionization mode (adding polarity), retention time (RT), characteristic ions, and potential adducts (i.e. H^+ or NH_4^+). For this, an intermediate standard solution of each compound (100 μg L^{-1}) was injected into the system. In relation to the chromatographic variables, generic chromatographic condition based on previous work developed by the research group was used, resulting in a total run time of 14 min. Logarithm of n-octanol-water partition coefficient (log K_{ow}) values for the targeted compounds included in this study ranged from 0.5 for aflatoxin G1 (PubChem, 2021) to 6.9 for etofenprox pesticide (EURL, 2021), so there are high polar compounds (log $K_{ow} < 2.5$), intermediate polar compounds ($2.5 \leq \log K_{ow} < 4$) and low polar compounds (log $K_{ow} \geq 4$) (Eyring et al., 2021). Information of molecular formula, chemical group/use type, log K_{ow}, retention time, accurate mass, and characteristic ions of the compounds analyzed is shown in Table 1, where it can be observed that different classes of pesticides were monitored.

3.2. Extraction

In relation to sample preparation, our main aim was the application of a generic and simple extraction method, that would support the simultaneous determination of pesticides (with a wide range of polarities) and mycotoxins in baby food samples with different compositions. The method should involve a simple and easy sample preparation that efficaciously eliminates interferents and guarantee adequate analytical sensitivity and recoveries for twenty-one pesticides and four aflatoxins. Thus, two different extraction procedures based on the literature, WAHSPE (Eyring et al., 2021) and QuEChERS (Petrarca et al., 2016) were tested. For that purpose a complex representative baby food matrix, mostly composed by fish and vegetables, was selected. Both extraction procedures followed a common pathway involving the release of the analytes from the matrices. The selected extraction methods were generic (Eyring et al., 2021; Petrarca et al., 2016) and they were tested following the procedures described in Sections 2.4.1 and 2.4.2. Initially for QuEChERS-based method, 0.1 g of PSA, 0.1 g of C18, and 0.6 g of $MgSO_4$ were added to 4 mL of extracted supernatant to perform the clean-up step.

The WAHSPE method allows the screening of multiple compounds in a single sample extraction due to different polarities of the involved solvents. According to Eyring et al. (2021), it is possible to get higher rates of recovery for both highly- and non-polar analytes, while QuEChERS method is more efficient to compounds of moderate polarity. The recoveries obtained, when both methods were tested in spiked samples at 200 μg kg^{-1} of the targeted pesticides, are shown in Table S1 (see supplementary material).

For QuEChERS-based extraction, recoveries between 70 and 120% were achieved for all the evaluated pesticides. On the contrary, for the method adapted from WAHSPE, only 4 pesticides were recovered

Table 2
Method performance characteristics obtained using a representative baby food sample composed of meat and vegetables.

Meat and vegetables based baby food										
Compounds			Linearity, R^2 (range of 2–100 µg kg^{-1})		Recovery Intra-day (%), *n = 5* (Inter-day, *n = 10*)			Precision, RSD % Intra-day, *n = 5* (Inter-day, *n = 10*)		
	LOD (µg kg^{-1})	LOQ (µg kg^{-1})	Solvent [a]	Matrix-matched	2 µg kg^{-1}	10 µg kg^{-1}	100 µg kg^{-1}	2 µg kg^{-1}	10 µg kg^{-1}	100 µg kg^{-1}
Pesticides										
λ-Cyhalothrin	1.0	2.0	0.9904	0.9974	106 (110)	106 (103)	94 (94)	20 (20)	7 (17)	10 (12)
Atrazine	0.4	2.0	0.9949	0.9993	105 (99)	77 (83)	76 (83)	7 (19)	13 (15)	2 (13)
Azoxystrobin	0.02	2.0	0.9913	0.9994	84 (89)	81 (85)	75 (81)	4 (12)	13 (14)	7 (14)
Chlorpyrifos	0.1	2.0	0.9911	0.9904	97 (106)	118 (110)	92 (92)	6 (17)	10 (16)	6 (7)
Cypermethrin	4.0	10.0	0.9907	0.9918	n.a.	112 (104)	104 (95)	n.a.	17 (19)	14 (17)
Deltamethrin	4.0	10.0	0.9958	0.9957	n.a.	98 (91)	104 (103)	n.a.	15 (13)	17 (15)
Difenoconazole	0.02	2.0	0.9926	0.9934	106 (103)	88 (91)	99 (96)	3 (6)	9 (16)	10 (11)
Dimethoate	1.0	2.0	0.9917	0.9986	93 (93)	98 (97)	90 (89)	11 (10)	7 (6)	5 (4)
Etofenprox	4.0	10.0	0.9977	0.9846	n.a.	99 (97)	115 (102)	n.a.	16 (10)	11 (17)
Imazalil	0.2	2.0	0.9930	0.9951	72 (70)	71 (71)	80 (79)	8 (7)	4 (4)	2 (5)
Kresoxim-Methyl	1.0	2.0	0.9978	0.9986	82 (91)	97 (95)	98 (97)	4 (17)	8 (7)	5 (7)
Malathion	0.1	2.0	0.9916	0.9998	110 (103)	88 (91)	91 (93)	3 (8)	15 (12)	6 (7)
Methidathion	2.0	4.0	0.9933	0.9961	n.a.	84 (89)	78 (81)	n.a.	8 (14)	7 (9)
Phosalone	0.4	2.0	0.9937	0.9972	88 (97)	111 (105)	89 (89)	11 (19)	9 (10)	11 (10)
Phosmet	4.0	10.0	0.9922	0.9954	n.a.	80 (85)	80 (82)	n.a.	7 (20)	6 (7)
Pirimicarb	0.2	2.0	0.9908	0.9991	100 (99)	93 (94)	91 (92)	6 (5)	4 (5)	1 (4)
Pirimiphos-methyl	4.0	10.0	0.9940	0.9945	n.a.	95 (95)	94 (92)	n.a.	4 (4)	7 (8)
Pyraclostrobin	0.1	2.0	0.9909	0.9905	96 (98)	111 (103)	84 (88)	9 (12)	13 (13)	2 (6)
Tebuconazole	4.0	10.0	0.9912	0.9998	n.a.	67 (65)	71 (75)	n.a.	7 (7)	2 (7)
Tetraconazole	0.2	2.0	0.9942	0.9954	103 (99)	95 (96)	89 (89)	10 (10)	15 (13)	7 (10)
Trifloxystrobin	0.1	2.0	0.9950	0.9931	113 (108)	100 (99)	107 (99)	4 (10)	7 (9)	8 (12)
Mycotoxins										
Aflatoxin B1	0.4	1.0	0.9977	0.9994	108 (100)	85 (89)	97 (92)	9 (12)	4 (8)	4 (7)
Aflatoxin B2	0.4	1.0	0.9919	0.9955	94 (94)	90 (91)	87 (86)	7 (7)	3 (4)	4 (5)
Aflatoxin G1	0.4	1.0	0.9996	0.9992	102 (97)	83 (86)	83 (83)	10 (11)	4 (5)	5 (5)
Aflatoxin G2	0.4	1.0	0.9999	0.9967	92 (91)	85 (90)	84 (81)	11 (9)	4 (12)	2 (5)

LOD: limit of detection; LOQ: limit of quantification; R^2: coefficient of determination; RSD: relative standard deviation; n.a.: not applicable because the spiked level is lower than the LOQ established for the compound.
[a] Acetonitrile solvent.

between 70 and 120% (sum of recoveries for two evaluated phases). In general, recoveries above 120% were obtained for this method. According to Eyring et al. (2021), matrix compounds can interfere with the results when are integrated into the signals obtained from each of these pesticides, bringing on recoveries >120%.

QuEChERS methodology was selected because it simplifies the extraction of analytes without adversely affecting their recovery. Besides, the method demonstrated to have a simple sample preparation, reducing the number of procedures and minimizing both time and sources of error, as well as it requires less separate analyses.

Although extraction with acetonitrile has a low extraction of ordinary matrix components such as fats and proteins (Prata et al., 2021), a cleaning step is necessary when contaminants and residues are extracted from baby food. For this, the d-SPE method was applied due to its low cost, speed, simplicity, repeatability and large applicability to different types of samples and analytes, such as pesticides and other contaminants, in comparison with traditional solid phase extraction (SPE) (Molina-Ruiz et al., 2015). However, the selection of sorbents is crucial due to its effect on the cleanup and recoveries. Thus, different sorbents were tested to evaluate the matrix effect in two representative baby foods, one composed of meat and vegetables and one composed of fish and vegetables. For this, representative baby foods were extracted using the QuEChERS procedure described in Section 2.4.1, including the d-SPE clean-up stage. The d-SPE was performed using a mixture of 1.5 mL extract and the same amount (0.05 g) of different sorbents (PSA, C18, florisil, GCB and Z-sep+). These were chosen because of their individual abilities, where, according to (Lawal et al., 2018), C18 sorbent can remove non-polar interferences such as lipids and fats, improving the detection of analytes in complex matrices without significant adverse effects on their responses. Moreover, PSA sorbent eliminates sugar molecules, polar, organic, and fatty acids while keeping a high recovery and repeatability for various compounds with different properties (Lawal et al., 2018; Zhang et al., 2019). Z-Sep + sorbent has the ability to reduce lipids from animal and plant tissue extracts, improving sample clean-up over traditional PSA/C18 (Musarurwa et al., 2020). Florisil sorbent is used for samples with high sugars, acids, pigments, and organic ingredients (Łozowicka et al., 2017).

In Table S2 it is possible to verify the matrix effect, as well as analyte losses caused by different sorbents using d-SPE technique as clean-up step. The response obtained for standards of the same concentrations (200 µg kg^{-1}) in solvent and matrix extracts were used to evaluate the matrix-effect, using equation (1), whereas the analyte loss during extraction was calculated following equation (2):

$$\text{Losses } (\%) = \left[\left(\frac{\text{Fortified analyte response in the extract before cleaning}}{\text{Fortified analyte response in the extract after cleaning}}\right) - 1\right] x\ 100 \quad \text{(Eq. 2)}$$

Matrix effect is negligible if the result is equal to or lower than ±20%. On the other hand, values higher than 20% and lower than −20%, indicates strong matrix enhancement and significant matrix suppression, respectively (Hergueta-Castillo et al., 2022).

Table S2 shows that the matrix effect was similar for most of the

compounds in both tested samples. It has been recognized that, for the UHPLC-Q-Orbitrap-MS method, ion suppression was more usually observed.

In general, for fish and vegetables baby food, a negligible ME% was observed for most of the analyzed compounds (between 13 and 16 compounds for each sorbent). On the other hand, for meat and vegetable baby food, the tested compounds showed different behaviors for each sorbent. When Z-Sep+ and GCB were tested, 13 and 12 pesticides presented negligible ME%, respectively, while for PSA, C18 and florisil, negligible matrix effect was observed for approximately 6 compounds. Despite this, in both baby food samples, GCB sorbent presented bigger losses of different analytes. This can be seen for phosalone and pyraclostrobin pesticides, which achieved loss values of approximately 46 and 70%, respectively. Probably, the GCB sorbent removed some pesticides that contain Cl, F, O, and N with aromatic rings or conjugated carbon chains (planar structures) (Ly et al., 2020; Musarurwa et al., 2019). Additionally it was observed that several pesticides are strongly adsorbed by GCB, resulting in low recoveries (Cabrera et al., 2016). Thereby, it was not selected in further experiments. For PSA, C18, and florisil, similar results were observed. However, QuEChERS multi-residue procedure followed by d-SPE clean up with PSA + C18 sorbents was successfully applied for pesticide residue analyses in food matrices with different compositions (Hercegová et al., 2007). Therefore, among the three sorbents tested, PSA and C18 were selected for this work. Z-Sep + sorbent showed a lower ME % in both baby food samples (Table S2) and therefore it was also included in the developed method.

Finally, after the evaluation of the efficiency of d-SPE clean-up step applying different sorbents, the best analytical performance was achieved using PSA, C18, and Z-Sep+. These sorbents produced good results for both representative baby food samples. In addition, for complex matrices as baby food samples that presented potential analytical interferences in the final extract (Petrarca et al., 2017), a mixture of two or three different sorbents can be used to obtain a sufficient clean-up of various types of co-extractives (Trevisan et al., 2017). Therefore, it was performed a d-SPE cleanup method based on mixed-mode using PSA, C18, and Z-Sep + sorbents.

On the other hand, the amount of sorbent used must be adequate. The use of high amounts of sorbent(s) increases the risk of obtaining unacceptable recoveries and cleanup performances for pesticides (Trevisan et al., 2017; Zhao et al., 2012). Thus, compounds were extracted from the two representative baby foods according to QuEChERS procedure described in Section 2.4.1 The obtained extracts were submitted to d-SPE clean-up. The d-SPE was performed using two different amounts of sorbent: (i) 1.5 mL of extract and 0.05 g of PSA, 0.05 g of C18 and, 0.05 g of Z-Sep+; and (ii) 1.5 mL extract and 0.03 g of PSA, 0.03 g of C18 and, 0.03 g of Z-Sep+.

The response obtained for standards at the same concentrations (200 μg kg) in solvent and matrix extracts were used to assess the matrix effects. Eq. (1) was used for the calculation of the percentage of matrix effect. Furthermore, the extraction efficiency of the method was also determined by the mean recovery (%) acquired from three replicates of spiked samples at 200 μg kg^{-1}. Aflatoxins B1, B2, G1, and G2 were also evaluated for ME% and recovery experiments.

Increasing the amount of sorbents from 0.03 to 0.05 g did affect neither the ME% nor the recovery of pesticides and aflatoxins in both representative baby foods analyzed (Fig. S1 and Fig. S2). When these results are taken into account, it was found that the cleanup method based on mixed-mode sorbents using 0.03 g PSA, 0.03 g C18, and 0.03 g Z-Sep + sorbents guarantee a good extraction efficiency, providing recoveries between 70 and 120% for almost all pesticides and aflatoxins. Furthermore, it ensures an efficient and robust cleanup to remove unwanted matrix interferences with minor losses of analytes. Therefore, the minimal amount tested (0.03 g) of sorbents PSA, C18 and Z-Sep + per 1.5 mL of extract, was set for d-SPE cleanup.

3.3. Method validation

The suitability of QuEChERS-based method for analysis of pesticides and mycotoxins in baby foods was assessed by in-house validation, evaluating the performance criteria indicated in Section 2.5.

A suitable linearity throughout the studied range was obtained for all targeted compounds, obtaining coefficient of determination (R^2) higher than 0.9900 for most of compounds in the two studied matrices (Table 2 and Table S3). The extraction efficiency of the proposed method was measured by calculating mean recovery (%) by intra- and inter-day conditions. Therefore, a representative "blank" baby food sample was spiked with a multi-analyte working solution before the extraction method (1 h), to guarantee a better interaction between the analytes and the matrix. It was achieve acceptable mean recoveries for the 21 pesticides and 4 mycotoxins for both representative baby food matrices.

Recoveries were within the ranges fixed by the SANTE/2020/12830 guidance, in which, 60.0–120.0% for concentrations from ≤10.0 μg kg^{-1} and 70.0–120.0%, for levels between >10.0 and ≤ 100.0 μg kg^{-1} (SANTE, 2021).

When intra-day precision was evaluated, RSD values ranged from 2 to 20% for meat and vegetables baby food and between 1 and 17% for fish and vegetables baby food. For inter-day precision, the RSD values ranged between 4 and 20%, and from 3 to 20% for meat and vegetables baby food and fish and vegetables baby food, respectively. According to the SANTE/2020/12830 guidelines, RSD values ≤ 30.0% and ≤20.0%

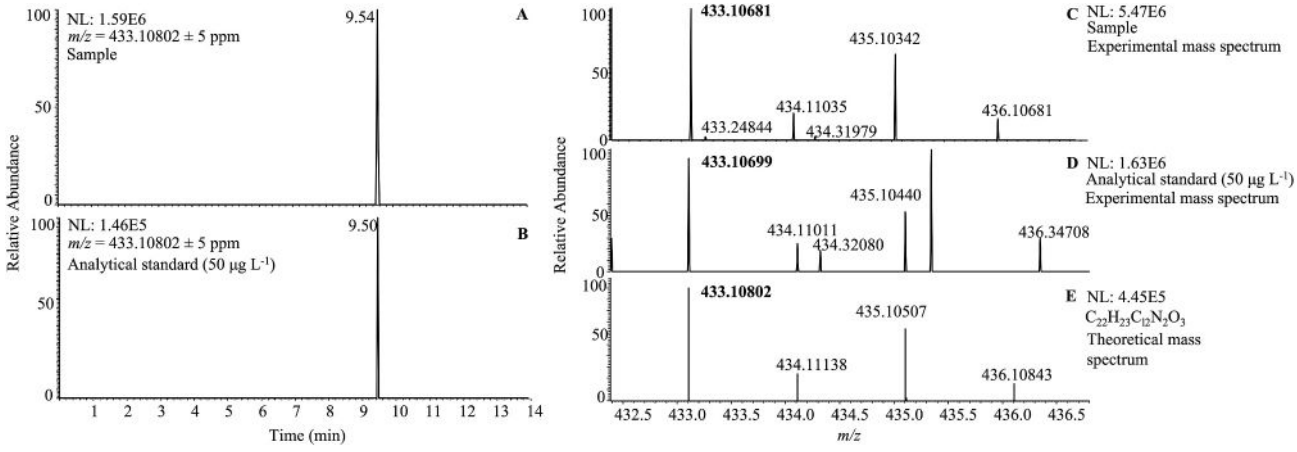

Fig. 1. UHPLC-Q-Orbitrap-MS extracted ion chromatograms of cypermethrin in (a) fruit-based baby food composed of yam, banana and strawberry (10.3 μg kg^{-1}) and (b) analytical standard (50 μg L^{-1}); Experimental mass spectrum of (c) analytical standard (50 μg L^{-1}) and (d) fruit-based baby food composed of yam, banana and strawberry (10.3 μg kg^{-1}); (e) Theoretical mass spectrum of cypermethrin.

are acceptable for concentrations ≤10.0 μg kg^{-1} and 10.0–100.0 μg kg^{-1}, respectively (SANTE, 2021).

LODs and LOQs are among the most sensitive analytical parameters for baby food control due to the strict MRL set by EU (Petrarca et al., 2017). Most of compounds had LODs lower than 2 μg kg^{-1} for meat and vegetables baby food and 0.4 μg kg^{-1} for fish and vegetables baby food. It can be observed that LOQ value had 2 μg kg^{-1} for most of the pesticides included in this study, for both types of samples analyzed. Furthermore, LOQ values for pesticides not exceed the MRL authorized for pesticide residues in baby foods. In addition, low LOQs were obtained for aflatoxins for the two matrices evaluated (1 μg kg^{-1}). The levels achieved are adequate to guarantee the monitoring of pesticides at the MRL established by the EU for baby foods (10 μg kg^{-1}) and for aflatoxins B1, B2, G1 and G2 for cereal-based baby food (1 μg kg^{-1}) established by ANVISA from Brazil, proving the high analytical sensitivity of the developed method (ANVISA, 2011; European Commission, 2006).

To guarantee that the selected matrices were representative enough to all kinds of samples, the extraction efficiency was also tested by recovery experiments in blank baby food samples composed of only fruits and vegetables. For this, pea and broccoli purée-based baby food and fruit purée-based baby food (composed of banana, apple, peach, orange, and, apricot) were spiked with working standard solutions at 10 and 100 μg kg^{-1} levels and extracted following the procedure explained in Section 2.4.1.

Among the analyzed compounds, recoveries were not suitable for only five compounds (etofenprox, phosmet, tebuconazole, aflatoxin B1 and aflatoxin G2) in at least one level and in an analyzed sample according to the SANTE/2020/12830 guidance (SANTE, 2021), as can be seen in Fig. S3. In both analyzed samples, high recoveries were obtained for phosmet pesticide (between 179 and 191%). Despite this, the method showed robustness and can be applied to the determination of pesticides and aflatoxins in baby food samples composed of only fruits or vegetables.

3.4. Analysis of commercial samples of baby foods

The developed method (optimized QuEChERS-based method) was applied to the analysis of 21 pesticides and 4 aflatoxins in 50 different baby food samples of two main types (meat and/or vegetables, and fruit-based baby food) available in the Brazilian market, and the results are indicated in Table S4 and Table S5.

In fruit-based baby food, about 47% of the samples presented at least one pesticide residue. For meat and vegetable-based baby food, at least one pesticide residue was detected in 85% of the analyzed samples. The highest number of residues detected in one sample was 4 (spaghetti bolognese and chicken breast, vegetables, and pasta baby food). However, the concentrations obtained for the different pesticides are low, with some exceptions.

The pyrethroid insecticide cypermethrin was detected in a sample composed of yam, banana, and strawberry at a level of 10.3 μg kg^{-1} (it was intended for infant consumption over 6 months old). Fig. 1 shows the extracted ion chromatogram (XIC) for this positive baby food sample. This result might be not compliant with legislation since the EU establishes MRLs for pesticides in processed baby food at 10 μg kg^{-1}, but considering RSD values, the set MRL would be included within the confidence interval. In Brazil, strawberry present a high MRL when compared to that allowed for baby foods (1000 μg kg^{-1} for alfa-cypermethrin). For yam, alfa- and zeta-cypermethrin, have a MRL of 20 and 50 μg kg^{-1}, respectively (ANVISA, 2021).

The pesticides difenoconazole and pirimiphos-methyl were detected in both kinds of matrices studied. The triazole fungicide difenoconazole was detected in 50% of analyzed samples with levels between < LOQ (2.0 μg kg^{-1}) and 9.0 μg kg^{-1} (in apple purée based baby food). In Fig. S4, it can be observed XIC of difenoconazole in apple purée based baby food. In pear purée based baby food difenoconazole was also found at level of 2.1 μg kg^{-1}. Moreover, λ-cyhalothrin was also detected in pear purée based baby food (6.5 μg kg^{-1}). This is in disagreement with the Brazilian Sanitary Surveillance Agency (ANVISA, 2021), where difenoconazole is not authorized to be used in pear, only in apple and other crops, with a MRL of 500 μg kg^{-1}. The compound pirimiphos-methyl was detected in 12% of analyzed samples, where, in bean soup baby food sample composed of beans, chicken, pasta, squash, carrot, and kale, was detected at levels of 3.5 μg kg^{-1}. This insecticide is autorized in wheat flour with MRL of 5000 μg kg^{-1} (ANVISA, 2021). The insecticide chlorpyrifos was detected in 48% of meat and vegetable-based baby food samples, being detected at 2.8 μg kg^{-1} in a baby food sample composed of rice, bean, meat, and vegetables. In Fig. S5 it can be observed the XIC of this positive baby food sample. In fruit-, cereal- and milk-based baby foods, cyhalothrin and etofenprox were detected in different samples at a level of 0.7 and 0.6 μg kg^{-1}, respectively (Petrarca et al., 2017).

Recently, imazalil, tetraconazole, difenoconazole, among others pesticides, were found at levels up to 20 μg kg^{-1}, in baby foods from Spain (Díaz-Galiano et al., 2021). Imazalil was also detected at a level up to 2.9 μg kg^{-1} (Gilbert-López et al., 2007, 2012). In France, similar results to our study were observed, where pesticide residues were detected in 67% of the baby food samples analyzed (tebuconazole and difenoconazole were detected at levels of 2.9 μg kg^{-1} and 1.3 μg kg^{-1}, respectively) (Nougadère et al., 2020). Azoxystrobin fungicide was detected in baby food from China (Jia et al., 2014). The same fungicide was detected in 40% of meat and vegetables-based baby foods analyzed in this study.

In Serbia, pyraclostrobin was detected at a level that exceeded MRL established by EU (13.0 μg kg^{-1}), (Vuković et al., 2012). Chlorpyrifos and phosalone were detected in apple-based baby foods (Štěpán et al., 2005).

In this study, the investigated aflatoxins were neither detected (level below the LOD) nor quantified in any sample. However, in Brazil, vegetables and pasta baby food sample was contaminated with aflatoxin B1 at 0.08 μg kg^{-1} (da Silva et al., 2020). In the study mentioned above, lower values of LOD and LOQ for aflatoxins compared to our study were observed. However, that method was aimed at analyzing 4 aflatoxins.

Table 3
Pesticides and metabolite detected in baby food samples by suspect screening.

Compound	Sample composition [a]
Aldicarb-sulfoxide	Bean broth, meat and rice
	Vegetables and meat
	Squash, black bean, and chicken breast
Allethrin	Sweet potato purée, corn and ora-pro-nobis
Clorantraniliprole	Banana, apple and raspberry
Diethofencarb	Chicken risotto
	Guava and banana (brand A)
	Guava and banana (brand B)
	Banana pureé
	Pear, banana and blueberry (plastic bag)
Dodine	Pear pureé
	Pear and mango (plastic bag)
Isoprocarb	Squash, black bean, and chicken breast
	Beet, bean and vegetables
	Sweet potato, black bean and meat
	Sweet potato, black bean and chicken
Piperonyl-butoxide	Spaghetti Bolognese
	Pasta, meat, and vegetables [b]
	Pasta, meat, and vegetables [b]
	Mixed fruits
Promecarb	Egg yolk, meat and vegetables
Propamocarb	Pasta, meat, and vegetables
	Beet, bean and vegetables
Propoxur	Sweet potato purée, corn and ora-pro-nobis
	Beet, bean and vegetables
Trinexapac-ethyl	Egg yolk, meat and vegetables

[a] Main ingredients.
[b] Different concentrations of each ingredient (same brand).

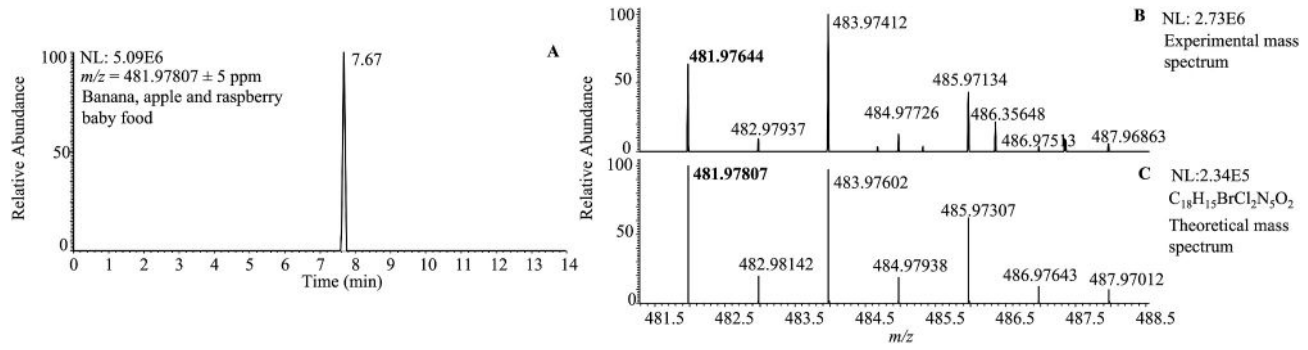

Fig. 2. UHPLC-Q-Orbitrap-MS (a) extracted ion chromatograms of clorantraniliprole (*m/z* 481.97807); (b) Experimental and (c) theoretical mass spectrum to clorantraniliprole.

Thus, it is expected lower values for LODs and LOQs compared to a multiresidue method.

In Iran and United States, aflatoxin B1 was detected at higher levels in rice-based baby food samples (Al-Taher et al., 2017; Mottaghianpour et al., 2017). Furthermore, aflatoxin B1 was found in 22 of commercial baby foods, analyzed in Qatar (Ul Hassan et al., 2018).

3.5. Suspect analysis

A suspect screening analysis was also performed to detect other contaminants not included in the initial study. For that purpose, a suspect analysis was performed using a homemade database containing 2424 compounds such as pesticides, mycotoxins, hormones, veterinary drugs and their metabolites. The name of the compounds, molecular formula and theoretical exact mass of the characteristic ion and one fragment were included. Suspect screening was carried out filtering theoretical exact masses in the total ion chromatogram. The following criteria, as exact mass, with a mass error lower than 5 ppm and at least two fragment ions, with a mass error lower than 10 ppm, were used to tentatively identified one compound. Furthermore, to confirm the compounds tentatively identified, analytical standards of the compounds identified in meat and vegetables-based baby food extract (250 μg L^{-1}) were injected. The retention time of the compounds in this extract and in the tested samples was also compared. When this study was performed, contaminants were detected in 20 out of a total of 50 baby food samples analyzed, showing the detected compounds in Table 3.

The occurrence of 5 insecticides (allethrin, clorantraniliprole, isoprocarb, promecarb, and propoxur) was detected in 7 baby food samples, showing in Fig. 2 an example of a baby food sample containing clorantraniliprole. Diethofencarb, dodine and propamocarb fungicides have been observed in 9 baby food samples. One growth regulator (trinexapac-ethyl) and one synergist (piperonyl-butoxide) were detected in one and 4 baby food samples, respectively. Additionally, one aldicarb metabolite (aldicarb-sulfoxide) was presented in 3 baby food samples.

4. Conclusions

An analytical multiresidue method was developed and fully validated for the simultaneous determination of pesticides and aflatoxins in Brazilian baby foods. QuEChERS extraction combined with d-SPE followed by UHPLC-Q-Orbitrap-MS analysis achieved suitable performance. Validation criteria (selectivity, matrix effect, linearity, recovery, precision and lower limits) were evaluated in compliance with SANTE guidelines, to guarantee the suitability of the method. The method achieved low LODs and LOQs to meet the MRL f 10 μg kg^{-1} set by EU for pesticide residues and 1 μg kg^{-1} for aflatoxins in baby food established by ANVISA. Subsequently, 50 samples were analyzed, and several pesticides were detected, obtaining that cypermethrin reached the highest concentration (10.3 μg kg^{-1}) in a yam, banana, ans strawberry baby food sample. The detection of this insecticide in one of the samples analyzed at a level above the established MRL indicates the importance of residue monitoring of pesticides in baby foods to guarantee the food safety and the proposed method can be implemented to ensure quality control and assurance of these products in relation to the presence of pesticide residues and mycotoxins. In addition, the application of the developed method to commercial meat and vegetables-based baby foods marketed in Brazil contributes to the first set of data on pesticides in this kind of sample. Furthermore, about 68% of the samples presented pesticide residues, but at low concentrations. Other pesticides (10) and one metabolite were detected when post-targeted analysis was performed. Despite of being at low concentration, these data can be useful for Brazilian regulatory authorities, and specific regulation for pesticide residues in baby foods could be proposed.

CRediT authorship contribution statement

Rafaela Prata: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Rosalía López-Ruiz:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Mateus Henrique Petrarca:** Supervision, Conceptualization, Writing – review & editing. **Helena Teixeira Godoy:** Supervision, Conceptualization, Resources, Funding acquisition. **Antonia Garrido Frenich:** Project administration, Supervision, Resources, Funding acquisition, Writing – review & editing. **Roberto Romero-González:** Conceptualization, Methodology, Data curation, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2022.109072.

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