UNIVERSIDADE ESTADUAL DE CAMPINAS
Faculdade de Engenharia de Alimentos

RAFAELA PRATA

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS FOR THE DETERMINATION OF CONTAMINANTS AND PESTICIDE RESIDUES IN BABY FOODS

DESENVOLVIMENTO E VALIDAÇÃO DE MÉTODOS ANALÍTICOS PARA A DETERMINAÇÃO DE CONTAMINANTES E RESÍDUOS DE AGROQUÍMICOS EM ALIMENTOS DESTINADOS AO PÚBLICO INFANTIL

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Tese apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de ou Doutor(a) em Ciência de Alimentos.

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Que a tua vida não seja uma vida estéril. — Sê útil. — Deixa rasto.

São Josemaría Escrivá

RESUMO

O propósito principal desta pesquisa consistiu em elaborar e validar procedimentos analíticos, que empregam métodos de preparo de amostras em conjunto com as técnicas de cromatografia gasosa (CG), cromatografia líquida (CL) e espectrometria de massas (EM), com o objetivo de identificar e quantificar contaminantes em alimentos destinados a crianças, incluindo produtos à base de frutas e outros à base de carne e/ou vegetais, que estão disponíveis no mercado brasileiro. Resíduos de agroquímicos, contaminantes ambientais, contaminantes de processamento térmico, além de aflatoxinas foram analisados utilizando diferentes técnicas otimizadas. O trabalho foi dividido em quatro capítulos. No primeiro, a cromatografia a líquido de alta aficiência com detector espectrofotométrico com arranjo de diodos (DAD) foi utilizada na determinação dos contaminantes de processamento térmico, como o hidroximetilfurfural (HMF), furfural e 4-hidroxi-2,5-dimetil-3(2H)-furanona (HDMF). Um método simples de extração com acetonitrila, seguido de partição com n-hexano e diluição com água deionizada (*dilute-and-shot*) apresentou adequadas características de desempenho e sensibilidade. A ocorrência desses compostos foi investigada em 60 amostras (comerciais e caseiras) de alimentos infantis. Furfural foi predominante em alimentos infantis à base de vegetais e/ou carnes. HMF e HDMF foram mais prevalentes em alimentos infantis industrializados do que em amostras caseiras. O segundo capítulo trata-se do monitoramento de 21 agroquímicos e 4 aflatoxinas em 50 alimentos infantis comercializados no Brasil, aplicando a cromatografia líquida de ultra alta eficiência acoplada à espectrometria de massa do tipo quadrupolo-Orbitrap (UHPLC-Q-Orbitrap-MS). Além disso, uma análise de triagem de suspeitos (*non-target*) também foi realizada para detectar outros contaminantes não incluídos no estudo inicial. Para isso, o método QuEChERS (*quick, easy, cheap, effective, rugged, and safe*) combinado com a limpeza de extração em fase sólida dispersiva (d-SPE - *dispersive solid-phase extraction*) utilizando amina secundária primária (PSA), octadecilsilano (C18) e C18 com sílica revestida com dióxido de zircônio (Z-Sep+) foi aplicado. Agroquímicos foram encontrados em 68% das amostras analisadas e o composto cipermetrina foi detectado em uma amostra em um nível que excedeu o limite máximo de resíduos (LMR) estabelecido pela União Europeia (EU). Além disso, outros 10 agroquímicos e um metabólito foram detectados quando a análise *non-target* foi realizada. O terceiro capítulo apresenta o desenvolvido e validação de um método para monitorar a presença de acrilamida em alimentos infantis, envolvendo um processo de extração simples usando uma mistura de acetonitrila:água:ácido fórmico (69:30:1, v/v/v) em combinação com d-SPE usando alumina. Após a extração, um total de 60 amostras foram analisadas utilizando a cromatografia líquida acoplada ao espectrômetro de massas em tandem (LC-QqQ-MS/MS). O método desenvolvido reduziu custos e etapas demoradas normalmente usadas na análise de acrilamida em amostras de alimentos, além disso, permitiu detectar a presença de acrilamida em 13% das amostras compostas principalmente por frutas e 37% das amostras contendo carne e/ou vegetais. No quarto capítulo, a cromatografia gasosa acoplada à espectrometria de massa de alta resolução (GC-Q-Orbitrap-MS*)* foi utilizada para conduzir a análise de 14 HPAs e 04 agroquímicos em alimentos infantis. Para isso, métodos otimizados de preparo de amostras baseados no método QuEChERS seguido de d-SPE foram utilizados. Como resultado, 24% das amostras analisadas apresentaram ao menos um agroquímico em sua composição. De maneira geral, este trabalho forneceu informações valiosas relacionadas à presença de diversos contaminantes em alimentos infantis, sobretudo para os alimentos infantis a base de carne e vegetais comercializados no Brasil, uma vez que foram apresentados dados inéditos oriundos dos monitoramentos efetuados.

ABSTRACT

The main purpose of this research was to develop and validate analytical procedures that utilize sample preparation methods in conjunction with gas chromatography (GC), liquid chromatography (LC), and mass spectrometry (MS) techniques to identify and quantify contaminants in baby food products, including fruit-based and meat/vegetable-based items available in the Brazilian market. Residues of pesticides, environmental contaminants, heat processing contaminants, as well as aflatoxins, were analyzed using different optimized techniques. The work was divided into four chapters. In the first chapter, high-performance liquid chromatography with a diode array detector (DAD) was used to determine heat processing contaminants such as hydroxymethylfurfural (HMF), furfural, and 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF). A simple extraction method with acetonitrile, followed by partitioning with n-hexane and dilution with deionized water (dilute-and-shot), exhibited suitable performance and sensitivity characteristics. The occurrence of these compounds was investigated in 60 samples (commercial and homemade) of baby foods. Furfural was predominant in vegetable and/or meat-based baby foods. HMF and HDMF were more prevalent in processed baby foods than in homemade samples. The second chapter involved the monitoring of 21 pesticides and 4 aflatoxins in 50 baby food products marketed in Brazil, using ultra-high-performance liquid chromatography coupled with quadrupole-Orbitrap mass spectrometry (UHPLC-Q-Orbitrap-MS). Additionally, a non-target screening analysis was performed to detect other contaminants not included in the initial study. For this purpose, the QuEChERS (quick, easy, cheap, effective, rugged, and safe) method combined with dispersive solid-phase extraction (d-SPE) using primary secondary amine (PSA), octadecylsilane (C18), and C18 with zirconium dioxide-coated silica (Z-Sep+) was applied. Pesticides were found in 68% of the analyzed samples, and the compound cypermethrin was detected in one sample at a level exceeding the maximum residue limit (MRL) established by the European Union (EU). Furthermore, 10 other pesticides and one metabolite were detected during the non-target analysis. The third chapter presents the development and validation of a method for monitoring the presence of acrylamide in baby foods, involving a simple extraction process using a mixture of acetonitrile:water:formic acid (69:30:1, v/v/v) in combination with alumina-based d-SPE. After extraction, a total of 60 samples were analyzed using liquid chromatography coupled with tandem mass spectrometry (LC-QqQ-MS/MS). The developed method reduced costs and time-consuming steps typically used in acrylamide analysis in food samples, and it detected the presence of acrylamide in 13% of the samples, primarily composed of fruits, and 37% of the samples containing meat and/or vegetables. In the fourth chapter, gas chromatography coupled with high-resolution mass spectrometry (GC-Q-Orbitrap-MS) was used to analyze 14 PAHs and 4 pesticides in baby foods. Optimized sample preparation methods based on the QuEChERS method followed by d-SPE were employed. As a result, 24% of the analyzed samples contained at least one pesticide. Overall, this work provided valuable information regarding the presence of various contaminants in baby foods, especially for meat and vegetable-based baby foods marketed in Brazil, as it presented unprecedented data from the conducted monitoring efforts.

SUMÁRIO

INTRODUÇÃO GERAL

A segurança alimentar no mercado de alimentos é uma das principais áreas de foco em saúde pública, porque afeta pessoas de todas as idades, raças, gêneros e níveis de renda em todo o mundo (GIZAW, 2019). No entanto, a presença de resíduos de agroquímicos em alimentos destinados ao público infantil, bem como de contaminantes de processamento e ambientais implica em um potencial risco para esse grupo de consumidores. Lactentes (de 0 a 12 meses incompletos) e crianças de primeira infância (1-3 anos) são grupos vulneráveis à toxicidade dos contaminantes, uma vez que apresentam um maior consumo de alimentos por unidade de peso corpóreo. Além disso, as vias metabólicas ainda são imaturas e assim a capacidade de metabolizar substâncias químicas tóxicas é diferente quando comparada à de um adulto (PETRARCA et al., 2016; AKOTO; OPPONG-OTOO; OSEI-FOSU, 2015; HULIN et al., 2014). Portanto, é necessário que os alimentos consumidos por esse grupo sejam constantemente examinados e avaliados para contaminantes, a fim de representar um menor risco para eles (MOAZZEN et al., 2022).

Os agroquímicos representam uma ampla gama de compostos químicos, extensivamente utilizados na agricultura para controlar organismos nocivos e aumentar a produtividade das culturas e, portanto, podem ser encontrados nos alimentos infantis. Seu uso generalizado tem causado sérias preocupações em todo o mundo em relação à qualidade e segurança dos alimentos e, portanto, é necessário avaliar os riscos da exposição crônica aos agroquímicos através da alimentação (MAKNI et al., 2022). Assim, os limites máximos de resíduos (LMRs) de agroquímicos em alimentos infantis, estão se tornando cada vez mais rigorosos, e desta forma programas de monitoramento eficazes e regulamentos rígidos são cada vez mais necessários (PANSERI et al., 2020). No entanto, diferentemente de outros países que estabeleceram LMRs de 0.01 mg kg^{-1} (COMISSÃO EUROPEIA, 2006), o Brasil não possui legislação específica para alimentos destinados a este vulnerável grupo de consumidores frente a esses contaminantes.

A presença de contaminantes de processamento térmico em alimentos tem chamado a atenção devido aos seus prováveis efeitos tóxicos. Acrilamida e derivados furânicos, são alguns desses compostos indesejáveis que são formados após o tratamento térmico (KOCADAĞLI; GÖKMEN, 2022). A acrilamida, formada pela degradação da asparagina livre na presença dos açúcares, é classificada como um provável carcinógeno humano no grupo 2A pela Agência Internacional de Pesquisa sobre o Câncer (IARC) (IARC, 2022).

Outro composto com alto potencial toxicológico, e que também é classificado como provável carcinógeno humano no grupo 2A pela IARC, é o benzo(a)pireno (IARC, 2022) pertencente ao grupo dos hidrocarbonetos policíclicos aromáticos (HPAs). Os HPAs são contaminantes ambientais que podem estar presentes no ar, solo e água e podem ser transportados a produtos agrícolas (como os cereais) especialmente quando as fazendas estão localizadas perto de locais industriais ou fábricas. Além disso, com o processamento (como grelhar, defumar, fritar e cozinhar), os HPAs são produzidos em uma grande variedade de alimentos (MOAZZEN et al., 2022; BADIBOSTAN et al., 2019). Portanto, as fontes alimentares representam a principal via de exposição de HPAs em humanos.

Com base em seu potencial toxicológico, benzo(a)antraceno, criseno, benzo(b)fluoranteno e benzo(a)pireno também são HPAs muito importantes, já que estão associados com o câncer gástrico, problemas pulmonares e alterações citogenéticas e bioquímicas (MOAZZEN et al., 2022; BADIBOSTAN et al., 2019).

Outros importantes compostos com alta toxicidade e que representam um risco potencial para a saúde humana e animal são as micotoxinas. Esses, metabólitos secundários, produzidos por espécies de fungos como a *Alternaria, Aspergillus, Claviceps, Fusarium* e *Penicillium,* têm sido associados a efeitos carcinogênicos, teratogênicos e imunossupressores (REBELLATO et al., 2021). Dentre as micotoxinas, a aflatoxina B1 é reconhecida universalmente como o tipo mais tóxico e abundante desse grupo de micotoxinas. Por isso, é fundamental realizar um monitoramento rigoroso de sua presença, visando reduzir a exposição a essas micotoxinas durante fases cruciais do desenvolvimento infantil (ALVITO; PEREIRA-DA-SILVA, 2022).

No Brasil, são poucos os trabalhos conduzidos a respeito de contaminantes ambientais, formados durante o processamento térmico, e resíduos de agroquímicos em alimentos infantis. Até o presente momento, podemos destacar o estudo sobre furano em alimentos infantis (ARISSETO; VICENTE; DE FIGUEIREDO TOLEDO, 2010), a determinação de fumonisinas (CALDAS; SILVA, 2007; DE CASTRO et al., 2004) e cloropropanóis (ARISSETO et al., 2013) em cereal matinal; em papinhas a base de frutas, foram realizadas análises de resíduos de agroquímicos (PETRARCA et al., 2017a, 2016), hidrocarbonetos policíclicos aromáticos (HPAs) (PETRARCA; GODOY, 2018), acrilamida e derivados furânicos, como a 4-hidroxi-2,5-dimetil-3(2H)-furanona (DMHF) e 5-hidroxi-metilfurfural (HMF) (MEINHART; GODOY, 2020; PETRARCA et al., 2017c; PETRARCA), além de poliaminas (PETRARCA et al., 2017b). Aflatoxinas foram analisadas em alimentos infantis destinados a crianças acima de 06 meses (DA SILVA et al., 2020).

Assim, torna-se necessário a otimização e validação de metodologias que possibilitem a determinação de contaminantes em nível traço em alimentos infantis, de modo a obter resultados inéditos sobre a contaminação em alimentos infantis com diferentes composições (a base de frutas e a base de carne e vegetais). Neste sentido, o presente estudo foi realizado a partir da otimização, desenvolvimento e análise de métodos de preparo de amostra para a análise de resíduos de agroquímicos e aflatoxinas, contaminantes de processamento térmico como a acrilamida e derivados furânicos, assim como de HPAs em alimentos infantis comercializados na cidade de Campinas, SP. Por fim, os métodos analíticos foram avaliados garantindo seu desempenho, de modo a garantir a fiabilidade dos resultados.

OBJETIVOS

Objetivo geral

Desenvolver e validar métodos analíticos utilizando abordagens de preparo de amostra em conjunto com técnicas cromatográficas para avaliar a presença e a quantidade de contaminantes de processamento e ambientais, bem como resíduos de agroquímicos e micotoxinas em produtos alimentares destinados a bebês (papinhas) disponíveis no mercado no estado de São Paulo.

Objetivos específicos

- Estabelecer e confirmar a eficácia de um método por LC-DAD para a avaliação quantitativa da presença de 5-hydroxymethylfurfural (HMF), furfural e 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF), gerados durante o processamento térmico, em alimentos industrializados e chamados caseiros destinados a bebês;
- Certificar a eficácia de um procedimento multi-resíduos utilizando a abordagem QuEChERS em conjunto com UHPLC-Q-Orbitrap-MS, com o objetivo de quantificar a presença de agroquímicos e aflatoxinas em papinhas infantis;
- Elaborar e validar um procedimento empregando uma técnica de extração simplificada, seguida pela determinação por cromatografia líquida acoplada a um espectrômetro de massas em tandem (LC-QqQ-MS/MS), com o propósito de monitorar a presença de acrilamida em alimentos infantis;
- Desenvolver e validar um método QuEChERS-GC-MS para determinação de hidrocarbonetos policíclicos aromáticos (HPAs) em papinhas infantis.

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CAPÍTULO 1 - Simultaneous determination of furfural, 5-hydroxymethylfurfural and 4-hydroxy-2,5-dimethyl-3(2H)-furanone in baby foods available in the Brazilian market

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Abstract

A liquid chromatography-diode array detection (LC-DAD) method is described for the simultaneous quantification of 5-hydroxymethylfurfural (HMF), furfural, and 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF) in different types of baby foods. Furan derivatives were extracted with acetonitrile, followed by partitioning with n-hexane. The extract was then diluted with deionized water to a large volume to minimize the matrix interferences. The fitness-of-purpose of the developed method was verified by analytical selectivity, linearity in solvent and matrix-matched calibration curves and adequate recoveries (83–105 %) and precision (RSDs ≤ 6 %), under repeatability and within-laboratory reproducibility conditions. The occurrence of furan derivatives was investigated in 60 commercial baby food samples composed of fruits, vegetables and/or meats, including both homemade and industrialized samples. A wide variation in the mean contents of furan derivatives was observed, with the highest contents of HMF detected in baby foods made of plum (343.10 μg g^{-1}).

Keywords: Furan derivatives; LC-DAD; Food processing contaminants; Infant foods; Dilute-and-shoot; Fruit-based baby food; Vegetables- and/or meat-based baby food.

1. Introduction

Children's growth and development largely depend on the quantity and quality of food that is eaten and when different food items are introduced. The nutritional needs of infants and young children change rapidly, and their eating patterns progress from simple breast milk and infant formula diets to more varied food (Devaney et al., 2004). According to the recommendations of the World Health Organization (WHO), infants should be exclusively breastfed for the first six months of life and thereafter should receive appropriate complementary feeding with continued breastfeeding up to two years or beyond. A wide variety of food products intended for infants and young children are formulated from fruits, cereals, meats, and vegetables and provide energy and nutrients, including vitamins and minerals, which are important for the initial stage of growth and development (European Commission, 2006; Piacentini et al., 2019).

Different heat treatments are employed during the manufacture of foodstuffs to improve their sensory qualities, such as desired flavours and colours as well as their digestibility, safety, and shelf-life; however, potentially toxic compounds can be generated, more precisely heat-induced process contaminants such as some furan derivatives (Capuano and Fogliano, 2011; Hu et al., 2013). These compounds are essentially produced by the Maillard reaction (MR) due to the presence of carbohydrates and certain amino acids, as well as they are also generated from lipid oxidation and caramelization (Capuano and Fogliano, 2011; Mesias et al., 2019). Additionally, the generation of furan derivatives in food is influenced by the concentration and type of sugar, pH, water activity and baking temperature (Petisca et al., 2014).

5-Hydroxymethylfurfural (HMF) is one of the most common intermediate products of the MR (Teixidó et al., 2008) and is recognized as an indicator of the deterioration in quality caused by excessive heating or inadequate storage. HMF is considered to be an irritant to the eyes, upper respiratory tract, skin and mucous membranes. Animal-based studies indicate that HMF may have diverse harmful effects on human health and may possess potential carcinogenic properties (Kowalski et al., 2013; Mesias et al., 2020). The genotoxic potential of HMF is associated with its metabolic 5-[(sulphoxy)methyl]furfural (SMF). SMF can react with DNA and produce mutagenic effects (EFSA, 2011). Another compound generated by the MR is 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF), an important aroma chemical and key flavour compound in many fruits (Schwab, 2013). However, DNA-breaking activity was demonstrated for HDMF. This effect can be explained by its prooxidant properties through the

reduction of transition metals resulting in the generation of reactive oxygen species, in particular, hydroxyl radical causing DNA base damage and cytotoxic effects (Murakami et al., 2007; Schwab, 2013). Furthermore, furfural (formed in processed food from ascorbic acid during thermal treatment or storage, as also from the degradation of pentoses) has also been reported to be a mutagen through its reaction with DNA (Hu et al., 2013; Mesías-García et al., 2010).

Numerous methods have been developed to analyse furan derivatives in food samples. For the analysis of furan derivatives in baby foods, the use of Carrez solutions as a clean-up and extraction step has been commonly observed (Alper et al., 2014; Fernández-Artigas et al., 1999; Guerra-Hernández et al., 1992; Madani-Tonekaboni et al., 2015; Rada-Mendoza et al., 2004, 2002; Ramírez-Jiménez et al., 2003; Švecová and Mach, 2017; Vorlová et al., 2006). Most of the laboratories working in the field of furan derivative analysis in food matrices employ a liquid chromatography–diode array detector system (LC-DAD) for identification and quantification (Fernández-Artigas et al., 1999; Guerra-Hernández et al., 1992; Mesías-García et al., 2010; Petrarca et al., 2020; Rada-Mendoza et al., 2004, 2002; Vorlová et al., 2006); however, other analytical techniques, such as capillary zone electrophoresis (Bignardi et al., 2014), LC or gas chromatography (GC) coupled with mass spectrometry systems (MS) (Condurso et al., 2018; Gökmen and Şenyuva, 2006; Petrarca et al., 2017), and spectrophotometric methods (Vella and Attard, 2019) have also been used.

Therefore, with the growing interest in green methodologies that minimize or eliminate the production of by-products, replacing toxic solvents with non-toxic ones (de Andrade et al., 2017), simplified approaches such as micro-extraction techniques have been applied in routine food analysis. Micro-extraction techniques are based on the miniaturization of conventional sample preparation procedures (Casado et al., 2020), and due to their many operating advantages, they have been considered a “journey into next-generation analytical chemistry” (Pedersen-Bjergaard, 2019). These techniques are usually classified into solid-phase and liquid-phase microextractions (Habibi et al., 2017). Solid phase microextraction (SPME) is a solvent-free sample preparation technique in which microquantities of a solid sorbent or liquid polymer in an appropriate format are exposed to the sample (Xu et al., 2016). Headspace-solid phase microextraction (HS-SPME) has been used in the analysis of furan and furan derivatives in baby foods (Condurso et al., 2018) and infant formulas (Kamalabadi et al., 2015). In addition, headspace liquid-phase microextraction (HS-LPME), a solvent-minimized sample preparation method, has been utilized for the determination of furan derivatives in baby foods (Habibi et al., 2013). For this same purpose, a high performance and powerful preconcentration method

was developed: dispersive liquid–liquid microextraction (DLLME), which is based on partitioning the analytes of interest using small volumes of a mixture of solvents (disperser and extractor) (Rezaee et al., 2006). DLLME has been utilized for the determination of furfural and HMF in baby formulas (Madani-Tonekaboni et al., 2015) and infant foods, such as milk-based infant formula, ready-to-eat soups, fruit purees and fruit juices (Habibi et al., 2017).

Furthermore, as an attractive approach for routine food analysis, the dilute-and-shoot strategy has been used for different food matrices (López-García et al., 2018; Petrarca et al., 2020; Sulyok et al., 2020), for which small amounts of sample, minimal consumption of toxic solvents, simple and fast operation, and the absence of clean-up steps comprise its principal advantages (Petrarca et al., 2020). This strategy has been used particularly for substances with high detection levels, which consists in the dilution of an aqueous or liquid matrix with a suitable solvent before direct injection into the chromatographic system to minimize the interference from co-extracted matrix compounds (Deventer et al., 2014; Petrarca et al., 2020; Phonchai et al., 2020). In this context, the aim of this work is to develop and validate a simple and fast HPLC-DAD method based on the dilute-and-shoot approach for the simultaneous quantification of furan derivatives, namely, 5-hydroxymethylfurfural, furfural, and 4-hydroxy-2,5-dimethyl-3(2H)-furanone, in different types of baby foods commercialized in Brazil.

2. Material and methods

2.1. Chemicals and standard solutions

Analytical standards of furfural (99 % purity) and 4-hydroxy-2,5-dimethyl-3(2H)-furanone (>99.8 %) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). The 5-hydroxymethylfurfural standard (98 %) was acquired from Carbosynth Ltd. (Compton Berkshire, UK). Individual stock standard solutions were prepared by dissolving 0.01 g of each standard in 10 mL of deionized water (1000 μg mL–1). A working standard solution of 10 μg mL–1 was obtained by diluting the stock solution with deionized water. All standard solutions were stored at 4 °C and protected from light. Acetonitrile and hexane, both HPLC grade, were acquired from Merck (Darmstadt, Germany) and Fisher Scientific (Waltham, Massachusetts, USA), respectively. Deionized water was obtained from Milli-Q system (Millipore, Milfold, MA, USA), and PVDF syringe filters (0.2 μm pore size, 13 mm i.d.) were purchased from GVS (Brazil).

2.2. Sampling

Commercial baby food samples, including vegetables- and/or meat-based baby foods (containing vegetables, beef, chicken, pasta, rice and/or other ingredients) and fruit-based baby foods (all containing fruit purées in their composition and other ingredients such as cereals) were purchased in the city of Campinas, located in the southeastern region of Brazil, between September and October 2019. A total of 60 samples were randomly collected from six supermarkets, and all of the samples were maintained in their original packaging, glass jars (120 g and 170 g each) or plastic pots (113 g each) at −22 °C until analysis. These commercial samples were classified into two categories on the basis of their manufacture process: industrialized baby foods (47 samples) and homemade baby foods (13 samples).

2.3. Simultaneous determination HMF, Furfural, and HDMF by LC-DAD

2.3.1. Sample preparation method

The samples presented smooth and homogeneous consistency, therefore the content was mixed manually with the use of a spatula until completely homogenized. Then, five grams of homogeneized baby food, was weighed and placed into a 15 mL polypropylene centrifuge tube, followed by the addition of 5 mL of acetonitrile and vortex agitation for 1 min. Then, 5 mL of hexane was added to the mixture, followed by vortex agitation for 1 min and centrifugation at 3000 × g for 15 min. The upper hexane phase was discarded, and the bottom phase (acetonitrile) was transferred to a volumetric glass flask (50 mL). The matrix pellet was re-extracted with 5 mL of acetonitrile and 5 mL of hexane under the same conditions; then, the supernatants were combined, and the 50 mL volume in the glass flask was completed with deionized water. Prior to LC-DAD analysis, the aqueous extracts were filtrated through a 0.2 μm PVDF syringe filter.

2.3.2. Liquid chromatography analysis

An Agilent 1260 Infinity quaternary liquid chromatography system with a diode array detector (DAD) was employed. Chromatographic separation was achieved on a reversed phase column (100 mm x 4.6 mm i.d., 3.5 μm particle size; Eclipse plus C18 Agilent) that was kept at 30 °C. The mobile phase consisted of deionized water (A) and acetonitrile (B), and the elution gradient was as follows: 0–6 min, 5% B; 6.1–9 min, 100 % B; 9.1–14 min, 5% B, at a flow rate of 1 mL min^{-1}, resulting in a total run time of 14 min. The DAD was set at a

wavelength of 284 nm, and the injection volume was between 1 and 100 μL according to the HMF, HDMF and furfural concentrations in the sample.

Furan derivatives were identified by their retention times (t_R) with a maximum tolerance of ±0.1 min within that obtained with standard solutions, as well as by a comparison of the UV spectrum with the characteristic spectra of the standard solutions at a maximal absorption at 284 nm, within a margin of ±2 nm. To assist with the identification, a co-chromatography procedure was also performed in which the extract previous to LC analysis was divided into two parts: one part was chromatographed as such and the other was added to a standard solution that was also chromatographed, and the enhanced peak height was equivalent to the amount of added analyte (European Commission, 2002).

2.4. In-house validation

The suitability of the proposed method was verified through in-house validation procedure, according to the Eurachem Guide recommendations (Magnusson and Örnemark, 2014). The method was validated for selectivity, limit of detection (LOD), limit of quantification (LOQ), linearity in solvent and matrix-matched calibration curves, matrix effects, recovery, and precision, under repeatability and within-laboratory reproducibility conditions. For this, two "blank baby foods", free of target furan derivatives, a rice-based baby food and another including fresh papaya and orange fruits were used as representative matrices.

3. Results and discussion

3.1. Sample preparation

Sample preparation is usually the most critical step in the development of methods for residue analysis due to the complexity of several foodstuff matrices (Pérez-Rodríguez et al., 2018). Regarding the sample preparation strategy adopted in the present study, our main goal was to obtain a simplified and easy sample preparation method for the simultaneous quantification of HMF, furfural, and HDMF in baby foods, with a minimal generation of residues and a lower number of steps to reduce both time and error sources. In this context, the dilute-and-shoot procedure is an attractive sample preparation strategy for routine analysis due to its easy, fast, and simple operation. In this approach, liquid or aqueous samples are generally diluted with acetonitrile and water, followed by mass spectrometry analysis (Guo et al., 2020). Recently, a sample preparation approach based on dilute-and-shoot and LC-DAD was proposed for the monitoring of HMF in baby foods (Petrarca et al., 2020); however, in this work, only fruit-based baby foods were studied. Thus, to investigate the occurrence of HMF in both fruit-

based baby foods and vegetables- and/or meat-based baby foods, as well as to evaluate the levels of other furan derivatives in the samples, the dilute-and-shoot LC-DAD method proposed by Petrarca et al. (2020) was modified and improved to obtain a more efficient and adequate sample preparation method for the simultaneous analysis of HMF, furfural and HDMF in several types of baby foods.

Although furan derivatives such as HMF and furfural occur widely in aqueous foods, they are relatively less polar than water. Thus, it is expected that in foods rich in lipids, these compounds could be dissolved in the oily phase (Durmaz and Gökmen, 2010). Vegetables- and/or meat-based baby foods have a higher content of fat than fruit-based baby foods, mainly due to the presence of vegetable oils, such as olive and sunflower oils, in their composition. According to Akpınar et al. (2011), the use of organic solvents is necessary for the extraction of HMF from high-fat foods; furthermore, this compound is strongly adsorbed on hydrophobic functional surfaces (Gökmen and Şenyuva, 2006). A mixture of methanol and water was successfully used for the extraction of furan derivatives from fat (Durmaz and Gökmen, 2010). Petrarca et al. (2020) tested three extraction solvents (methanol, water, and acetonitrile) for the determination of HMF in baby food, and it was observed that acetonitrile presented a visually lower content of co-extracted matrix components than water or methanol. Additionally, acetonitrile results in little extraction of common matrix components such as non-polar lipids (Koesukwiwat et al., 2010; Wenzl, 2009). Moreover, an advantage associated with the use of acetonitrile is the precipitation of proteins, an important feature, particularly for the analysis of meat-based baby foods. Therefore, acetonitrile was our first choice for the extraction of furan derivatives and partitioning with hexane was indispensable in eliminating the lipophilic matrix co-extracts (Wenzl, 2009).

As HMF, furfural, and HDMF could not be completely recovered from the matrices in a single-stage extraction process, the number of extraction cycles using acetonitrile as the extraction solvent was studied. After the re-extraction of the matrix with another 5 mL of acetonitrile, an increase in the recovery of all compounds was observed for both the representative homemade baby foods, especially for the vegetables- and/or meat-based samples. Thus, two extraction cycles were fixed for the sample preparation. In the literature, it has been observed that furan derivatives cannot be completely recovered from oil matrices using a single stage extraction process, thus three extraction cycles have been applied (Ariffin et al., 2014; Durmaz and Gökmen, 2010).

With the aim of minimizing the interference from other co-extracted matrix components and obtaining a clean extract, two procedures were compared after two extraction

cycles and partitioning with hexane: dispersive solid-phase extraction (D-SPE), in which 200 mg of primary secondary amine (PSA), 200 mg of octadecyl (C18) and 30 mg of graphitized carbon black (GCB) sorbent were directly added to the acetonitrile extract followed by vortex agitation for 1 min and centrifugation at 3000 × g for 10 min; and a dilute-and-shoot strategy, in which the obtained acetonitrile extract was transferred to a glass volumetric flask and the volume was completed to 50 mL with deionized water, followed by HPLC-DAD analysis.

A comparison between the d-SPE and dilute-and-shoot sample preparation is shown in **Fig. 1**, and the dilute-and-shoot strategy resulted in a better peak shape and detectability of all compounds at 284 nm. In addition to the cost of the sorbents, the d-SPE procedure was not efficient for the complete removal of an intense co-extracted matrix compound at the retention time of 1.2 min. Although GCB removes chlorophyll and carotenoid pigments, considerable losses have been observed for different compounds in food matrices due to their retention on the GCB sorbent (Lawal et al., 2018; Tsagkaris et al., 2019; Wang et al., 2019). Therefore, the dilute-and-shoot strategy with deionized water was validated and then applied to the fruit-based baby foods and vegetable- and/or meat-based baby food samples. This approach reduces sample preparation time, improves laboratory efficiency, and contributes to a lower final cost and residue generation, a current trend in the analysis of complex food samples (Ballesteros-Gómez and Rubio, 2019).

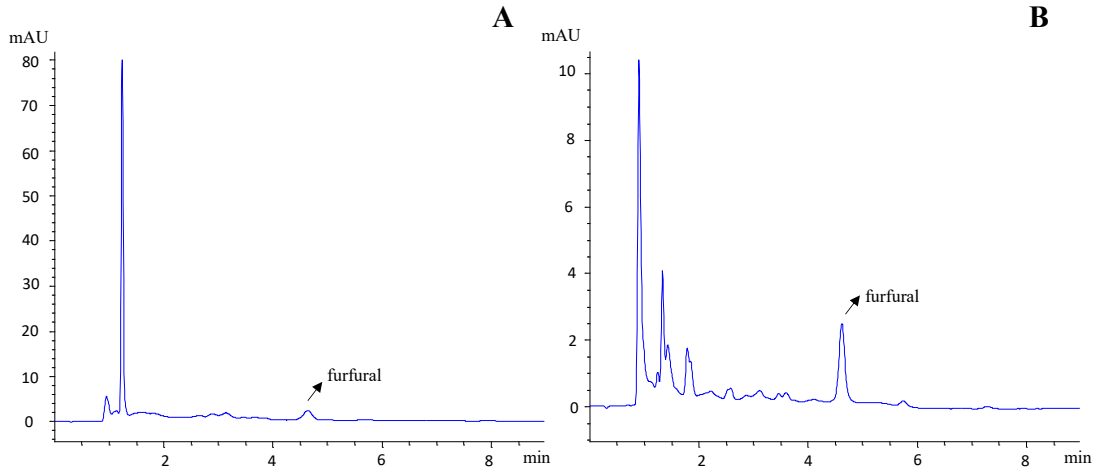

Fig. 1. HPLC-DAD chromatograms (λ =284 nm) obtained from extracts of meat-based baby food containing furfural (1.56 μg g^{-1}) after (**A**) d-SPE clean-up with PSA, C18, and CGB sorbents, and (**B**) dilute-and-shoot clean-up strategy.

3.2. Method performance characteristics

The performance characteristics of the method were evaluated using "blank" homemade baby foods in which the investigated analytes were not detected, as seen in **Figs. 2A**

and **2B**. These homemade samples, one rice-based baby food and one including fresh fruits, were used as representative matrices in trueness (recovery) and precision studies and to obtain matrix-matched calibration curves used for quantification purposes.

Furfural and HMF are not commonly detected in fresh and untreated food; however, they can rapidly accumulate during heat treatment and storage of carbohydrate-rich products. Therefore, fresh fruits were used to obtain representative fruit-based baby foods. For this purpose, papaya and orange fruits, purchased from a local market, were washed and peeled, and the seeds were removed; then, approximately 400 g of papaya and 200 mL of orange juice were homogenized in a cutter (Skymsen CR-4 L), and the obtained mixture was not submitted to heating. Although HDMF has been reported at high levels in fresh fruits, such as strawberries, mangoes and pineapples (Zabetakis et al., 1999), this compound was not detected in the homemade fruit-based baby food used as the "blank" representative matrix (**Fig. 2B**).

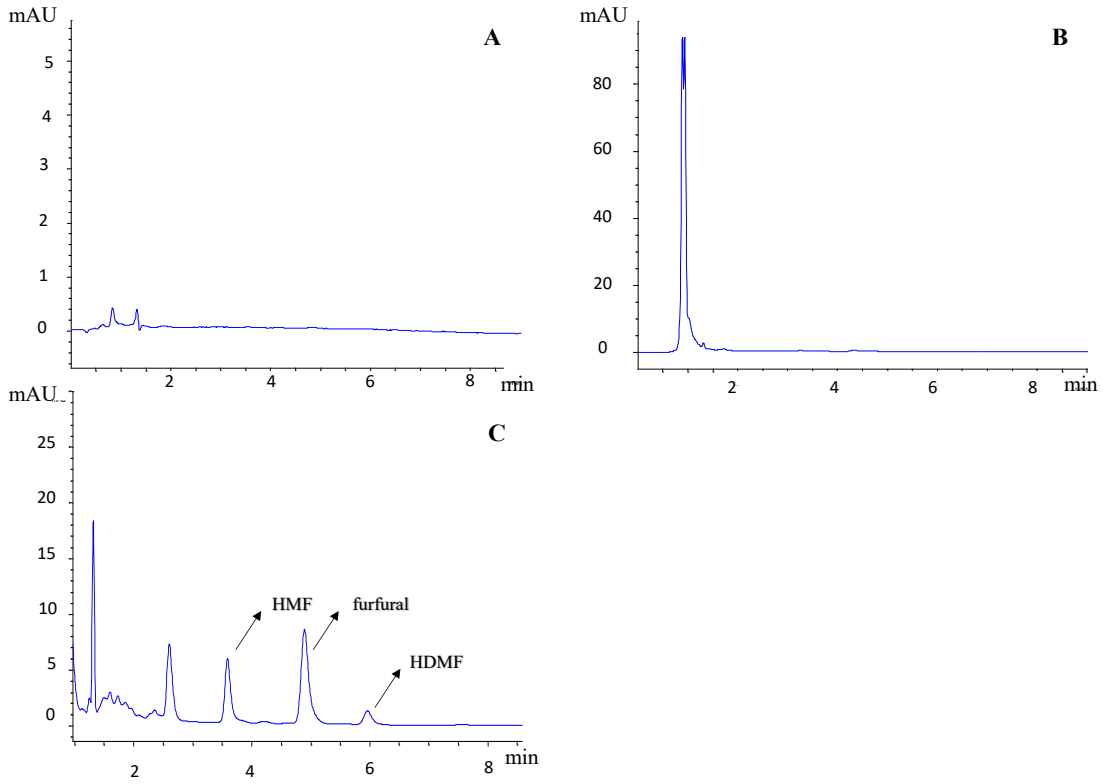

Fig. 2. HPLC-DAD chromatograms (λ =284 nm). (**A**) Blank matrix composed of boiled rice; (**B**) blank matrix composed of papaya purée and orange juice; (**C**) commercial meat- and vegetables-based baby food containing naturally HMF, furfural and HDMF.

Unlike fruit-based baby food, baby foods made with vegetables and/or meat must be subjected to heat treatment to be consumed. Thus, finding a "blank" representative matrix, free of the investigated analytes, that contained heat-treated ingredients was a great challenge. The formation of these compounds from the MR depends directly on the temperature,

processing time, and type of heat treatment applied (Delgado-Andrade et al., 2010); boiling and steaming are less aggressive than frying, roasting or grilling (Pérez-Burillo et al., 2019). After several tests using different boiled vegetables, cereals and meats, it was observed that the compounds of interest were not detected in only one homemade baby food, which was made with boiled rice (Fig. 2A); therefore, this homemade baby food was also used as a "blank" representative matrix for the validation procedure. In this case, approximately 350 g of industrialized rice, purchased from local stores and 700 mL of deionized water were heated in an open pan for 10 min, reaching a temperature of 95 °C; then, it was constantly agitated under heating to guarantee uniformity in the distribution of heat. After cooking, the homemade baby food was homogenized in a cutter (Skymsen CR-4 L) and, despite the heat treatment applied, no signal was detected at the retention time of the analytes. HMF was also not detected in japonica rice after cooking and heating at 100 °C (Feng et al., 2019); however, low levels of HMF were reported in rice-based breakfast and baby cereals (García-Villanova et al., 1993; Guerra-Hernández et al., 1992). For both "blank" representative matrices, the samples were prepared on the same day the validation procedure was conducted.

The selectivity of the method was assessed based on its ability to determine the target analytes accurately in the presence of matrix co-extractives (Petrarca et al., 2020). Complete chromatographic separations of HMF (t_R =3.5 min), furfural (t_R =4.8 min) and HDMF (t_R =5.8 min) from matrix interferences was observed for all the baby food samples analysed, which allowed the unequivocal identification of the analytes and easy manual integration of the chromatographic peaks (**Fig. 2C**).

The limits of detection (LOD) and quantification (LOQ) were established by means of an analysis of the baby food extracts spiked with decreasing concentrations of HMF, HDMF, and furfural until obtaining signal-to-noise ratios of 3:1 and 10:1, respectively. Using a representative rice-based baby food, an LOD of 0.15 μg g^{-1} and LOQ of 0.30 μg g^{-1}were achieved for HMF and furfural and an LOD of 0.50 μg g^{-1} and LOQ of 1 μg g^{-1} were achieved for HDMF. For the representative fruit-based baby food, an LOD of 0.10 μg g^{-1} and LOQ of 0.20 μg g^{-1} were obtained for HMF; an LOD of 0.15 μg g^{-1} and LOQ of 0.30 μg g^{-1} were obtained for furfural; and an LOD of 0.25 μg g^{-1} and LOQ of 0.50 μg g^{-1} were obtained for HDMF.

Linearity was assessed in the solvent and matrix-matched calibration curves, which included seven points each with concentration levels ranging from the LOQ to 25 μg g^{-1}. Over the calibration ranges selected, all the calibration curves presented adequate linearity (solvent and matrix-based calibration curves) with coefficients of determination (R^2) exceeding 0.9996

in all cases (**Table 1, Table 2**). In addition, an analysis of variance of residuals of the calibration curve was also run to evaluate the significance of regression. The analysis of variance (ANOVA) demonstrated the significance of the regression ($p < 0.05$) in the concentration range studied for both the solvent and matrix-matched calibration curves. The slopes obtained for the calibration curves of the same concentration in solvent (deionised water) and matrix extracts ("blank" baby foods) were employed to estimate the matrix-induced effects using the following equation: Matrix Effect (%) = [(matrix-matched calibration slope – solvent calibration slope)/solvent calibration slope] x 100 (Petrarca et al., 2020). A low matrix effect (< 6.7 %) was observed for all compounds, indicating a negligible matrix effect in the LC-DAD analysis.

The extraction efficiency of the developed method was verified by means of recovery and precision experiments. For these experiments, representative "blank" baby foods were spiked at three levels (LOQ, 10 and 25 μg g^{-1}). Before the extraction procedure, the fortified samples were kept at room temperature for 1 h to achieve better interactions between the analytes and the matrix. A total of twelve independent replicates at each level were analysed on two days, with mean recoveries between 83 and 105 % for the representative baby foods (**Table 1, Table 2**). According to the requirements of the quantitative analysis methods stated by the Commission Decision 2002/657/EC, recoveries within the range of 80–110 % are acceptable for samples spiked at levels ≥ 10 μg kg^{-1} (European Commission, 2002).

Precision was expressed in terms of the relative standard deviation (RSD %). The RSD values, under repeatability conditions, ranged from 1 to 6 % for the representative baby foods (**Table 1, Table 2**) and were obtained from six independent replicates at each level of the spiked samples analysed on the same day. Under within-laboratory reproducibility conditions, a total of twelve independent replicates was analysed on two different days (six replicates per day) for each spiked level, and the RSD values varied between 2 and 5 % for the representative baby foods (**Table 1, Table 2**). All the RSD values were consistent with the criteria established by the Commission Decision 2002/657/EC (European Commission, 2002).

Table 1. Performance characteristics of HPLC-DAD method for the determination of HMF, furfural and HDMF using a representative vegetable- and/or meat-based baby food matrix[a].

	Linearity, R^2 *Solvent (Matrix-matched)*	LOD µg g^{-1}	LOQ µg g^{-1}	Spiked Level µg g^{-1}	Mean recovery (%, *n = 6*)	Precision (RSD %) Intra-day, *n = 6* (Inter-day, *n = 12*)
HMF	0.9999 (0.9999)	0.15	0.3	0.3	99	2 (4)
				10	96	2 (3)
				25	95	1 (2)
Furfural	0.9999 (0.9999)	0.15	0.3	0.3	105	5 (5)
				10	92	1 (3)
				25	91	1 (2)
HDMF	0.9996 (0.9996)	0.5	1	1	100	2 (5)
				10	83	2 (2)
				25	86	1 (3)

LOD: limit of detection; LOQ: limit of quantification; R^2: coefficient of determination; RSD: relative standard deviation. [a] Baby food composed of boiled rice.

Table 2. Performance characteristics of HPLC-DAD method for the determination of HMF, furfural and HDMF using a representative fruit-based baby food matrix[a].

	Linearity, R^2 *Solvent (Matrix-matched)*	LOD µg g^{-1}	LOQ µg g^{-1}	Spiked Level µg g^{-1}	Mean recovery (%, *n = 6*)	Precision (RSD %) Intra-day, *n = 6* (Inter-day, *n = 12*)
HMF	0.9999 (0.9999)	0.1	0.2	0.2	91	3 (2)
				10	101	2 (2)
				25	100	1 (4)
Furfural	0.9999 (0.9999)	0.15	0.3	0.3	87	1 (2)
				10	90	1 (2)
				25	92	4 (2)
HDMF	0.9999 (0.9997)	0.25	0.5	0.5	103	6 (5)
				10	104	5 (5)
				25	102	4 (5)

LOD: limit of detection; LOQ: limit of quantification; R^2: coefficient of determination; RSD: relative standard deviation. [a] Baby food composed of papaya purée and orange juice.

3.3. Analysis and occurrence of HMF, furfural and HDMF in baby food samples

With the aim of checking the suitability of the proposed method for measuring HMF, furfural, and HDMF in baby foods, 60 samples of the most popular baby food brands available in Brazilian markets were analysed. As shown in **Table 3**, **Table 4**, HMF, furfural, and HDMF were detected and quantified at different concentration levels depending on the matrix (vegetables- and/or meat-based baby foods and fruit-based baby foods) and manufacture process (industrialized baby foods and homemade baby foods). Furfural was present in all the samples of vegetables- and/or meat-based baby foods analysed, with levels varying between 1.44 ± 0.07 μg g^{-1} (sample composed of meat, black bean, rice, and vegetables) and 5.71 ± 0.04 μg g^{-1} (sample composed of beet, bean broth and vegetables). In the literature, red beet purée has demonstrated be the vegetables-based purée most susceptible to furan formation (0.011 μg g^{-1}) after sterilisation treatment (Palmers et al., 2015). The fruit-based baby foods showed slightly lower concentrations of furfural than the vegetables- and/or meat-based samples. According to Mesías-García et al. (2010), the high values of furfural is probably due to the natural ascorbic acid decomposition during the vegetable processing (cutting and warming), increased at the high pH. Higher furfural levels have been reported in vegetables-based baby foods (from 0.58 ± 0.08 to 2.87 ± 0.04 μg g^{-1}) than in fruit-based baby foods (up to 1.82 ± 0.25 μg g^{-1}) purchased in Spain (Mesías-García et al., 2010).

The highest HMF contents were detected in fruit-based baby foods, in comparison with vegetables- and/or meat-based baby foods (**Table 3, Table 4**). A wide variation in the mean content of HMF was observed, between not detectable to 343.10 ± 3.55 μg g^{-1}, with the highest levels detected in plum-based baby foods (Table 4). Therefore, plum-based samples have been identified as a potential source of HMF for infants and young children (Petrarca et al., 2020). The high carbohydrate content as also the sugar degradation increased at low pH, support the formation of this furan derivative in fruit-based baby foods (Mesías-García et al., 2010). Regarding to the vegetables-based baby food (Table 3), the carrot purée sample presented the highest HMF content (4.91 ± 0.18 μg g^{-1}).

Vella and Attard (2019) observed a significant difference in the HMF content of prune-based infant food (99.10 ± 11.45 μg g^{-1}) compared to other infant foods, such as meat-based infant food, in which the HMF contents ranged from 1.86 ± 0.18 to 3.13 ± 0.22 μg g^{-1}. Similar results were found in samples sold in the city of Granada, Spain, with HMF concentrations between 0.14 ± 0.002 and 9.59 ± 0.29 μg g^{-1} in fruit-based baby foods and concentrations up to 2.88 ± 0.18 μg g^{-1} in vegetables-based samples (Mesías-García et al.,

2010). Contents from 0.17 to 57.18 μg g^{-1}were reported in milk- and cereal-based baby food from Turkey (Gökmen and Şenyuva, 2006). In samples from Parma, Italy, low HMF concentrations were reported in cereal-based baby foods (Bignardi et al., 2014). HMF has also been reported in commercial fruit-based infant foods in Spain (contents between 0.30 and 65 μg g^{-1}) (Rada-Mendoza et al., 2002, 2004), the Czech Republic (from 2.06 to 28.90 μg g^{-1}) (Čížková et al., 2009; Vorlová et al., 2006), and Brazil (between 2.30 and 195.40 μg g^{-1}) (Petrarca et al., 2020).

Table 3. Furan derivatives content in vegetable and/or meat-based baby food samples.

Baby food [a]	Mean ± standard deviation, n = 3 (μg g^{-1})		
	HMF	Furfural	HDMF
	Industrialized baby food		
Arracacha purée	0.46±0.01	4.99±0.05	n.d.
Bean broth, meat, and rice	0.29±0.04	4.15±0.07	1.38±0.08
Beet, bean broth and vegetables	1.45±0.03	5.71±0.04	n.d.
Carrot purée	4.91±0.18	2.54±0.02	n.d.
Chicken breast and vegetables	<LOQ	5.35±0.03	1.64±0.00
Chicken breast, vegetables, and pasta	<LOQ	4.34±0.09	<LOQ
Chicken risotto	0.45±0.03	1.56±0.01	<LOQ
Creamed corn, carrot, and chicken breast	n.d.	4.55±0.06	1.35±0.06
Meat, vegetables and arracacha *	0.48±0.01	5.29±0.64	1.60±0.05
Meat, vegetables and arracacha *	0.41±0.11	4.82±0.12	1.44±0.01
Minced beef meat	1.04±0.01	4.83±0.03	1.61±0.04
Pasta, meat, and vegetables *	0.40±0.03	4.23±0.08	1.07±0.03
Pasta, meat, and vegetables *	0.56±0.02	4.29±0.03	1.12±0.03
Pasta, meat, and vegetables *	<LOQ	4.12±0.14	1.09±0.09
Pasta, vegetables, and chicken breast	0.29±0.01	4.30±0.06	<LOQ
Spaghetti Bolognese	1.07±0.03	3.67±0.25	<LOQ
Stroganoff and rice	1.11±0.07	1.94±0.07	1.16±0.14
Vegetables and meat *	0.32±0.03	4.96±0.07	1.17±0.04
Vegetables and meat *	0.61±0.04	3.91±0.03	<LOQ
Vegetables and meat *	1.37±0.04	4.66±0.02	<LOQ
	Homemade baby food		
Arracacha purée	n.d.	4.25±0.21	n.d.
Beef liver, chickpeas, and vegetables	n.d.	4.89±0.07	n.d.
Chicken, potato, and vegetables	n.d.	4.34±0.07	n.d.
Fish, pinto bean, and vegetables	n.d.	3.66±0.24	n.d.
Meat, black bean, rice, and vegetables	n.d.	1.44±0.07	n.d.
Vegetables and egg yolk	0.48±0.02	3.76±0.13	n.d.
Vegetables purée	n.d.	3.19±0.29	n.d.

n.d.: not detected. [a] Principal ingredients. * Same brand and ingredients, but different concentration of each ingredient.

Table 4. Furan derivatives content in fruit-based baby food baby food samples.

Baby food [a]	Mean ± standard deviation, $n = 3$ (μg g^{-1})		
	HMF	Furfural	HDMF
	Industrialized baby food		
Apple	7.97 ± 0.01	2.21 ± 0.03	n.d.
Apple and banana	0.93 ± 0.03	n.d.	n.d.
Apple and oat	1.33 ± 0.01	0.92±0.02	<LOQ
Apple, pear, cinnamon, and oat	8.58 ± 0.63	1.73±0.02	n.d.
Banana and apple (brand A)	3.80 ± 0.08	0.48±0.01	n.d.
Banana and apple (brand B)	1.40 ± 0.01	n.d.	n.d.
Banana and apple (brand C)	0.79 ± 0.06	0.42±0.00	n.d.
Banana and oat *	1.11 ± 0.01	n.d.	n.d.
Banana and oat *	1.83 ± 0.02	<LOQ	n.d.
Mango and apple	7.22 ± 0.09	0.49 ± 0.02	0.64 ± 0.03
Mango, apple, and carrot	5.00 ± 0.19	0.47 ± 0.03	n.d.
Mango, passion fruit and oat	3.70 ± 0.18	1.48 ± 0.03	3.35 ± 0.21
Mixed fruits	6.77 ± 0.11	1.69 ± 0.15	n.d.
Organic apple	3.81 ± 0.26	0.55 ± 0.04	n.d.
Organic banana	1.47 ± 0.04	n.d.	n.d.
Organic grape and banana	3.88 ± 0.02	n.d.	0.72 ± 0.02
Organic guava and banana	1.32 ± 0.01	n.d.	0.82 ± 0.17
Papaya and Orange	7.15 ± 0.15	1.14±0.04	n.d.
Peach and apple	1.94 ± 0.03	0.52 ± 0.03	n.d.
Pear	10.22 ± 0.08	0.93 ± 0.05	0.75 ± 0.02
Pear, grape and apple	0.85 ± 0.15	1.88 ± 0.04	n.d.
Plum	343.1 ± 3.55	10.15 ± 0.29	n.d.
Plum, banana, and sweet potato	97.70 ± 1.30	0.60 ± 0.02	n.d.
Red fruits, pear, and oat	5.10 ± 0.06	2.51 ± 0.12	n.d.
Strawberry, raspberry, and apple	2.38 ± 0.15	0.76 ± 0.06	2.12 ± 0.17
Tropical fruits	1.74 ± 0.01	<LOQ	1.47 ± 0.18
Yellow fruits and chia seeds	1.00 ± 0.10	0.30 ± 0.01	0.78 ± 0.14
	Homemade baby food		
Apple and guava	3.59 ± 0.05	<LOQ	0.93±0.04
Apple, papaya, carrot, and e beet	1.35 ± 0.06	n.d.	n.d.
Banana and guava	5.38 ± 0.13	0.83±0.01	4.59±0.22
Banana, watermelon, and apple	14.06 ± 0.25	0.55 ± 0.02	n.d.
Papaya, banana, and apple	0.33 ± 0.00	n.d.	n.d.
Strawberry and pear	n.d.	n.d.	n.d.

n.d.: not detected. [a] Principal ingredients. * Same brand and ingredients, but different concentration of each ingredient.

HDMF was detected in 41 % of the samples analysed at levels ranging from 0.64 ± 0.03 to 4.59 ± 0.22 μg g^{-1}. The highest HDMF level was observed in homemade fruit-based baby food (banana and guava) (Table 4). However, the investigated furan derivatives were not detected in the strawberry- and pear-based baby food (homemade samples). The occurrence of HDMF was also reported in fruit-based baby food commercialized in Brazil with contents

varying from 0.025 to 0.262 μg g^{-1} (Petrarca et al., 2017). Moreover, HDMF was detected in baby foods containing meat in their composition (levels from 1.07 to 1.64 μg g^{-1}). According to Schwab (2013), the furanones have been detected in cooked beef as Maillard products.

In general, the measured results are consistent with the literature and the industrialized baby foods showed higher levels of furan derivatives than the homemade products. The considerable variations in the furan-derivative contents among the baby food samples may be indicative of differences in the processing conditions. Industrialized baby foods are sterilized, which means they are exposed to heat treatments in closed containers, such as glass jars or plastic bags, whereas, the freshly homemade baby food is cooked in an open pan or bowl. Therefore, the volatile analyte can be more easily released from the matrix. In consequence, domestic cooking in open vessels allows furan derivatives to escape; thus, it represents a feasible way to reduce the furan derivative content in foods (Anese and Suman, 2013).

4. Conclusion

In the present work, a simplified sample preparation approach combined with HPLC-DAD was successfully applied for the simultaneous determination of 5-hydroxymethylfurfural (HMF), furfural, and 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF) in different types of baby foods. Adequate performance characteristics, such as good sensitivity and high analytical precision and recovery, were presented. In addition, a low consumption of organic solvents and short extraction time were achieved, allowing the extraction of different analytes at low concentration levels in complex matrices rich in carbohydrates, pigments, proteins, and fats and with very divergent compositions (fruits, meats, and/or vegetables). The dilution step used in the sample preparation minimized the interference of other components of the matrix, eliminating the demand for additional clean-up procedures. In summary, this study contributes to the first data on furan derivatives in vegetables- and/or meat-based baby food marketed in Brazil, with furfural prevalent in these samples, and HMF and HDMF more prevalent in industrialized baby foods than homemade samples.

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CAPÍTULO 2 - 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.

Keywords: Contaminants; Residues; Suspect screening; LC-Q-Orbitrap-MS; Baby food; QuEChERS.

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 (International 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 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 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):

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

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 $\leqslant$ 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 QuEChERS-based method, 2.4.2 WAHSPE (water,

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

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.

			Precursor ions (quantifier ions)				Fragment ions (qualifier ions)			
Compound	**Log *Kow* [a]**	**Chemical Group/ Use type [a]**	**Elemental composition**	**Monitored ion**	**Theoretical mass (*m/z*) [b]**	**Mass error (ppm)**	**Theoretical mass (*m/z*)**	**Mass error (ppm)**	**Elemental composition**	**RT [c]**
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 96.05562	-0.45 -2.88	$C_5H_9ClN_5$ $C_4H_6N_3$	7.67
Azoxystrobin	2.50	Strobilurin/Fungicide	$C_{22}H_{17}N_3O_5$	$[M + H]^+$	404.12410	-2.03	372.09788 344.10297	-0.96 -0.95	$C_{21}H_{14}N_3O_4$ $C_{20}H_{14}N_3O_3$	7.71
Chlorpyrifos	4.70	Organophosphorous/Insecticide	$C_9H_{11}Cl_3NO_3PS$	$[M + H]^+$	349.93356	-1.92	197.92747 321.90226	-0.66 4.75	$C_5H_3Cl_3NO$ $C_7H_8Cl_3NO_3PS$	9.36
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	$C_{19}H_{17}Cl_2N_3O_3$	$[M + H]^+$	406.07197	-2.18	251.00250 337.03928	-3.49 -4.17	$C_{13}H_9Cl_2O$ $C_{17}H_{15}Cl_2O_3$	8.67
Dimethoate	0.70	Organophosphorous/Insecticide	$C_5H_{12}NO_3PS_2$	$[M + H]^+$	230.00690	-1.82	198.96470 170.96978	-2.05 -2.54	$C_4H_8O_3PS_2$ $C_3H_8O_2PS_2$	6.72
Etofenprox	6.90	Pyrethroid/Insecticide	$C_{25}H_{28}O_3$	$[M + NH_4]^+$	394.23767	-2.24	177.12739 349.17982	-4.53 7.39	$C_{12}H_{17}O$ $C_{23}H_{25}O_3$	10.51
Imazalil	3.82	Imidazole/Fungicide	$C_{14}H_{14}Cl_2N_2O$	$[M + H]^+$	297.05560	-1.82	158.97628 200.98685	-2.28 -2.87	$C_7H_5Cl_2$ $C_9H_7Cl_2O$	7.20
Kresoxim-Methyl	3.40	Strobilurin/ Fungicide	$C_{18}H_{19}NO_4$	$[M + H]^+$	314.13868	-2.00	222.09134 282.11247	-2.39 0.00	$C_{15}H_{12}NO$ $C_{17}H_{16}NO_3$	8.40
Malathion	2.75	Organophosphorous/Insecticide	$C_{10}H_{19}O_6PS_2$	$[M + H]^+$	331.04334	-1.76	99.00767 257.00656	-1.82 -3.74	$C_4H_3O_3$ $C_7H_{14}O_4PS_2$	8.00
Methidathion	2.20	Organophosphorous/Insecticide	$C_6H_{11}N_2O_4PS_3$	$[M + H]^+$	302.96913	-2.12	145.00662 85.03964	-2.45 -0.82	$C_4H_5N_2O_2S$ $C_3H_5N_2O$	7.73
Phosalone	4.01	Organophosphorous/Insecticide	$C_{12}H_{15}ClNO_4PS_2$	$[M + H]^+$	367.99414	-2.01	182.00033 138.01050	-2.65 -2.05	$C_8H_5ClNO_2$ C_7H_5ClN	8.60

Table 1. (continued)

Compound	Log *Kow* [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	
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 72.04439	-2.46 2.35	$C_9H_{16}N_3O$ C_3H_6NO	7.21
Pirimiphos-methyl	4.20	Organophosphorous/Insecticide	$C_{11}H_{20}N_3O_3PS$	$[M + H]^+$	306.10358	-1.70	164.11822 108.05562	-1.67 -0.87	$C_9H_{14}N_3$ $C_5H_6N_3$	8.69
Pyraclostrobin	3.99	Strobilurin/ Fungicide	$C_{19}H_{18}ClN_3O_4$	$[M + H]^+$	388.10586	-1.90	163.06278 149.04713	-1.60 4.96	$C_9H_9O_2N$ $C_8H_7NO_2$	8.51
Tebuconazole	3.70	Triazole/Fungicide	$C_{16}H_{22}ClN_3O$	$[M + H]^+$	308.15242	-2.07	70.03997 125.01525	-5.71 -1.88	C_2H_4N3 C_7H_6Cl	8.48
Tetraconazole	3.56	Triazole/Fungicide	$C_{13}H_{11}Cl_2F_4N_3O$	$[M + H]^+$	372.02881	-0.63	158.97628 184.99193	-2.15 -2.82	$C_7H_5Cl_2$ $C_9H_7Cl_2$	8.09
Trifloxystrobin	4.50	Strobilurin/ Fungicide	$C_{20}H_{19}F_3N_2O_4$	$[M + H]^+$	409.13697	-1.95	186.05251 206.08117	-2.74 -2.28	$C_9H_7F_3N$ $C_{11}H_{12}O_3N$	8.67
Aflatoxin B1	1.23 [d]	Mycotoxin	$C_{17}H_{12}O_6$	$[M + H]^+$	313.07066	-1.90	285.07575 270.05227	-0.88 -2.93	$C_{16}H_{13}O_5$ $C_{15}H_{10}O_5$	7.14
Aflatoxin B2	1.45 [d]	Mycotoxin	$C_{17}H_{14}O_6$	$[M + H]^+$	315.08631	-1.79	287.05501 259.06010	-0.38 -1.05	$C_{15}H_{11}O_6$ $C_{14}H_{11}O_5$	7.05
Aflatoxin G1	0.50 [d]	Mycotoxin	$C_{17}H_{12}O_7$	$[M + H]^+$	329.06558	-2.03	311.05501 243.06519	-3.10 -1.30	$C_{17}H_{11}O_6$ $C_{14}H_{11}O_4$	6.90
Aflatoxin G2	0.71 [d]	Mycotoxin	$C_{17}H_{14}O_7$	$[M + H]^+$	331.08123	-1.72	245.04445 313.07066	1.43 -3.27	$C_{13}H_9O_5$ $C_{17}H_{13}O_6$	6.84

[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).

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):

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

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

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

Compounds	Meat and vegetables based baby food									
			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.

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).

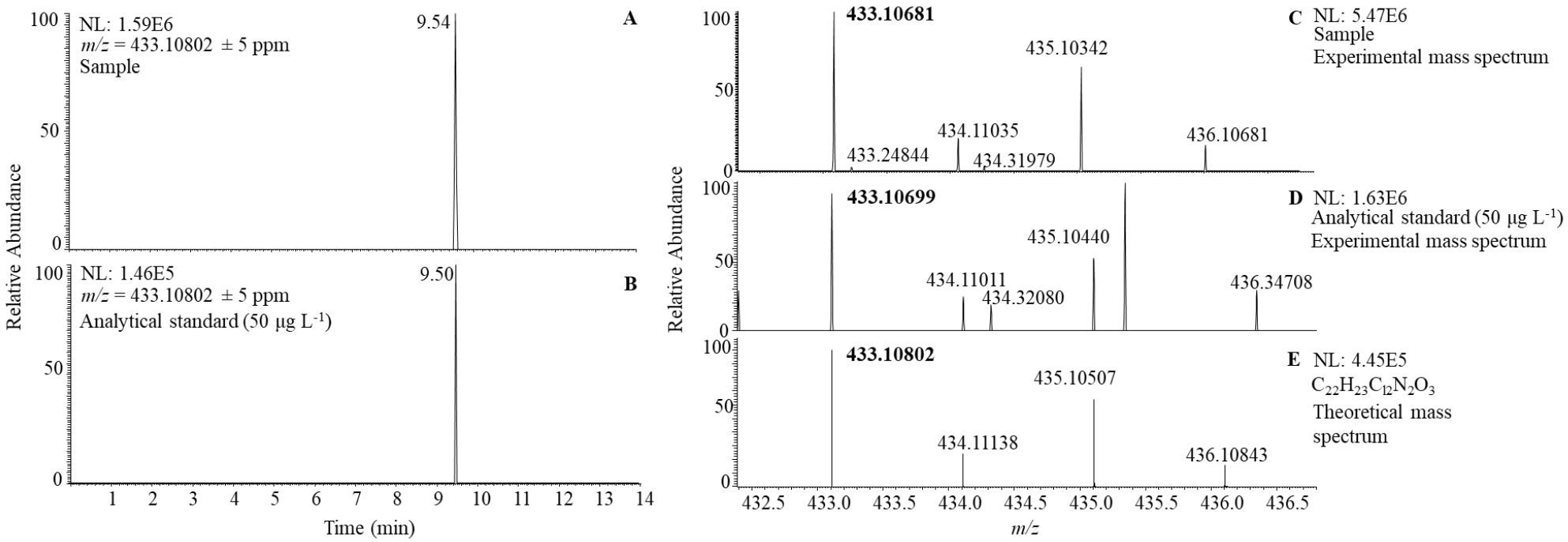

Fig. 1. UHPLC-Q-Orbitrap-MS extracted ion chromatograms of cypermethrin in (a) fruit-based baby food composed of yam, banana and strawberry (10.3 µg kg^{-1}) and (b) analytical standard (50 µg L^{-1}); Experimental mass spectrum of (c) analytical standard (50 µg L^{-1}) and (d) fruit-based baby food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

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. 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.

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).

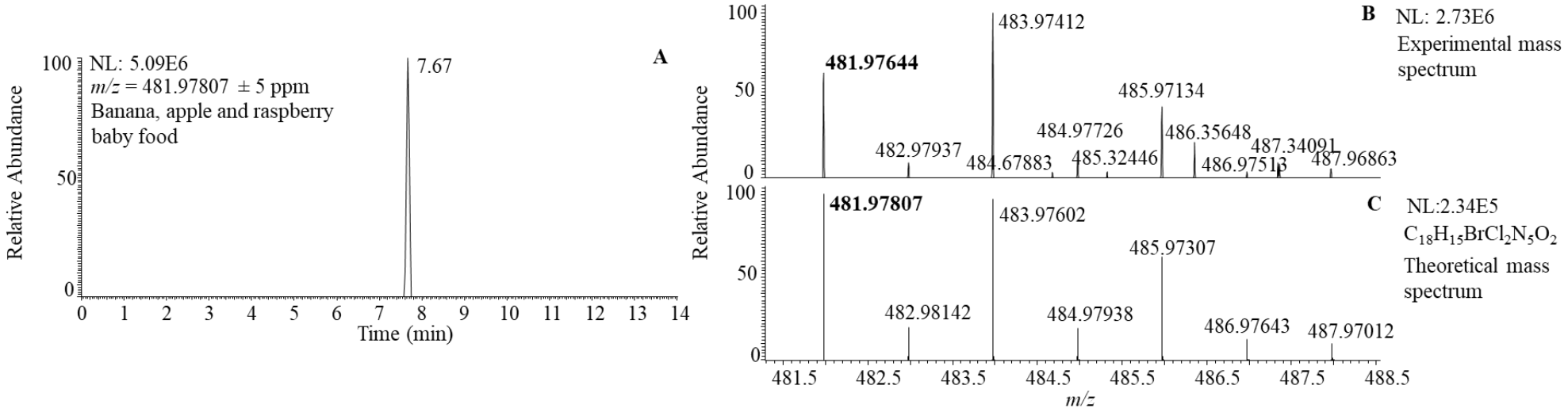

Fig. 2. UHPLC-Q-Orbitrap-MS (a) extracted ion chromatograms of clorantraniliprole (*m/z* 481.97807); (b) Experimental and (c) theoretical mass spectrum to clorantraniliprole.

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, and 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.

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Supplementary data

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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Table S1. Recoveries using QuEChERS and WAHSPE procedure. Analysis of pesticides of an extract of fish and vegetables based baby food (spiked samples at 200 μg kg^{-1}).

Compound	Recovery from QuEChERS - based method (%)	WAHSPE	
		Recovery from the acetonitrile phase (%)	Recovery from the water phase (%)
λ - Cyhalothrin	83.7 (3.8)	119.3 (12.2)	n. e.
Atrazine	91.2 (6.3)	138.4 (2.7)	n. e.
Azoxystrobin	87.7 (3.0)	159.2 (6.1)	n. e.
Chlorpyrifos	81.5 (4.1)	61.3 (7.7)	n. e.
Cypermethrin	95.4 (3.4)	99.2 (4.6)	n. e.
Deltamethrin	103.3 (4.3)	105.3 (7.4)	n. e.
Dimethoate	84.5 (5.9)	157.1 (3.8)	n. e.
Difenoconazole	94.9 (5.2)	126.0 (3.2)	n. e.
Etofenprox	74.7 (13.0)	46.8 (7.8)	n. e.
Imazalil	89.4 (9.3)	160.4 (2.9)	n. e.
Kresoxim-methyl	90.6 (8.5)	150.2 (4.4)	n. e.
Malathion	92.6 (2.8)	158.5 (0.9)	n. e.
Methidathion	70.1 (2.5)	148.8 (7.0)	n. e.
Phosalone	95.8 (9.5)	149.1 (2.3)	n. e.
Phosmet	90.5 (3.4)	251.7 (3.7)	n. e.
Pirimicarb	88.1 (6.7)	154.8 (1.9)	n. e.
Pirimiphos methyl	94.1 (4.9)	86.6 (4.5)	n. e.
Pyraclostrobin	86.5 (6.4)	136.9 (1.3)	n. e.
Tebuconazole	93.2 (1.7)	147.8 (4.4)	n. e.
Tetraconazole	112.6 (9.5)	149.1 (2.2)	n. e.
Trifloxystrobin	96.0 (3.6)	124.0 (3.8)	n. e.

[a] n. e.: not extracted (recovery < 10 %); RSDs (%) are shown in parentheses;

Table S2. Effect of d-SPE sorbents on the matrix effect and loss of analyte for analysis of pesticides in baby foods.

Compound	Meat and vegetables baby food (Fish and vegetables baby food)									
	PSA		C18		Florisil		CGB		Z-sep+	
	ME %	Losses %	ME %	Losses %	ME %	Losses %	ME%	Losses %	ME%	Losses %
λ-Cyhalothrin	-29.2 (-27.4)	39.3 (13.4)	-18.2 (-13.7)	-15.9 (-5.4)	-19.4 (-12.4)	11.1 (-10.0)	-30.8 (-18.7)	5.2 (-5.2)	-19.5 (-10.9)	-3.1 (4.8)
Atrazine	-32.8 (-30.6)	-9.3 (-9.2)	-35.6 (-37.3)	-5.9 (-11.1)	-39.2 (-33.0)	10.9 (-2.4)	-39.0 (-32.2)	3.7 (-9.8)	-29.2 (-28.3)	-8.9 (-4.9)
Azoxystrobin	-35.2 (-40.7)	-14.3 (-6.8)	-33.3 (-39.9)	-17.5 (-22.9)	-40.7 (-36.4)	0.7 (-8.7)	-43.3 (-50.0)	2.0 (-9.4)	-33.7 (-33.6)	-23.3 (-18.2)
Chlorpyrifos	-35.1 (-28.2)	9.7 (5.0)	-27.1 (-17.7)	-22.6 (-10.6)	-27.0 (-21.0)	12.3 (-6.2)	-30.2 (-20.2)	-39.7 (-34.7)	-21.6 (-11.8)	-1.0 (-1.3)
Cypermethrin	-37.7 (-14.2)	59.5 (-4.7)	-25.1 (-4.9)	0.2 (-18.7)	-32.0 (-3.3)	28.0 (-5.9)	-15.0 (-15.3)	-12.0 (-13.2)	-9.3 (-7.3)	-4.8 (-5.8)
Deltamethrin	-32.9 (-5.2)	51.3 (0.8)	-25.9 (-3.6)	20.1 (-10.0)	-18.8 (8.1)	12.4 (-2.6)	0.4 (-18.9)	-18.3 (-14.2)	1.6 (5.3)	1.2 (-7.0)
Dimethoate	-37.3 (-30.7)	4.7 (-9.8)	-43.4 (-39.5)	-6.2 (-10.2)	-38.2 (-36.2)	-4.8 (-3.6)	-42.7 (-40.6)	3.6 (-6.1)	-28.1 (-20.8)	-5.5 (-16.4)
Difenoconazole	-22.3 (4.3)	-12.0 (-16.1)	-29.2 (-0.7)	0.2 (-23.7)	-30.1 (-8.4)	3.7 (-8.3)	-10.5 (6.0)	-17.5 (-19.7)	-5.0 (14.7)	-12.7 (-7.6)
Etofenprox	3.3 (-16.9)	-2.5 (35.7)	4.9 (-26.6)	2.0 (13.4)	5.4 (15.0)	22.6 (-23.7)	8.7 (-18.6)	-44.9 (-24.1)	7.3 (-8.5)	19.7 (2.8)
Imazalil	-10.1 (-5.9)	-0.2 (-10.7)	-17.6 (-13.6)	-9.1 (-13.0)	-17.1 (-13.6)	2.9 (-0.5)	-17.7 (-12.1)	-13.0 (-15.8)	-7.0 (-21.7)	-11.4 (-19.0)
Kresoxim-methyl	-30.7 (-12.7)	22.4 (-4.0)	-23.7 (-18.6)	-13.5 (-6.2)	-27.7 (-14.4)	14.3 (-3.5)	-22.0 (-20.6)	-11.5 (-0.1)	-17.0 (-9.1)	4.0 (-12.2)
Malathion	-19.4 (-11.9)	1.6 (-8.3)	-16.6 (-19.1)	-8.3 (-11.5)	-21.3 (-16.7)	4.2 (-7.3)	-17.4 (-18.7)	-8.1 (-3.4)	-23.5 (-9.2)	16.2 (-4.2)
Methidathion	-86.3 (-86.6)	-28.5 (-35.9)	-87.4 (-87.8)	-27.1 (-38.8)	-87.0 (-87.6)	-29.0 (-31.6)	-88 (-90.3)	-17 (-25.1)	-85 (-87.1)	-38 (-33.1)
Phosalone	-31.0 (-16.2)	21.1 (-10.9)	-30.4 (-17.3)	0.8 (-21.0)	-26.5 (-17.6)	2.7 (-5.5)	-20.2 (-12.9)	-47.6 (-46.4)	-23.4 (18.3)	0.7 (8.1)
Phosmet	-60.1 (-59.5)	-28.6 (-40.1)	-63.3 (-64.1)	-12.4 (-34.9)	-59.8 (-60.6)	-25.8 (-30.1)	-64.3 (-61.6)	-48.7 (-38.6)	-57.0 (60.5)	-30.5 (-25.6)
Pirimicarb	-18.5 (-15.0)	1.4 (-10.8)	-25.7 (-23.8)	-1.5 (-10.2)	-25.1 (-17.0)	4.8 (-0.5)	-24.4 (-18.8)	-23.4 (-23.5)	-13.6 (13.0)	-2.2 (-3.4)
Pirimiphos methyl	-29.9 (-14.6)	2.8 (-0.2)	-26.9 (-9.1)	2.8 (-11.8)	-31.6 (-13.0)	2.6 (-1.3)	-19.7 (-9.5)	-19.6 (-12.0)	-11.0 (-3.7)	-12.5 (-5.6)
Pyraclostrobin	-16.5 (-12.8)	-12.0 (-5.9)	-21.4 (-9.1)	-7.2 (-30.3)	-14.4 (-10.0)	1.8 (-6.0)	-5.1 (1.4)	-76.9 (-72.8)	-3.7 (-6.0)	-11.2 (1.7)
Tebuconazole	-17.6 (3.9)	6.4 (-5.2)	-3.8 (5.1)	-14.4 (-20.8)	-9.3 (8.0)	8.3 (-10.4)	2.5 (10.1)	-8.4 (-14.7)	1.4 (4.9)	-9.3 (-12.1)
Tetraconazole	-21.9 (-16.6)	3.7 (4.4)	-21.2 (-15.9)	3.5 (-10.6)	-24.9 (-13.1)	2.6 (-9.6)	-12.7 (-32.9)	-6.4 (29.5)	-21.1 (-13.7)	7.6 (1.9)
Trifloxystrobin	-31.2 (-10.2)	-0.4 (-9.7)	-30.3 (-5.4)	9.1 (-19.4)	-33.2 (-11.2)	4.5 (-3.4)	-18.2 (-8.5)	-12.8 (-7.7)	-9.6 (3.7)	-17.0 (-3.2)

Table S3. Method performance characteristics obtained using a representative baby food sample composed of fish and vegetables.

Compounds	Fish and vegetables based baby food									
			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	0.9968	79 (89)	92 (96)	103 (98)	7 (18)	10 (14)	6 (9)
Atrazine	0.4	2.0	0.9949	0.9985	90 (98)	94 (96)	96 (94)	6(13)	5 (7)	3 (3)
Azoxystrobin	0.1	2.0	0.9913	0.9969	112 (108)	91 (96)	96 (96)	5 (10)	5 (10)	4 (6)
Chlorpyrifos	0.1	2.0	0.9911	0.9924	81 (88)	97 (96)	102 (96)	12 (14)	9 (9)	4 (9)
Cypermethrin	4.0	10.0	0.9907	0.9856	n.a.	86 (78)	73 (72)	n.a.	17 (20)	15 (15)
Deltamethrin	2.0	4.0	0.9958	0.9871	n.a.	97 (91)	77 (77)	n.a.	17 (18)	7 (11)
Difenoconazole	0.02	2.0	0.9926	0.9963	82 (79)	76 (78)	85 (90)	11 (16)	8 (15)	5 (10)
Dimethoate	1.0	2.0	0.9917	0.9981	113 (106)	99 (100)	89 (90)	11 (15)	6 (8)	1 (4)
Etofenprox	4.0	10.0	0.9977	0.9941	n.a.	78 (74)	82 (77)	n.a.	13 (13)	15 (17)
Imazalil	0.2	2.0	0.9937	0.9987	99 (97)	87 (95)	88 (90)	13 (11)	13 (15)	4 (8)
Kresoxim-Methyl	2.0	4.0	0.9922	0.9958	n.a.	92 (96)	92 (91)	n.a.	9 (12)	4 (6)
Malathion	0.1	2.0	0.9930	0.9980	70 (70)	75 (72)	84 (83)	6 (8)	4 (8)	2 (3)
Methidathion	0.4	2.0	0.9978	0.9941	99 (102)	104 (105)	82 (90)	6 (7)	4 (7)	3 (11)
Phosalone	2.0	4.0	0.9933	0.9958	n.a.	96 (94)	88 (90)	n.a.	13 (9)	4 (6)
Phosmet	4.0	10.0	0.9917	0.9940	n.a.	103 (105)	91 (102)	n.a.	16 (15)	7 (19)
Pirimicarb	0.2	2.0	0.9909	0.9972	99 (98)	91 (99)	94 (04)	3 (7)	5 (10)	10 (10)
Pirimiphos-methyl	0.2	2.0	0.9908	0.9979	101 (100)	97 (97)	95 (95)	4 (11)	4 (6)	6 (5)
Pyraclostrobin	4.0	10.0	0.9944	0.994	n.a.	117 (102)	115 (118)	n.a.	7 (19)	11 (10)
Tebuconazole	2.0	4.0	0.9912	0.9997	n.a.	73 (70)	70 (75)	n.a.	3 (11)	3 (9)
Tetraconazole	0.4	2.0	0.9942	0.9916	90 (100)	91 (96)	90 (94)	6 (14)	9 (10)	2 (8)
Trifloxystrobin	0.1	2.0	0.9950	0.9953	108 (105)	86 (93)	92 (9%)	8 (10)	5 (16)	6 (8)
Mycotoxins										
Aflatoxin B1	0.4	1.0	0.9999	0.9995	87 (87)	88 (89)	81 (79)	5 (9)	6 (5)	5 (5)
Aflatoxin B2	0.4	1.0	0.9996	0.9986	96 (94)	90 (89)	81 (78)	7 (7)	7 (5)	5 (5)
Aflatoxin G1	0.4	1.0	0.9919	0.9964	101 (94)	93 (92)	82 (82)	5 (11)	7 (7)	4 (4)
Aflatoxin G2	0.4	1.0	0.9977	0.9945	97 (94)	94 (91)	89 (87)	4 (8)	5 (6)	3 (5)

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

[a] Acetonitrile solvent.

Table S4. Concentration (μg kg^{-1}) of pesticides detected in different fruit-based baby food samples.

Sample	Composition [a] (Fruit based baby food)	Concentration (μg kg^{-1})			
		λ-Cyhalothrin	Cypermethrin	Difenoconazole	Pirimiphos-methyl
1	Acaí berry and banana	-	-	-	-
2	Apple and banana (brand A)	-	-	<LOQ	-
3	Apple and banana (brand B)	-	-	-	-
4	Apple and plum (plastic bag)	-	-	-	-
5	Apple and strawberry	-	-	-	-
6	Apple purée (plastic bag)	-	-	-	-
7	Apple purée (brand A)	-	-	9.0	-
8	Apple purée (brand B)	-	-	-	-
9	Banana and oat	-	-	<LOQ	<LOQ
10	Banana purée	-	-	-	-
11	Banana, apple and raspberry	-	-	<LOQ	-
12	Grape and banana	-	-	<LOQ	-
13	Guava and banana (brand A)	-	-	-	-
14	Guava and banana (brand B)	-	-	<LOQ	-
15	Mango, apple and banana (plastic bag)	-	-	-	-
16	Mixed fruits	-	-	<LOQ	-
17	Pear and mango (plastic bag)	<LOQ	-	<LOQ	-
18	Pear purée	6.5	-	2.1	-
19	Pear, banana and blueberry (plastic bag)	-	-	-	-
20	Plum	-	-	-	-
21	Yam, banana and Blackberry	-	-	-	-
22	Yam, banana and dragon fruit	-	-	<LOQ	-
23	Yam, banana and strawberry	-	10.3	<LOQ	-

< LOQ: Compound detected below LOQ but not quantified; -: Compound not detected; LOQ: 2.0 μg kg^{-1} (considering validation parameter for fish and vegetables based baby food); [a] Main ingredients.

Table S5. Concentration (μg kg^{-1}) of pesticides detected in different meat and vegetables-based baby food samples.

Sample	Composition[a] (meat and vegetables based baby food)	Concentracion (μg kg^{-1})				
		Azoxystrobin	Chlorpyrifos	Difenoconazole	Pirimiphos-methyl	Trifloxystrobin
1	Bean broth, meat and rice	<LOQ	--	<LOQ	-	<LOQ
2	Bean soup	-	-	<LOQ	3.5	-
3	Beet, bean and vegetables	<LOQ	<LOQ	<LOQ	-	-
4	Cassava, meat and kale	-	<LOQ	-	-	-
5	Chicken breast and vegetables	-	-	-	-	-
6	Chicken breast, vegetables, and pasta	<LOQ	<LOQ	<LOQ	<LOQ	-
7	Chicken risotto	<LOQ	<LOQ	<LOQ	-	-
8	Creamed corn, chicken, and yam	-	-	<LOQ	-	-
9	Egg yolk, meat and vegetables	<LOQ	-	<LOQ	-	-
10	Lentil, meat and vegetables	<LOQ	-	-	-	<LOQ
11	Meat, vegetables, and arracacha	<LOQ	-	-	-	-
12	Mushroom risotto and leek	-	<LOQ	<LOQ	-	-
13	Pasta, meat, and vegetables *	-	-	<LOQ	<LOQ	-
14	Pasta, meat, and vegetables *	<LOQ	-	-	<LOQ	-
15	Rice, bean, meat, and vegetables	-	2.8	-	-	-
16	Rice, chicken breast, and vegetables	-	-	-	-	-
17	Spaghetti Bolognese	<LOQ	<LOQ	<LOQ	<LOQ	-
18	Squash and chickpea	-	<LOQ	-	-	<LOQ
19	Squash, black bean, and chicken breast	-	-	-	-	-
20	Squash, meat, and vegetables	-	<LOQ	-	-	-
21	Stroganoff and rice	<LOQ	<LOQ	<LOQ	-	-
22	Sweet potato purée, corn and ora-pro-nobis	-	-	<LOQ	-	-
23	Sweet potato, black bean and chicken	-	<LOQ	-	-	-
24	Sweet potato, black bean and meat	-	-	-	-	-
25	Vegetables and meat *	-	<LOQ	<LOQ	-	-
26	Vegetables and meat *	<LOQ	-	<LOQ	-	-
27	Vegetables soup and meat	-	<LOQ	-	-	-

<LOQ: Compound detected below LOQ but not quantified; -: Compound not detected; LOQ: 2.0 μg kg^{-1} (considering validation parameter for fish and vegetables based baby food); * Different concentrations of each ingredient (same brand); [a] Main ingredients.

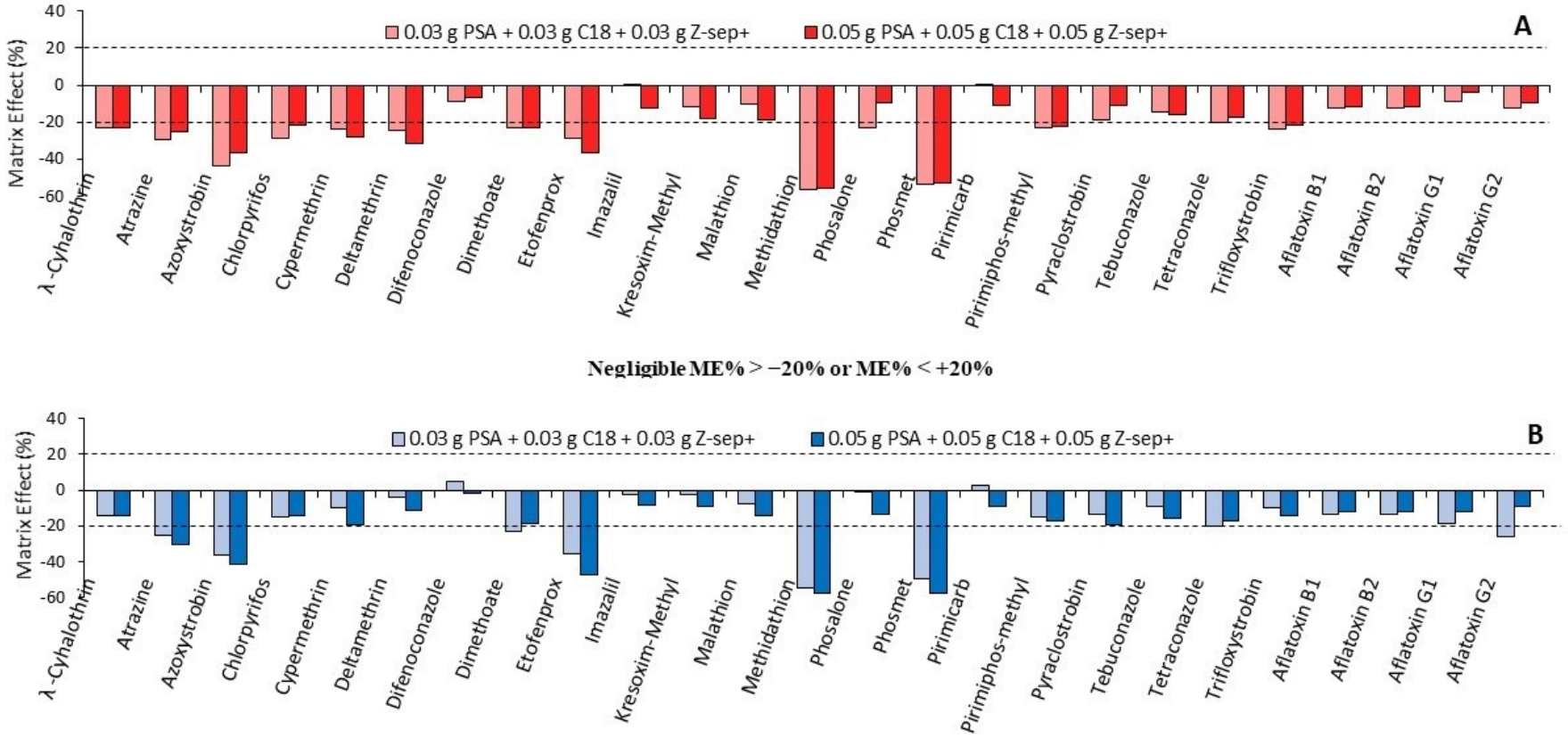

Fig. S1. Evaluation of the effect of amount sorbents during the clean-up step in matrix effect (ME) % for pesticides and aflatoxins in two spiked matrices at the level of 200 µg kg^{-1}: (a) meat and vegetables baby food and (b) fish and vegetables baby food.

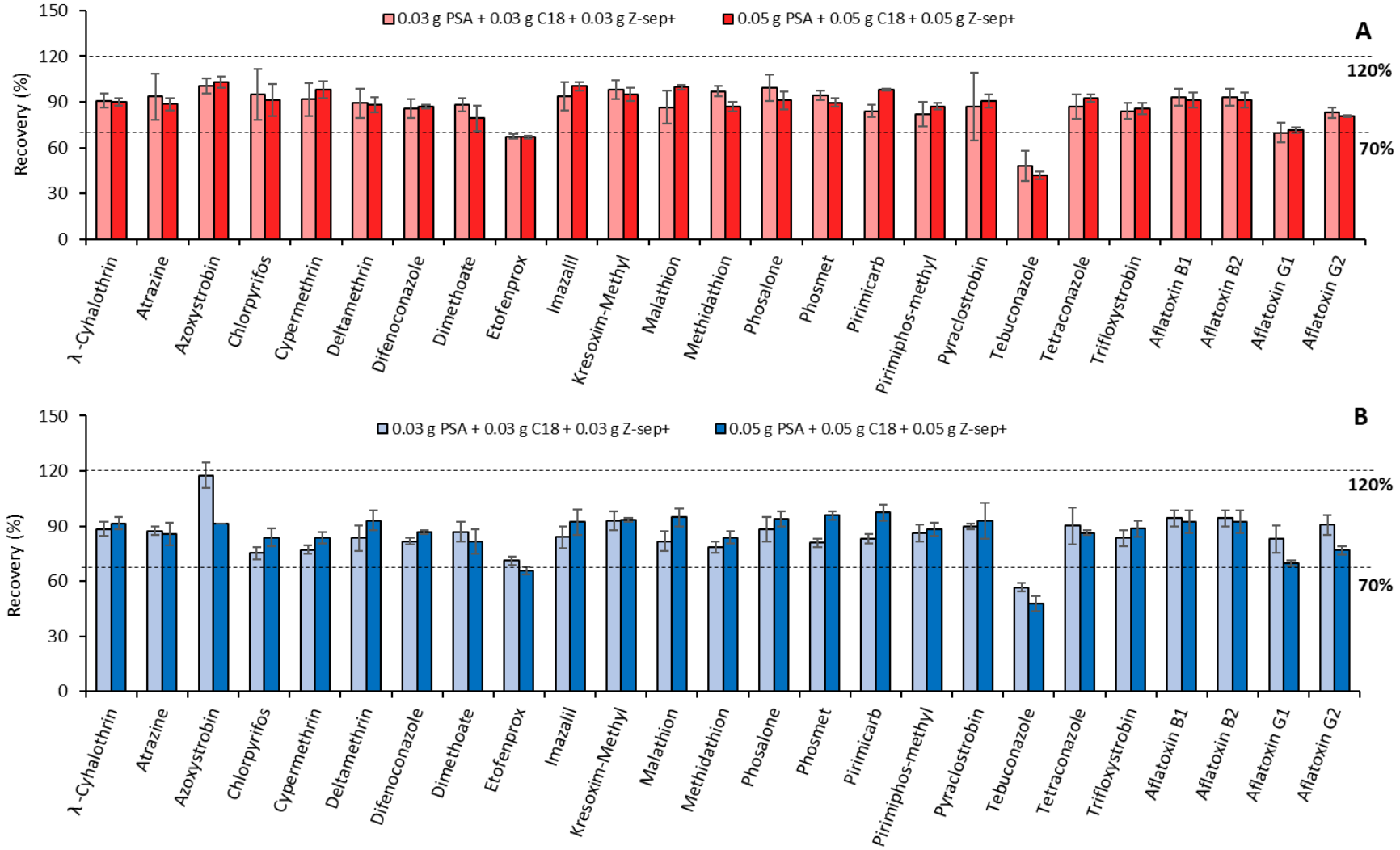

Fig. S2. Evaluation of the effect of amount sorbents during the clean-up step in recovery (%) for pesticides and aflatoxins in two spiked matrices at the level of 200 µg kg^{-1}: (a) meat and vegetables baby food and (b) fish and vegetables baby food (n = *3*).

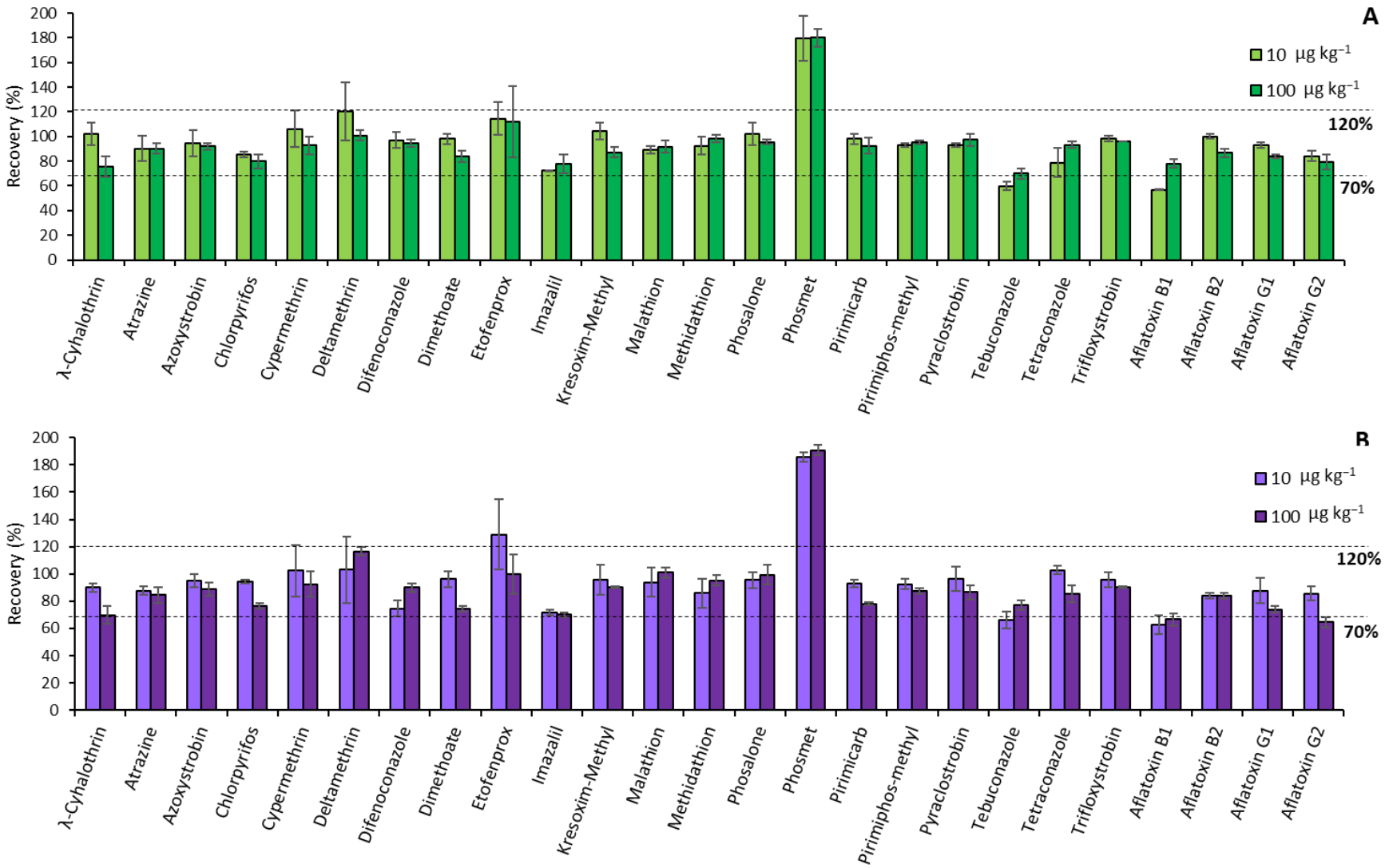

Fig. S3. Evaluation of the recovery (%) for pesticides and aflatoxin in two spiked matrices at 10 and 100 µg kg^{-1} levels: (a) pea and broccoli purée-based baby food and (b) fruit purée-based baby food (n = *3*).

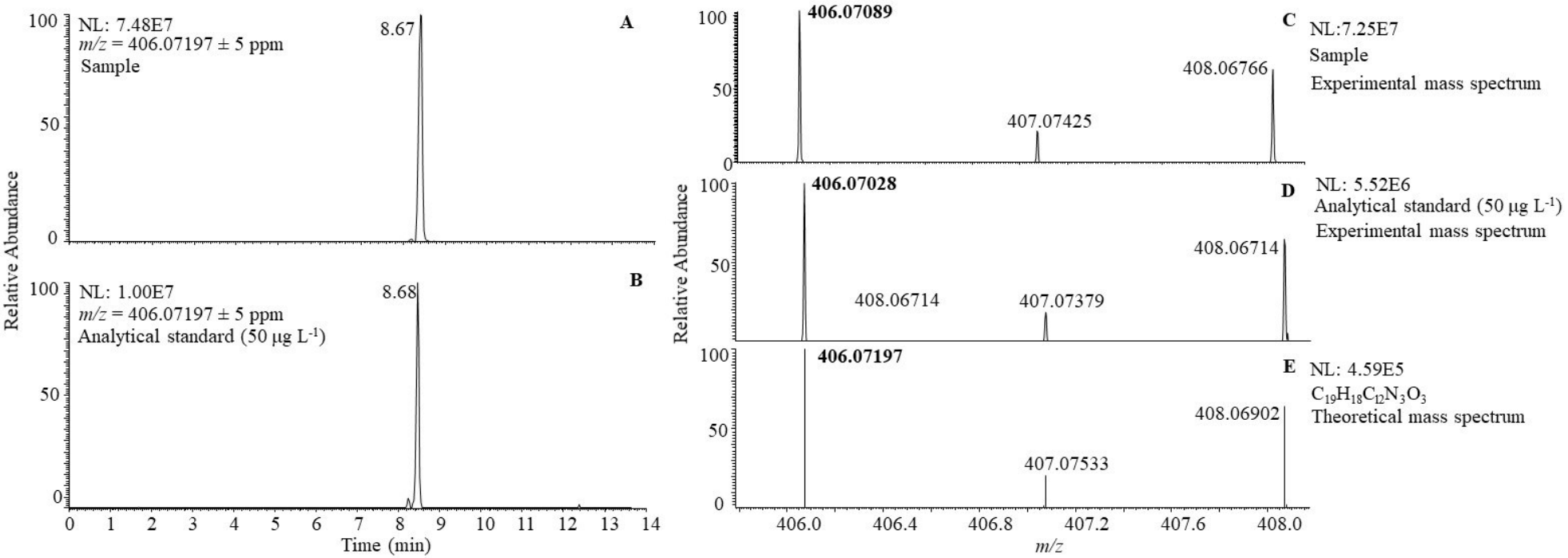

Fig. S4. UHPLC-Q-Orbitrap-MS extracted ion chromatograms of difenoconazole in (a) apple purée-based baby food (9.0 µg kg^{-1}) and (b) analytical standard (50 µg L^{-1}); Experimental mass spectrum of (c) analytical standard (50 µg L^{-1}) and (d) apple purée-based baby food (9.0 µg kg^{-1}); (e) Theoretical spectrum of difenoconazole.

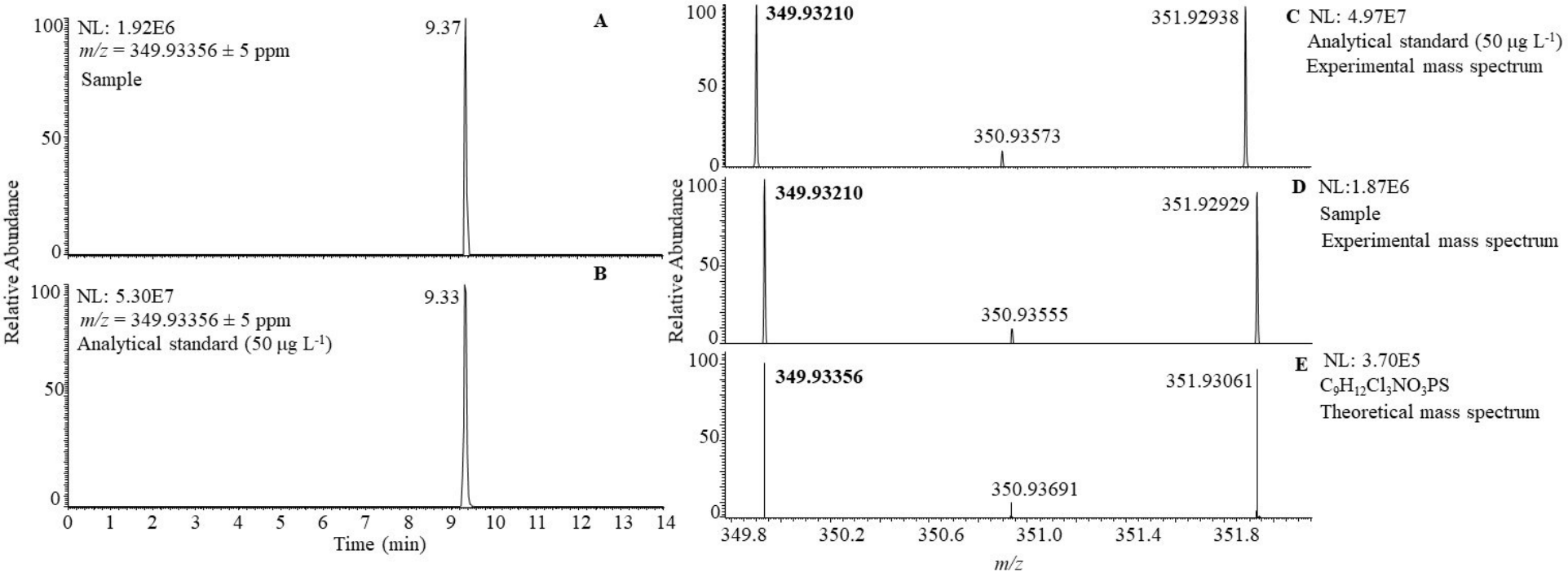

Fig. S5. UHPLC-Q-Orbitrap-MS extracted ion chromatograms of chlorpyrifos in (a) rice, bean, meat, and vegetables- based baby food (2.8 µg kg^{-1}) and (b) analytical standard (50 µg L^{-1}); Experimental mass spectrum of (c) analytical standard (50 µg L^{-1}) and (d) rice, bean, meat, and vegetables baby food (2.8 µg kg^{-1}); (e) Theoretical spectrum of chlorpyrifos.

CAPÍTULO 3 - Determination of acrylamide in commercial baby foods by LC-QqQ-MS/MS: a simple method for routine analyses

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Abstract

A method based on liquid chromatography-triple quadrupole tandem mass spectrometry was developed and validated for the analysis of acrylamide in baby foods. The sample preparation involves a simple extraction process using a mixture of acetonitrile:water:formic acid (69:30:1, v/v/v) in combination with dispersive solid-phase extraction (d-SPE) using alumina as sorbent. The method shown good linearity within the range 20 – 250 μg kg^{-1} through matrix-matched and solvent calibrations. The recovery rates for acrylamide ranged from 100 to 108% with coefficients of variation below 10%, under repeatability and reproducibility conditions (within-laboratory). The obtained limit of quantification (20 μg kg^{-1}) complies with the values indicated by the European Union for acrylamide analysis in baby foods. The developed and validated method was applied to 50 ready-to-eat baby foods available in the Brazilian market. Acrylamide was detected in 13% of samples primarily composed of fruits, whereas it was found in approximately 37% of baby food containing meat and/or vegetables. Additionally, in 2 samples, the detected levels exceeded the benchmark value established by the EU (40 μg kg^{-1}). The study showcases the applicability of this method for routine analysis of acrylamide detection.

Keywords: Processing contaminant; Heat-induced compound; LC-QqQ-MS/MS method; Baby food; Food analysis.

1. Introduction

According to the International Agency for Research on Cancer (IARC), acrylamide has been classified as "probably carcinogenic to humans" in group 2A (IARC 1994). It can be found in various heat-treated foods such as potato chips, dried fruits, biscuits, pastry, chocolate, bread, and coffee (Khan et al. 2017, 2018). This compound is primarily present in carbohydrate-rich foods that have undergone heat processing at temperatures exceeding 120 °C under low moisture conditions (Fernández et al. 2022; Boyaci-Gunduz 2022). The Maillard reaction is a signifcant pathway for the formation of this contaminant in foods, being asparagine the main precursor. Additionally, other pathways, including acrylic acid, acrolein, 3-aminopropionamide, and wheat gluten, have also been involved in the generation of acrylamide (Capuano and Fogliano 2011). It is well-known that glycidamide, one of the metabolites formed after acrylamide intake, represents the major route of genotoxicity and carcinogenicity associated with this contaminant (EFSA 2015).

Baby foods have been identified as the main contributor to the total exposure of infants, followed by other products made with potatoes and cereal-based ingredients (EFSA 2015). Furthermore, the exposure to acrylamide in infants, toddlers, and other children is higher compared to adults, owing to the higher daily food intake relative to body weight (Boyaci-Gunduz 2022). Therefore, this contaminant generated during thermal processes poses a significant risk to children's health, as it is commonly present in the diet of this vulnerable group of consumers (Boyaci-Gunduz 2022). Hence, it is needed to stablish measures for controlling and mitigating the presence of acrylamide in food, as well as setting maximum limits in foodstuffs.

The regulatory benchmark level for the presence of acrylamide in food intended for infants and young children is set at 40 μg kg−1 by the European Commission (European Commission, 2017). However, the acrylamide content in foods can vary significantly depending on the processing technique, ingredients used, storage methods, moisture content, and pH levels (Timmermann et al. 2021). Potato products, such as French fries, have traditionally been the main sources of acrylamide in the human diet (Elias et al. 2017). However, purple, and red varieties of potatoes are more prone to acrylamide formation compared to yellow potatoes due to the higher content of sugar (Orsák et al. 2022). Consequently, strategies can be used to reduce acrylamide formation in children's food by substituting ingredients and adjusting thermal processing conditions. In the literature, there are limited reports on acrylamide in baby foods, and the existing methods involve complex sample

preparation steps due to the nature of these matrices. For the extraction of acrylamide and other processing contaminants from fruit-based baby foods, a combination of acetonitrile, as extraction solvent, with dispersive primary secondary amine (PSA) and cation-exchange solid-phase extraction (SPE) cleanup was used (Petrarca et al. 2017). The QuEChERS (quick, easy, cheap, effective, rugged, safe) method followed by dispersive solid-phase extraction (d-SPE) cleanup step using PSA as sorbent was employed for the analysis of meat and/or vegetables and fruit-based baby foods (Elias et al. 2017). Another study applied water/n-hexane partitioning, followed by clarification using Carrez reagents and SPE cleanup, for the extraction of acrylamide in baby food containing meat and vegetables/cereals processed (Mojska et al. 2012). In the analysis of cereal-based baby food with fruit purees, methanol/water mixture was used as the extracting solvent, followed by defatting with n-hexane, freezing at − 18 °C, and SPE cleanup with Oasis HLB cartridges (Michalak et al., 2013). Additionally, for the analysis of acrylamide in meat-based baby foods, defatting with petroleum ether, extraction with an aqueous solution of sodium chloride, liquid-liquid extraction with ethyl acetate, and cleanup by Oasis HLB SPE cartridges were employed (Jiao et al. 2005). Fohgelberg et al. (2005) reported an extraction method using water, followed by filtration through an SPE column (Multimode, 1 g), and the filtered extract was loaded onto an SPE column (ENV+) for determination of acrylamide in meat, vegetables, and fruit-based baby foods. However, many of these methods reported employed SPE technique, which is time-consuming, expensive, and not easily applicable for sample preparation (Orlando and Simionato 2013; Tuzimski et al. 2016).

In the present study, a simple extraction approach followed by liquid chromatography triple quadrupole tandem mass spectrometry (LC-QqQ-MS/MS) was proposed for the detection of acrylamide in baby foods with different compositions. The analytical method fulfills the requirements for quantitative analyses of acrylamide in food matrices established by the Commission Regulation (EU) 2017/2158 (European Commission 2017). Furthermore, the feasibility of the developed method was demonstrated by analyzing 50 commercially available ready-to-eat baby food samples from the Brazilian market.

2. Material and Methods

2.1 *Equipment, Material, and Reagents*

Analytical standard of acrylamide (purity, 99.9%) and deuterium-labeled acrylamide-d3 (500 mg L^{-1} in acetonitrile), as internal standard (IS), were obtained from Sigma-Aldrich (St. Louis, MO, USA). Stock and working standard solutions were prepared in water

at concentrations of 1000 μg mL^{-1} and 1 μg mL^{-1}, respectively, and stored at 5 °C. MilliQ water was acquired from a Milli-Q system (Millipore, Milfold, MA, USA). HPLC-grade acetonitrile was obtained from Honeywell (Morristown, NJ, USA), and formic acid was purchased from Fisher Scientific (Pittsburgh, PA, USA). Primary secondary amine (PSA) and Al_2O_3 (alumina), used as sorbents, were supplied by Scharlab (Barcelona, Spain) and Bruker (Billerica, MA, USA), respectively. Filters (0.2- μm nylon syringe) were obtained from Agilent Technologies (Santa Clara, CA, USA). For the sample preparation, an analytical AB204-S balance (Mettler Toledo, Greifensee, Switzerland), a vortex mixer WX (Velp Scientific, Usmate, Italy), an Ohaus centrifuge model FC5718R (Parsippany, NJ, USA), and a Reax 2 overhead shaker (Heidolph, Schwabach, Germany) were used.

2.2 *LC-QqQ-MS/MS Analysis*

The LC-QqQ-MS/MS conditions were based on a previous study described by Ferrer-Aguirre et al. (2016). However, several modifications were made to enhance the analytical signal obtained for acrylamide. Chromatographic analysis was performed using a liquid chromatograph 1290 RRLC instrument (Agilent, Santa Clara, CA, USA) equipped with a Jet Stream electronic spray ionization (ESI) source (G1958-65138) and connected to an Agilent triple quadrupole mass spectrometer (6460 A). The MassHunter Workstation Quantitative Analysis for QQQ (Agilent) software was employed for data acquisition. The MS conditions were as follows: gas flow rate of 5 L min^{-1}; source temperature of 300 °C; sheath gas temperature of 400 °C; nebulizer pressure set at 45 psi; sheath gas flow rate of 11 L min^{-1}; capillary voltage of 3500 V; and nozzle voltage of 500 V. The dwell time for all selected reaction monitoring (SRM) transitions was set to 0.15 s. Nitrogen was used as nebulizing and collision gas. Chromatographic separation was achieved on an ACE Excel 3 SuperC18 column (150×4.6 mm, 3.0-μm particle size), maintained at 30 °C (Advanced Chromatography Technologies, Aberdeen, Scotland). The mobile phase consisted of an aqueous solution containing 0.1% of formic acid (v/v) (eluent A) and methanol (eluent B). The flow rate was set at 0.4 mL min^{-1}, and the gradient profile was as follows: 95% eluent A for 3 min, followed by a linear decrease to 0% in 4 min, and then held for 11 min. Subsequently, eluent A was restored to 95% within 1 min and maintained for 1 min. The total running time was 20 min, and the injection volume was 5 μL.

Data acquisition was performed in SRM mode using ESI in positive mode (Table 1). Acquisition of ion transitions was established for acrylamide and acrylamide-d3, and the protonated molecular ion $[M + H]^+$ were selected for each compound. A retention time (t_R) of

6.5 min (± 0.1 min of a maximum tolerance) was employed for the positive identification of acrylamide in baby food samples. The IS was added to the samples at a concentration of 100 μg kg^{-1} prior to the extraction procedure.

2.3 *Sampling*

Two groups of baby foods were obtained in the city of Campinas, SP, located in the South-Eastern region of Brazil. One group primarily consisted of baby foods made with fruits (n = 23), while the other group comprised meat- and/ or vegetable-based baby foods (n = 27). A total of 50 commercial baby food samples were randomly collected in local retail supermarkets and stored in their original packaging, such as glass jars (up to 170 g each) or plastic pots (99 g each), at room temperature until analysis.

2.4 *Sample Extraction*

For the extraction, 1 g of baby food (spiked with 100 μg kg−1 of IS), previously homogenized, and 9 mL of acetonitrile:water:formic acid (69/30/1, v/v/v) solution were added into a centrifuge tube (polypropylene). The mixture was agitated on a rotatory shaker for 60 min at 100 rpm, followed by centrifugation at 3061×g for 20 min. Then, the supernatant (1.5 mL) and 50 mg of alumina sorbent were vortexed for 1 min and then centrifuged at 3061×g for 5 min. The extract was filtered through a 0.2-μm nylon syringe filter and transferred to a glass vial for subsequent LC-QqQ-MS/ MS analysis.

2.5 Validation Procedure

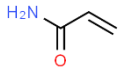
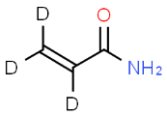
The analytical method was in-house validated according to the following documents: Eurachem Guide recommendations criteria (Magnusson and Örnemark, 2014) and Commission Regulation (EU) 2017/2158 (European Commission, 2017). Therefore, analytical selectivity, accuracy, limit of detection (LOD), limit of quantification (LOQ), linearity in solvent and matrix-matched calibration curves, and matrix effects were evaluated using a representative baby food matrix. Accuracy was evaluated through recovery and precision trials, which were carried out under repeatability and within-laboratory reproducibility conditions. The LOD and LOQ were determined by analyzing blank baby food samples spiked at different concentration levels. For this, the LOD was defined as the concentration that gives a signal-to-noise ratio of 3:1, while the LOQ was set at the concentration that produces a signal-to-noise ratio of 10:1 for both quantification (m/z 72.1 > 55.1) and qualification (m/z 55.1 > 43.9)

transitions. The matrix effect was estimated by comparing the slopes obtained from solvent and matrix-matched calibration curves within the range of 20 - 250 µg kg^{-1}, using Eq. 1:

$$\text{Matrix Effect (\%)} = \left[\frac{(\text{matrix slope} - \text{solvent slope})}{\text{solvent slope}}\right] x\ 100 \quad (1)$$

Linearity was evaluated using solvent and matrix matched calibration curves within the concentration range of 20 - 250 µg kg^{-1}, including the LOQ as the lowest concentration level. To assess repeatability, recovery (%) and precision (relative standard deviation—RSD, %) were determined by analyzing five replicates of spiked baby food samples at two levels (20 and 100 µg kg^{-1}). These replicates were extracted within a single day. To evaluate reproducibility, five replicates of spiked baby food samples at each concentration level (20 and 100 µg kg^{-1}) were analyzed on five different days.

Table 1. Selective reaction monitoring (SRM) transitions and LC–MS/MS operating parameters for the analysis of acrylamide in baby foods using an electrospray interface (ESI) in positive ionization mode.

Analyte	Chemical structure[a]	MW (g mol^{-1})[b]	Precursor ion, $[M + H]^+$	Product ion$_1$[c]	CE[d] (eV)	Product ion$_2$[e]	CE[d] (eV)
Acrylamide		71.1	72.1	55.1	7	43.9	25
Acrylamide-d3		74.1	75.1	58.1	10		

[a] (ChemSpider, 2022).
[b] MW: molecular weight (PubChem, 2022).
[c] Product ion used for quantification.
[d] CE: collision energy.
[e] Product ion used for qualification.

3. Results and Discussion

3.1 *Optimization of the Extraction Procedure*

To minimize the steps involved in the determination of acrylamide, a conventional solid–liquid extraction (SLE) was employed using a mixture of acetonitrile, water, and formic acid (69/30/1, v/v/v) as extraction solvent. This SLE procedure was based on a previous study (Tölgyesi and Sharma 2020), with some modifications considering the effectiveness of these solvents in extracting acrylamide at low levels from complex matrices such as high-sugar

content foods, bread, and fried potato (Tölgyesi and Sharma 2020). The use of acetonitrile minimizes the co-extraction of common matrix components like highly non-polar fats while also aiding in protein precipitation (Petrarca et al. 2017). Additionally, the acidified acetonitrile has proven to be more effective for the extraction of acrylamide from different types of food samples (Tölgyesi and Sharma 2020).

During the optimization of the extraction solvent volume, different amounts of acetonitrile, water, and formic acid solution (69/30/1, v/v/v) were tested, ranging from 3 to 9 mL. A baby food sample consisting of meat and vegetables (potato, carrot, tomato, and onion) was spiked at 200 μg kg−1. The results are shown in Fig. 1, and it was observed that as the volume of the extraction solvent increased, the recovery of acrylamide also increased. When a one-factor analysis of variance was used, significant difference was observed, observing similar results when an extraction volume higher than 5 mL was used. Moreover, a decrease in the matrix effect was observed when the extraction solvent increase, likely due to the dilution of co-extractives. Consequently, the largest volume tested was selected for the extraction of acrylamide from baby foods. Due to the significant amount of matrix interferences that can be co-extracted with acrylamide when water is used as the extraction solution (Ferrer-Aguirre et al. 2016), a cleanup step based on the dispersive solid-phase extraction (d-SPE) technique was studied. Thus, 1.5 mL of the extract obtained from a baby food sample containing meat and vegetables was submitted to two different cleanup procedures: (i) d-SPE with 50 mg of PSA and (ii) d-SPE with 50 mg of alumina (Fig. 1). Both tested sorbents provide suitable recoveries. However, a slightly higher recovery was observed when alumina was used as the cleanup sorbent in the d-SPE process, although when a two sample t-test was carried out, significant difference were not observed between the results obtained for both sorbents. Additionally, a low matrix effect of approximately 4% was also observed using this sorbent (Fig. 1). Consequently, 50 mg of alumina was selected for the cleanup step, effectively reducing the occurrence of co-extractives in the final extract, which could potentially affect the reproducibility and sensitivity of the analytical method. Moreover, it should be noted that alumina sorbent can adsorb amino acids (Omar et al. 2015). Considering that valine has been identified as a critical interference in the analysis of acrylamide in food matrices by LC-MS (Şenyuva and Gökmen 2006), the use of alumina sorbent can prevent the presence of this matrix interference.

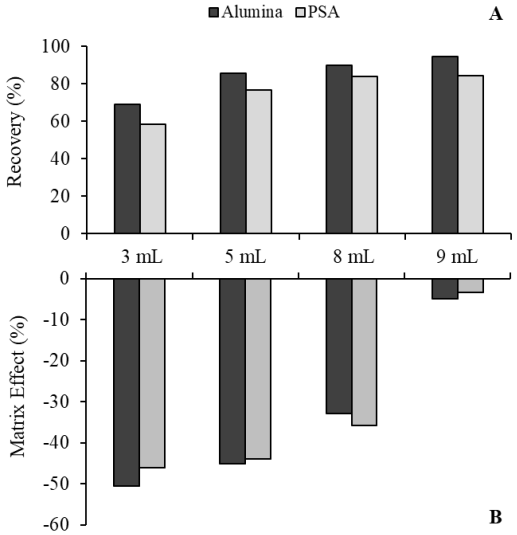


Fig. 1. Effect of the extraction solvent volume on (a) recovery (%) for acrylamide in a spiked matrix of baby food composed of meat and vegetables (potato, carrot, tomato, and onion) at 200 µg kg^{-1} level using alumina or PSA as sorbents and (b) matrix effect for acrylamide in a spiked matrix at 200 µg kg^{-1} after performing the clean-up with alumina or PSA.

3.2 *Method Validation*

Analytical selectivity was verified by analyzing a "blank" baby food and spiked baby food samples, and no interfering peaks were observed at the retention time of the acrylamide in the SRM chromatograms, allowing an unequivocal identification of the target compound (Fig. 2). Moreover, adequate linearity was observed in both solvent and matrix-matched calibration curves within the range 20 – 250 µg kg^{-1}, with coefficients of determination (R^2) ≥0.9932. The LODs and LOQs were 5 and 20 µg kg^{-1}, respectively. The achieved LOQ meets the requirements indicated by the Commission Regulation (EU) 2017/2158 (European Commission 2017), which mandates a minimum LOQ of 20 µg kg^{-1} for analytical methods used for the determination of acrylamide in baby foods, bearing in mind that the benchmark level is lower than 125 µg kg^{-1} (40 µg kg^{-1}).

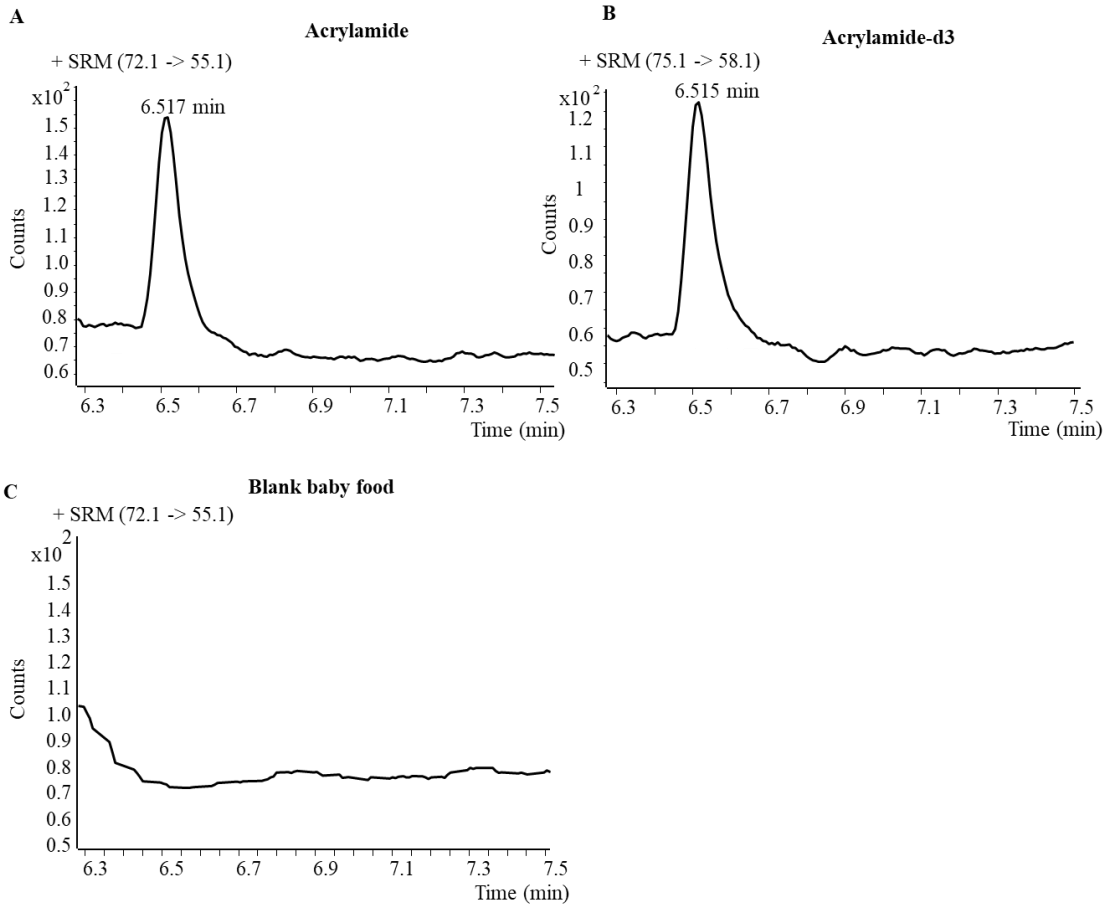


Fig. 2. LC–MS/MS chromatograms obtained in selective reaction monitoring (SRM) mode of a blank baby food (composed by meat and vegetables) sample, **a** spiked with acrylamide and **b** deuterium-labeled acrylamide-d3 both at 100 μg kg^{-1}, and a **c** blank baby food.

The extraction efficiency of the developed method was evaluated through recovery and precision studies. The average recoveries ranged from 100 and 108%, which fall within the acceptable range of 75 – 110% for analytical methods intended for acrylamide analysis (European Commission 2017). Furthermore, the precision of the method meets the criteria set by Commission Regulation (EU) 2017/2158 (European Commission, 2017). Under repeatability conditions, the RSD values ranged from 7 to 10%, while under within-laboratory reproducibility conditions, the RSD was 8% for both analyzed concentration levels. All these values were lower than the coefficient variation (CV) estimated at the level of 100 μg kg^{-1} (22.6%), which was calculated using the Horwitz equation: $CV = 2^{(1-0.5\log C),}$ where *C* is the mass fraction expressed as a power (exponent) of 10. For levels below 100 μg kg^{-1}, it is recommended to have CV values as low as possible (European Commission 2002).

Additionally, the extraction efficiency was also assessed using three other baby food samples with different formulations: (i) a fruit purée–based baby food composed of apple,

peach, banana, and apricot; (ii) a vegetable purée–based baby food composed of carrot, tomato, potato, onion, spinach, and chickpea; and (iii) a baby food sample composed of fish and vegetables. These samples were spiked at 20 μg kg^{-1} (LOQ level) and extracted following the procedure described in the "Sample extraction" section. The matrix effect and recovery were evaluated. The results showed a low matrix effect and high recoveries, ranging from 80 to 109% (Fig. 3), indicating that our proposed method is robust and suitable for the analysis of acrylamide in baby foods with diverse compositions.

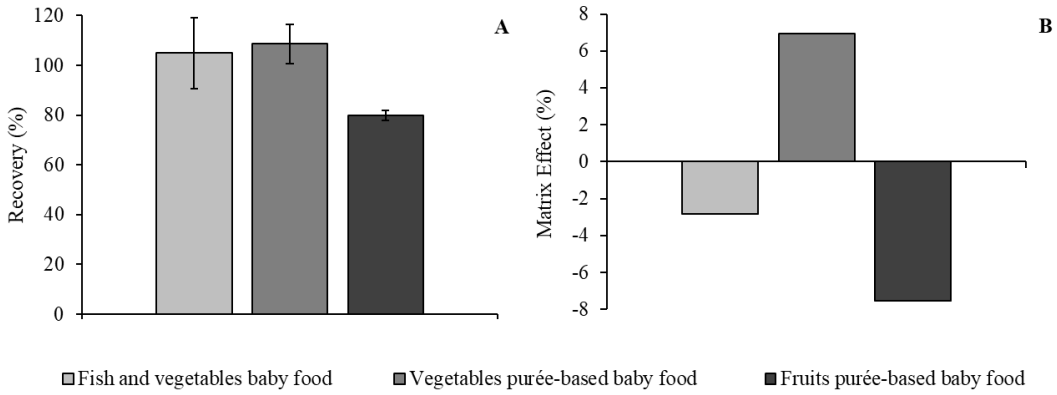


Fig. 3. Evaluation of the (a) recovery (%) for acrylamide in three spiked matrices at 20 μg kg^{-1} level (*n=3*) (mean value ± standard deviation) and (b) matrix effect for acrylamide in three matrices of baby food, composed of different ingredients.

3.3 *Analysis of Commercial Samples of Baby Foods*

To assess the suitability of the proposed method, a total of 50 commercial baby food samples available in the Brazilian market were analyzed but acrylamide was only detected in 13 samples as it was indicated in Table 2. The concentration of acrylamide varied depending on the ingredients of the sample. Among the fruit-based baby foods, acrylamide was detected in 13% of the samples, with concentration ranging from < LOQ to 37 μg kg−1. Furthermore, acrylamide was also detected in baby foods containing plum, albeit at levels < LOQ. In the case of baby foods containing apples and strawberries, the contaminant was detected at a concentration of 20 μg kg^{-1}. Figure 4 displays the SRM chromatograms of the samples naturally contaminated with acrylamide.

Table 2. Levels of acrylamide (μg kg^{-1}) detected in different baby food samples.

Sample	Composition [a]	Acrylamide
	Fruit-based baby food	
1	Apple and plum (plastic bag)	37
2	Apple and strawberry	20
3	Plum	<LOQ
	Meat and/or vegetable-based baby food sample	
4	Cassava, meat, and kale	<LOQ
5	Chicken risotto	56
6	Creamed corn, chicken, and yam	< LOQ
7	Egg yolk, meat, and vegetables	<LOQ
8	Meat, vegetables, and arracacha	<LOQ
9	Rice, bean, meat, and vegetables	<LOQ
10	Squash, meat, and vegetables	<LOQ
11	Stroganoff and rice	90
12	Purple sweet potato purée, ora-pro-nobis and corn	<LOQ
13	Vegetables and meat	<LOQ

< LOQ: Compound detected below LOQ but not quantified; LOQ: 20 μg kg^{-1}. [a] Main ingredient.

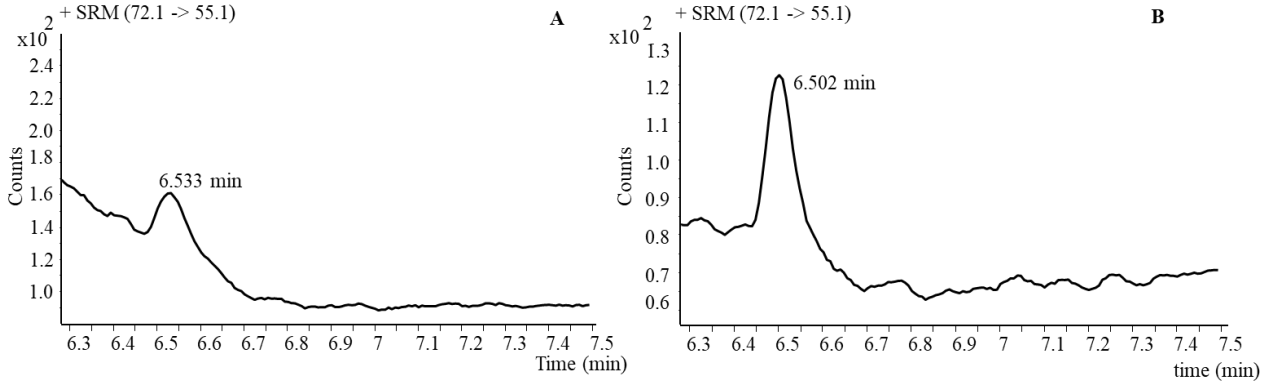


Fig. 4. Chromatograms obtained in the selective reaction monitoring (SRM) mode of baby food samples of A chicken risotto and B apple and plum (plastic bag), both containing naturally acrylamide at a concentration of 56 and 37 μg kg^{-1}, respectively.

The acrylamide levels found in this study were in accordance with those values reported in the literature. Similar content was reported in plum-based baby food from Brazil with a concentration of 35 μg kg^{-1} (Petrarca et al. 2017). In cereal-based baby foods containing fruit purees from Poland, acrylamide levels ranged from 10.8 and 15.7 μg kg^{-1} (Michalak et al. 2013). Fruit-based baby foods from Estonia had a mean acrylamide content < 30 μg kg^{-1} (Elias et al. 2017). Commission Regulation (EU) 2017/2158 has established measures to minimize the

presence of acrylamide in baby foods, particularly those with low acid and composed of prune-based purée. These measures include the use of varieties of prunes with minimal acrylamide precursors, and the modification of time/temperature parameters during the baking process (European Commission 2017). In baby food samples containing meat and/or vegetables, higher levels of acrylamide were observed compared to fruit-based baby foods. Approximately 37% of the analyzed samples contained detectable amounts of acrylamide.

Among them, the highest acrylamide level was found in a sample of stroganoff and rice (90 μg kg^{-1}) followed by chicken risotto (56 μg kg^{-1}) (Table 2). Both samples contained a high proportion of potatoes in addition to meat and rice. Cereal grains (such as rice and wheat) and potatoes are known to be rich in the precursors that contribute to the formation of acrylamide, which could explain the higher levels of the contaminant in these samples (Tateo et al. 2007). The formation of acrylamide is also influenced by processing conditions and food seasonings (Tateo et al. 2007). Regarding the samples made solely with vegetables, acrylamide was detected in one sample at a concentration < LOQ (20 μg kg^{-1}). Acrylamide was also reported in meat and/or vegetable-based baby foods from Poland, with an average level of 55 μg kg^{-1} (Mojska et al. 2012). In Sweden and China, levels up to 25 and 124.93 μg kg^{-1}, respectively, were also observed (Fohgelberg et al. 2005; Jiao et al. 2005). Acrylamide levels in samples from Estonia were similar to those obtained in the present study (Elias et al. 2017).

4. Conclusions

The combination of LC-QqQ-MS/MS with a simple and easy acidified aqueous acetonitrile-based extraction achieves adequate performance characteristics for monitoring acrylamide in baby foods with complex and diverse compositions. An attractive feature of the proposed method is its efficient removal of matrix co-extractives, as evidenced by the low matrix effect (approximately 4%), achieved through dispersive alumina sorbent cleanup, without compromising the analytical sensitivity and accuracy. Moreover, the sample preparation approach eliminates the need for expensive and time-consuming SPE techniques typically used in acrylamide analysis in food samples. The results obtained in this study further support previous findings that the acrylamide content in baby foods is influenced by their composition. Therefore, by selecting baby foods with ingredients that exhibit lower acrylamide formation during thermal processing, it is possible to achieve reduced dietary exposure to acrylamide in infants.

Acknowledgements

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CAPÍTULO 4 - Method validation for GC amenable pesticides and PAHs in baby foods using QuEChERS-based extraction procedure

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Abstract

This study evaluated 14 GC- amenable polycyclic aromatic hydrocarbons (PAHs) and 4 pesticides in fifty baby food samples commercialized in Brazil. Gas chromatography coupled to high resolution mass spectrometry was used to conduct this analyzes. The use of quick, easy, cheap, effective, rugged and safe (QuEChERS) method and dispersive solid-phase extraction (d-SPE) achieved suitable performance characteristics for both methods. For pesticides analysis recovery results ranged from 87 to 120% and the limit of quantification (LOQ) was 2 to 10 μg kg^{-1}. For the PAHs analysis method, recoveries between 71 and 120% were observed. Limit of quantification was established at 0.1 μg kg^{-1} for the different compounds. Furthermore, good linearity within the analyzed range through matrix-matched calibrations has been observed for both methods. As result, in 24% of analyzed samples pesticide residues were quantified or detected at a concentration ranging below LOQ to 4.1 μg kg^{-1} and PAHs were not detected in any sample. These results indicate that the need for greater oversight of protecting food intended for infants to avoid feeding baby food contaminated even at low concentrations.

Keywords: Pesticides; PAHs, Gas chromatography; Baby food; High-resolution mass spectrometers, QuEChERS.

1. Introduction

Residues of pesticides may be found in fruits and vegetables as a result of the application of protective agents designed to manage plant pests and enhance yield across various crops (Lozano et al., 2018; Prata et al., 2022). Furthermore, about 20% of the entire volume of pesticides used in the world is consumed in Brazil (Alcântara et al., 2019). Consequently, pesticide residues can be found in processed products, such as ready-to-eat baby food processed in Brazil (Prata et al., 2022).

Among pesticides, pyrethroids are synthetic pesticides derived from naturally occurring pyrethrins, taken *Chrysanthemum* flowers, and due to their extensive use, are frequently detected in the global environment as well as food products (Wongmaneepratip et al., 2022). Some studies proved that pyrethroids have an influence on oxidative stress, through the modification of DNA, RNA, proteins, lipids, and carbohydrates (Hołyńska-Iwan & Szewczyk-Golec, 2020). Furthermore, pyrethroids have been proven to bioaccumulate in humans, due to their lipophilicity (Aznar-Alemany & Eljarrat, 2020). Bifenthrin and permethrin are representatives of this subclass of pesticides, and according to the World Health Organization (WHO), are listed as moderately hazardous (Class II) (WHO, 2019).

Furthermore, other pesticide residues such as dicarboximide fungicide procymidone also cause great risks to the environment and human health, due to its extensive use (Zhang et al., 2022). In this sense, some pesticides, such as acaricide bromopropylate, were banned in European agriculture due to their high lipophilic properties and persistence (El Agrebi et al., 2020).

Hence, in order to control pesticide levels and safeguard consumers from potential health risks, the European Commission established maximum residue levels (MRLs) for pesticides in baby food (European Commission, 2006). The MRLs for pesticides in processed baby food have been set at 10 μg kg^{-1}. Additionally, lower maximum residue limits (MRLs) were established for particular pesticides, such as cadusafos (6 μg kg−1). The Directive 2006/125/EC also determines which pesticides are not authorized for use on crops intended for baby food (European Commission, 2006).

Polycyclic aromatic hydrocarbons (PAHs) refer to important contaminants composed of carbon and hydrogen atoms arranged in ring structures. They are organic compounds that originate at temperatures of 500–700 °C from the incomplete combustion or pyrolysis of organic matter. Their characteristics are determined by the dimensions (quantity of carbon atoms) and configuration (pattern of ring linkages) of each individual

molecule.(Jinadasa et al., 2020; Petrarca & Godoy, 2018; Yebra-Pimentel et al., 2015). Thus, molecules containing more than 5 aromatic rings are categorized as "heavy" PAHs, while those with fewer than 5 rings are denoted as "light" PAHs. (Sadowska-Rociek & Surma, 2021).

PAHs are present in the atmosphere, soil, water, and a variety of food items. As a result, human exposure to PAHs primarily occurs through the consumption of food. These compounds are primarily formed during thermal food processes like drying, smoking, grilling, roasting, and frying, as well as due to environmental pollution (Bansal et al., 2017; Sadowska-Rociek & Surma, 2021; Yebra-Pimentel et al., 2015).

Several PAHs have been proven to have toxic, mutagenic, and carcinogenic properties, because of their potential to bind to cellular proteins and DNA, posing a great threat to human health (Bansal et al., 2017; Kim et al., 2013). Evidence suggests that certain compounds, such as benzo[a]pyrene (BaP), prompt the development of digestive-tract tumors in experimental animals (IARC, 2010). As stated by the International Agency for Research on Cancer (IARC), benzo[a]pyrene (BaP) is recognized as one of the most hazardous PAHs, and it has been categorized as Group 1, signifying its status as a human carcinogen. Additionally, dibenz[*a,h*]anthracene (DBahA) as probably carcinogenic (group 2A), and benz[*a*]anthracene (BaA), benzo[*b*]fluoranthene (BbFA), benzo[*k*]fluoranthene (BkFA), chrysene (CHR) and indeno[*1,2,3-cd*]pyrene (IP) as possible carcinogenic to humans (group 2B) (IARC, 2022).

The CONTAM Panel (Scientific Panel on Contaminants in the Food Chain) of European Food Safety Authority (EFSA), based on the currently available data relating to occurrence and toxicity, that a combination of four distinct substances (PAH4 - BaA, CHR, BbFA and BaP) or eight specific substances (PAH8 - BaP, CHR, BaA, BbFA, BkFA, benzo[*ghi*]perylene (BghiP), DBahA, and IP) would be the most appropriate indicators of PAH in food. EFSA also concluded that PAH8 not offering much added value compared to PAH4 (European Food Safety Authority, 2008). Thus, in the European Union, the existing law recommends using the PAH4 sum as a PAH marker in food (European Commission, 2011). In baby foods, maximum levels have been established for PAHs, including $1\ \mu g\ kg^{-1}$ for BaP individually or combined with BaA, BbFA and CHR (European Commission, 2011).

The methods of extracting PAHs are significantly influenced by the characteristics of the food substances. As a result, numerous analytical techniques have been devised to accurately measure the minute concentrations of PAHs present in different food items (Bansal et al., 2017). Different approaches for extraction, separation, and identification have reported. Traditionally, Historically, the extraction of PAHs from food items has followed a three-step

approach, encompassing saponification, liquid-liquid extraction (LLE), and subsequent purification using column chromatography or solid-phase extraction (SPE) (Plaza-Bolaños et al., 2010). Nonetheless, as noted by Martinez et al. (2004), there have been documented instances of benzo[a]pyrene (BaP) losses due to partial partitioning into the alcoholic phase during saponification. This phenomenon could potentially have adverse implications for the stability of more delicate compounds.

For instance, in ground coffee, Pressurized Liquid Extraction (PLE) with *n*-hexane/acetone, followed by alkaline saponification, was used for the determination of PAHs, however, a cleaning silica gel step was necessary (Houessou et al., 2006). In infant formula, a method that included extract the fat under alkali conditions, saponification step (to remove the fat content), followed by solid-phase extraction purification step, was developed to analyze the PAH4 content (Cai et al., 2020). The analysis of infant formulas also determined eight PAHs through the utilization of saponification, liquid-liquid extraction, and subsequent purification via solid-phase extraction cartridges (Han et al., 2014).

Consequently, an increasing demand exists for precise identification of trace-level PAHs and pesticides within food items. A basic analysis of existing literature reveals a substantial volume of investigations conducted by various researchers worldwide, focusing on the extraction and analysis of pesticides and PAHs. However, more recently, the method described as quick, easy, cheap, effective, rugged, and safe (QuEChERS) has demonstrated numerous benefits for analyzing pesticides in diverse matrices (Musarurwa et al., 2019). Furthermore, as a substitute for conventional extraction approaches, this paper reports techniques of clean-up step carried out after QuEChERS extraction for the determination of PAHs in baby foods by GC.

2. **Material and methods**

2.1 *Material and reagents*

Analytical standards (purity ≥ 99.8%) of bifenthrin, bromopropylate, permethrin, and procymidone, were obtained from Agilent (Santa Clara, CA, USA). Acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*k*]fluoranthene, benzo[*b*]fluoranthene, benzo[*a*]pyrene, indeno[*1,2,3-cd*]pyrene, dibenz[a,h]anthracene and the deuterated internal standard (chrysene-d12), all with a standard purity of > 98%, were obtained from Supelco (Bellefonte, PA, USA), and were prepared in acetonitrile or acetone, at 1000 μg mL^{-1}. The multi-analyte working standard

solution at a concentration of 1 μg mL^{-1} was prepared by diluting the stock solution with acetone. All standard solutions are stored at –18ºC.

Water (LC-MS grade) was supplied by Supelco (Darmstadt, Germany). Acetone was acquired from Fluka (St. Louis, MO, USA) and acetonitrile, LC MS grade, was acquired from Honeywell, (Morriston, NJ, USA). Ethyl acetate (purity ≥ 99.8%, HPLC grade) was purchased from Chem-Lab (Zedelgem, Belgium). Hexane (HPLC grade) were acquired from Fisher Scientific (Waltham, Massachusetts, USA). Deionized water was obtained from a Milli-Q reagent water system (Millipore, Milfold, MA, USA).

The filters (0.2 μm nylon syringe) and C18 sorbent were acquired from Agilent Technologies (Santa Clara, CA, USA). Z-Sep+ sorbent was purchased from Supelco (Bellefonte, PA, USA). Florisil and PSA sorbents were acquired from Scharlab (Barcelona, Spain). Sodium chloride and anhydrous magnesium sulfate were purchased by Sigma-Aldrich (St. Louis, MO, USA).

For HRMS analyzer calibration, perfluorotributylamine from Thermo Fisher Scientific (San Jose, CA, USA) was employed. The extraction procedure involved the utilization of an analytical balance PX124 (Nänikon, Switzerland), a vortex mixer brand Velp Scientifica (Usmate, Italy), and centrifuge Consul 21 high-volume (Madrid, Spain).

2.2 GC-Q-Orbitrap-MS Parameters

2.2.1 GC-amenable pesticides

For the separation of the target compounds, a Trace 1310 GC system was used. A TriPlus RSH autosampler (Thermo Scientific™) was employed. A capillary column Varian VF-5 ms (30 m × 0.25 mm, 0.25 μm film thickness) provided by Agilent Technologies (Santa Clara, CA, USA) were used for the chromatography analysis. Helium (99.999%) was employed as carrier gas at a constant flow rate of 1 mL min^{-1}. The GC system was coupled to a Q-Exactive Orbitrap (Thermo Fisher Scientific, Bremen, Germany). Electron ionization mode (EI) at -70 eV was selected. A range of m/z 30 to 500 (full scan mode) was set. The injector temperature was set at 250 °C. The injector split ratio was set at 20:1 when the instrument was in standby mode. Upon inserting the syringe into the injector (with an injection volume of 2 μL), the splitless mode was activated for a duration of 2 minutes. Subsequently, the split valve was opened at a flow rate of 50 mL min^{-1} for the purpose of cleansing the glass liner and preventing any carry-over effects. The flow rate was then reduced to 20 mL min^{-1} after 2 minutes. During the analysis, the septum purge was 5 mL min^{-1}. The total running time was 23 min. The injection

volume was 2 µL. First, the column temperature was kept at 40 °C for 2 min and, it was increased at 20 °C min^{-1} to 310 °C, and this temperature was kept for 8 min. Both the transfer line and the ionization source were established at a temperature of 250 °C. A 5-minute filament delay was implemented to safeguard the instrument from potential damage. External calibration mode was employed for result acquisition, and subsequent data processing was conducted using Xcalibur™ version 4.3.73. (Les Ulis, France). For quantification purposes and to perform suspect screening, TraceFinder 5.1 (Thermo 183 Fisher Scientific) was applied.

2.2.1 GC-amenable PAHs

In general, the same conditions used for the separation of the target compounds in the pesticide analysis were used in the PAHs analyzes. However, for this analysis, the injector was comprised by a single taper liner of 78.5 mm × 4 mm ID (Thermo Fisher Scientific). Liner performed hot spitless injections of 1 µl, at 280 ºC, and 1 min spitless time. Furthermore, the column temperature was kept at 50 °C for 1 min and, it was increased at 20 °C min^{-1} to 170 °C. Posteriorly, it was increased at 10 °C min^{-1} to 310 °C, and this temperature was kept for 8 min.

To identify the distinctive ions associated with each compound, a portion of each standard solution (100 µg L^{-1}) was introduced into the system. The spectral library NIST 2.0 (National Institute of Standards and Technology) was utilized to determine the ions relevant to each analyte. Details encompassing elemental composition, log Kow, retention time, theoretical mass, and error of the targeted pesticides and PAHs included in this study are shown in **Table 1.**

2.2 *Sampling*

Fifty samples of commercial baby food products originating from various manufacturers in Brazil were procured in Campinas, São Paulo, Brazil, during the 2021 year, in the period of March to April. These samples were categorized into two distinct groups: one with products containing vegetables and/or meat, and another consisting of fruit and other components like cereals. The samples were maintained and analyzed directly from their original packaging, ensuring protection against light exposure. A temperature of 20 ºC was adopted to conserve the samples, which were presented in plastic bags (99 g each) or either glass jars (ranging between 115 g and 170 g each).

Table 1. Exact mass database including, log K_{ow}, retention time windows (RTWs), theoretical accurate masses, molecular formula, and fragments of the detected ions of target PAHs and pesticides determined by GC-Q-Orbitrap- HRMS.

	Precursor ions (quantifier ions)				Fragment ions (qualifier ions)			
Compound	**Log *Kow* [a]**	**Molecular formula**	**Theoretical mass (*m/z*) [b]**	**Mass error (ppm)**	**Theoretical mass (*m/z*)**	**Mass error (ppm)**	**Molecular formula**	**RT [c]**
PAHs								
Acenaphthylene	3.93	$C_{12}H_8$	152.06202	-0.23	126.04649	-0.32	$C_{10}H_6$	12.22
Acenaphthene	3.92	$C_{12}H_{11}$	153.06977	-0.17	154.07752	-006	$C_{12}H_{11}$	12.61
Fluorene	4.18	$C_{13}H_9$	165.06982	-021	166.07757	-0.42	$C_{13}H_{10}$	13.82
Phenanthrene	4.46	$C_{14}H_{10}$	178.07775	-0.45	152.06222	-0.34	$C_{13}H_7$	16.03
Anthracene	4.45	$C_{14}H_{10}$	178.07775	-0.61	152.06222	-045	$C_{12}H_8$	16.16
Fluoranthene	5.16	$C_{16}H_{10}$	202.07763	-0.54	101.03864	-0.42	C_8H_5	18.72
Pyrene	4.88	$C_{16}H_{10}$	202.07763	-0.35	101.03864	-0.27	C_8H_5	19.21
Benz[*a*]anthracene + Chrysene	5.76/5.73	$C_{18}H_{12}$	228.09334	-0.18	113.03865	-0.49	C_9H_5	21.86
Benzo[*k*]fluoranthene + Benzo[*b*]fluoranthene	6.11/5.78	$C_{20}H_{12}$	252.09339	-0.28	125.03863	-0.37	$C_{10}H_5$	24.05
Benzo[*a*]pyrene	6.13	$C_{20}H_{12}$	252.09338	-0.24	126.04642	-0.35	$C_{10}H_6$	24.54
Indeno[1,2,3-cd]pyrene	6.70	$C_{22}H_{12}$	276.09390	-0.47	138.04695	-0.45	$C_{11}H_6$	26.23
Dibenz[*a,h*]anthracene	6.50	$C_{22}H_{14}$	278.10955	-0.28	139.05478	-0.54	$C_{11}H_7$	26.32
Pesticides								
Bifenthrin	6.60	$C_{14}H_{13}$	181.10118	-0.18	166.07770	-0.19	$C_{13}H_{10}$	15.01
Bromopropylate	5.40	$C_{13}H_9Br_2O$	338.90147	-0.19	182.94400	-0.05	C_7H_4BrO	15.13
Permethrin	6.10	$C_{13}H_{11}O$	183.08044	-0.03	163.00758	-0.01	$C_7H_9Cl_2$	16.03
Procymidone	3.14	$C_{13}H_{11}Cl_2NO_2$	283.01614	-0.01	96.05697	-0.05	C_6H_8O	13.42

[a] extracted from the NIH PubChem (EURL, 2021); [b] *m/z*: mass-to-charge ratio; [c] RT: Retention time (minutes).

2.3 *Sample extraction (QuEChERS-based method)*

First, a mass of five grams from a well-mixed infant food sample was precisely measured and placed into a 50 mL conical centrifuge tube (polypropylene). Subsequently, 10 mL of acetonitrile was introduced, and the mixture was subjected to vortexing for a duration of 1 min. After that, 4 g of $MgSO_4$ and 1 g of NaCl were supplemented to the mixture, which was again subjected to vortexing for another minute. The resultant mixture was then centrifuged at a force of 3061×g for a span of 10 min.

For the analysis of PAHs, 5 milliliters of the supernatant obtained during the initial step were carefully moved into a 15 mL conical centrifuge tube (polypropylene) containing 0.25 g of PSA, 0.25 g of C18, 0.25 g of florisil, and 0.4 g of $MgSO_4$, then the mixture was subjected to vortexing for 1 minute followed by centrifugation at 3061×g for 10 min. Next, 3 mL of the extract was gathered and evaporated to dryness using a nitrogen stream. Ultimately, the remaining substance was reconstituted in 500 µL of ethyl acetate. The extracts were filtered using a 0.2 µm nylon syringe filter, with 10 µL subsequently utilized for GC analysis.

However, the preparation process for pesticide analysis followed a precedent methodology outlined in the work by Prata et al. (2022), in which, 1.5 mL of the obtained supernatant from the initial step was transferred into a 15 mL conical centrifuge tube, within which 0.03 g of PSA, 0.03 g of C18, and 0.03 g of Z-Sep+. The mixture underwent a 1 min vortexing and was subsequently centrifuged at 3061×g for 10 min. The samples were subsequently filtered using a 0.2 µm nylon syringe filter. Following this, 1 mL of the filtered extract was subjected to evaporation under a nitrogen stream until dryness, after which 1 mL of ethyl acetate solvent was introduced. This resulting solution was then directly injected into the GC system.

2.4 *Analytical performance and method validation*

An internal validation of the methodology was conducted to ensure the credibility of the acquired outcomes. The validation process for the refined technique followed the guidelines outlined by SANTE (SANTE, 2021). Hence, various aspects were comprehensively assessed through selectivity, precision, and accuracy, as well as the determination of limits of detection (LOD) and quantification (LOQ), using a range of representative baby food matrices. Accuracy was gauged through recovery and precision trials, evaluating the repeatability and reproducibility conditions (intra and inter-day precision). The representative "blank" samples

of baby food were subjected to extraction procedures adhering to the ultimately optimized QuEChERS-based methods, specifically designed for the extraction of pesticides and PAHs.

2.5 *Statistical analysis*

The IBM SPSS (version 25.0, IBM Corporation, New York, USA) was utilized to perform the Analysis of variance (ANOVA). A one-way ANOVA was used to analyze all dependent variables. When the requirement of the homogeneity of variances was fulfilled Welch correction was utilized. If equal variances could be assumed, means were compared using Tukeýs. Dunnett T3 was used if equal variances were not assumed. A 5% significance level was performed at all statistical tests.

3. **Results and discussion**

3.1 *Clean-up efficiency and sample preparation method*

Usually, the aim of the extraction step is to separate all analytes of interest from the matrix without co-extractives (Cunha et al., 2007). To test the capability of PAHs extraction from a sample and the least possible extraction of interfering components, a representative baby food, constituted of a mixture of vegetables and chicken was submitted to three sets of procedures: (i) QuEChERS combined with low temperature followed by dispersive solid-phase extraction (d-SPE) clean up, (ii) QuEChERS with d-SPE clean up and (iii) QuEChERS combined with saponification.

For the first step of sample preparation (QuEChERS), a weight of 5 g of baby food sample was introduced into a 50 mL conical centrifuge tube (polypropylene) and added 10 mL of acetonitrile. The mixture was subjected to vortex mixing for 1 min. In the next step, 1 g of NaCl and 4 g of MgSO4 were introduced and the mixture was vortexed for 1 min. The conical centrifuge tube was centrifuged at 3000 x g for 10 min.

The freezing stage was used to verify the cleaning efficiency for fat removal by precipitation. After the extraction step by QuEChERS, the solution was freezing at a temperature of -22 ± 2º C for 1 hour (QuEChERS combined with low temperature and d-SPE clean up). At this temperature, only the fat content is frozen out. Thus, the extracted organic solvent could be separated and used for the next sample preparation step. The next step, d-SPE was performed using adsorbent salts such as PSA, C_{18}, and florisil.

In previous studies, PSA and C18 sorbents used in clean-up stage did not affect the recovery of analyzed PAHs (Petrarca & Godoy, 2018). Furthermore, florisil is recommended

for matrices with high pigment content in the QuEChERS method(Słowik-Borowiec et al., 2022).

For this, in the freezing-out extract (5 mL) were added 0.25 g of PSA, 0.25 g C18, 0.25 g of florisil sorbent, and 0.4 g of MgSO4 were added to the freezing-out extract (5 mL) followed by vortex agitation for 1 min and centrifugation at 3000 × g for 10 min. Then, stream nitrogen was utilized to evaporate 3 mL of clean-up extract to dryness. Finally, 75 µL of acetonitrile was introduced to dissolve the residue.

For the second procedure (QuEChERS with d-SPE), the obtained extract without freezing was transferred directly to d-SPE step, using the same conditions established in the first procedure.

To evaluate the use of saponification as a possible cleansing step, QuEChERS extraction method followed by saponification was also evaluated. For this, 3 mL of extract from QuEChERS was subjected to the addition of 1 ml potassium hydroxide (2 M in methanol). The obtained solution was shacked 30 s on vortex. The mixture was extracted was mixed with 1 mL of n-hexane, vortexed for 30 seconds, and subsequently centrifuged at 3000 x g for 5 minutes in two consecutive cycles. A stream of nitrogen was used to evaporate the combined upper organic layers (n-hexane) to dryness. Finally, the residue was dissolved in 75 µL of acetonitrile.

The gravimetric measurements were utilized to assess the removal of co-extracted matrix components (Sapozhnikova & Lehotay, 2013). The trial was carried out in triplicates. Following the purification phase for the three distinct methods under examination, the obtained extracts were carefully transferred to glass tubes with known weights. These tubes had been preheated to 100°C for a minimum of 1 hour to eliminate any moisture content. Subsequently, using a stream of nitrogen, the extracts were evaporated to dryness. Afterward, the glass tubes containing the desiccated residues were subjected to an additional 100°C heating for at least 1 hour and then weighed. The efficacy of removing co-extractives was assessed by calculating the variance in weight of co-extractives before and after the purification processes, using the equation (1) (Sapozhnikova & Lehotay, 2013):

$$\text{Matrix co} - \text{extractive removal efficiency (\%)} = \frac{[(\text{co} - \text{extractives weight before clean} - \text{up}) - (\text{co} - \text{extractives weight after clean} - \text{up})}{(\text{co} - \text{extractives weight before cleanup})]} \; X\; 100$$

It is worth mentioning, that for the QuEChERS procedure, acetonitrile was used as an extraction solvent. According to (Sapozhnikova & Lehotay, 2013), the use of acetonitrile as a solvent enables the concurrent extraction of both non-polar and moderately polar analytes in

a single extraction procedure. Therefore, it will extract larger molecular weight PAHs (≥ 4 rings) and the lower molecular weight PAHs (< 3 rings) since PAHs are soluble in lipid and the aqueous solubility decreases with increasing molecular size (Yebra-Pimentel et al., 2015). Moreover, the use of acetonitrile as an extraction solvent allows for the reduced quantity of co-extractive compounds (fat and lipids) in contrast to the utilization of non-polar solvents like *n*-hexane or ethyl acetate (Sapozhnikova & Lehotay, 2013).

As a result, the combination of low temperature with d-SPE clean up resulted in an expressive reduction of 89% in the co-extractives, demonstrating the efficiency of technique. On another hand, the use of only d-SPE technique as a cleaning step after QuEChERS extraction resulted in a reduction of 86% in the co-extractives. Thus, considering the addition of 1 hour in the sample preparation with the storage of the extract after QuEChERS at low temperature to precipitate fats, the addition of only 3% in the elimination of co-extracts of sample was not very considerable. There were no statistically significant differences between the two procedures ($p>0.05$) (oneway ANOVA). The procedure using saponification, presented the worst reduction in the % of co-extractives (67%) (**Fig. 1A**). Whereas the saponification is used in order to reduce the lipidic content (e.g. triacylglicerols), using KOH solutions in methanol solution, it is necessary to use another step to remove other interferents, duo to the complexity of baby food samples, including carbohydrate and proteins ingredients.

The gravimetric measurements were also used to evaluate the co-extractive removal efficiency of d-SPE using various sorbents. For this, obtained extracts for QuEChERS were submitted to seven different procedures of d-SPE, using PSA, C_{18} and Florisil sorbents (0.25 g of each sorbent plus and 0.4 g of $MgSO_4$); all procedure was carried out in triplicate). According to the **Fig. 1B**., the use of C18 in d-SPE The clean-up process led to a 60% decrease in the amount of co-extractives present in the extract, in comparison to the crude extract without undergoing clean-up. Indeed, the C18 sorbent is commonly utilized for purifying samples containing a significant concentration of fatty acids, duo to the elongated hydrocarbon chain structure, considered as a non-polar (El Husseini et al., 2018; Munaretto et al., 2013). A great effect of C_{18} sorbent on clean-up efficiency also was observed for baby food constituted by fruit purées, rice flour, starch and heavy cream, with an approximate reduction of 53% in the quantity of co-extractives (Petrarca & Godoy, 2018).

In this study, florisil (magnesium silicate) and PSA, an ion exchange sorbent (Muhammad et al., 2020) in d-SPE clean-up resulted in a 24% and 30% reduction in the content of co-extractives remaining, respectively. The PSA sorbent exhibits a dual capacity, functioning

both as a polar phase and a weak anion exchanger. This enables it to eliminate fatty acids, sugars, and other co-extractive components such as organic acids, and anthocyanin pigments from the matrix (Rutkowska et al., 2018). On the other hand, the florisil sorbent is used for polar interferences (Zhong et al., 2019), which explains why it is less efficient to remove co-extracts when compared to PSA. As a great result, the utilization of PSA, C18, and florisil sorbents in the d-SPE process led to a notable decrease of 76% in the presence of co-extractives, in comparison to the unprocessed raw extract. This underscores the effectiveness of the d-SPE technique in achieving efficient cleanup.

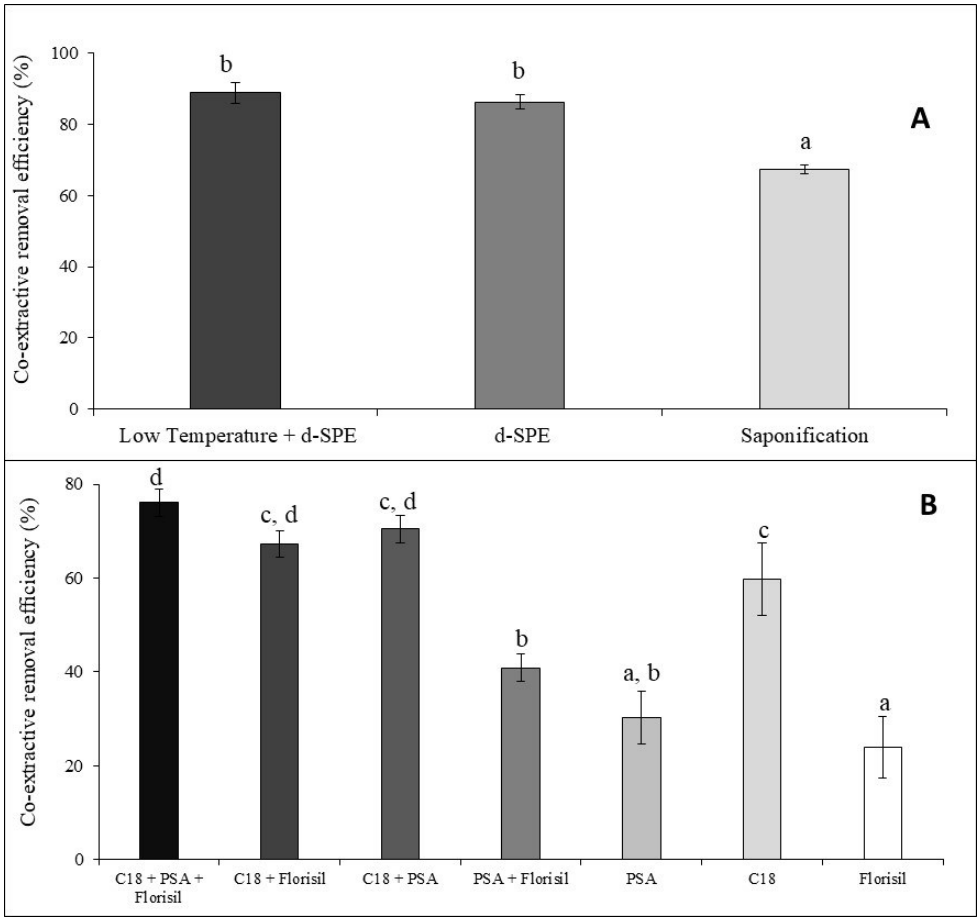

Fig. 1. Co-extractive removal efficiency obtained after three QuEChERS based different procedures (*%, n=3*) (A); Co-extractive removal efficiency of d-SPE with different sorbents (*%, n=3*) (B). Different small letters mean statistically significant differences, at a 5% significance level.

The blend of PSA and C18 also led to a substantial 70% decrease in co-extractives. The efficiency of co-extractives removal through d-SPE for catfish extracts using the C18 + PSA treatment exhibited an 80% removal rate by weight (Sapozhnikova & Lehotay, 2013).

However, to ensure greater efficiency, it was decided to use the three combined adsorbents for extraction protocol of PAHs in baby foods.

The next step was to investigate the influence of these sorbents amounts after removing the interferents content, in the decrease the recovery values. Recovery study was conducted using a representative baby food spiked at 5 μg kg^{-1} (normalized to the internal standard). For this purpose, three different amounts of PSA, C18, and florisil sorbents were studied (0.10, 0.25, and 0.40 g). The amounts of sorbent chosen to be used in the method were based mainly on the recovery obtained for the compound BaP, as it is the most nocive PAH. As result, lower recovery value was observed with the use of a smaller amount of sorbents (0.10 g) for BaP (39%). On the other hand, similar recoveries were observed for the sorbent amounts of 0.40 and 0.25 g (93 and 95%, respectively). Thus, it is possible to verify that 0.25 g of each sorbent, is the best option among the studied values (considering the recoveries values e and lower cost due to less use of sorbents).

Furthermore, sample preparation performed in this study for the determination of pesticides in baby food was based on previously validated and optimized method described by Prata et al. (2022). In addition, the effectiveness of the QuEChERS method has been evaluated numerous times for the analysis of pesticides in various types of food (Makni et al., 2022; Theurillat et al., 2021).

3.2 *In-house validation and matrix effect evaluation*

3.2.1. Method for pesticides

The analytical performance of QuEChERS-based method was assessed by in-house validation, evaluating the performance criteria indicated in Section 2.4.

The linearity of the method was evaluated by preparing calibration curves of standards in solvent and two matrix-matched (meat and vegetables baby food, and fish and vegetables baby food). For all targeted compounds a good linearity was obtained throughout the studied range (2 - 100 μg kg^{-1}). The coefficient of determination (R^2), exceeding 0.9916, was obtained in both analyzed sample matrices (**Table 2**).

Matrix effects depended on each individual pesticide, in addition to the matrix tested. The slopes acquired from the calibration curves using matrix-matched standards for both tested baby foods were contrasted with those derived from solvent-based standards. The results can be observed in **Table 2**.

Table 2. Method performance characteristics for the determination of pesticides in baby food.

Compounds	LOD (µg kg^{-1})	LOQ (µg kg^{-1})	ME (%)	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*)		
				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}
Meat and vegetables based baby food											
Pesticides											
Bifenthrin	1.0	2.0	122	0.9929	0.9983	120 (109)	102 (109)	112 (116)	17 (20)	14 (16)	16 (15)
Bromopropylate	1.0	2.0	127	0.9930	0.9964	96 (96)	96 (95)	103 (104)	15 (12)	1 (7)	16 (17)
Permethrin	4.0	10.0	73	0.9917	0.9971	n.a.	100 (100)	119 (119)	n.a.	9 (8)	4 (12)
Procymidone	4.0	10.0	19	0.9944	0.9986	n.a.	103 (111)	110 (107)	n.a.	3 (13)	2 (14)
Fish and vegetables based baby food											
Bifenthrin	1.0	2.0	76	0.9929	0.9952	117 (120)	106 (111)	120 (119)	6 (6)	9 (9)	9 (8)
Bromopropylate	0.4	2.0	82	0.9930	0.9968	89 (95)	100 (104)	106 (109)	18 (16)	18 (14)	9 (11)
Permethrin	0.1	2.0	43	0.9916	0.9957	92 (97)	95 (99)	87 (94)	5 (8)	4 (6)	10 (12)
Procymidone	0.4	2.0	-5	0.9940	0.9967	103 (103)	93 (94)	89 (92)	6 (6)	5 (15)	4 (7)

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] ethyl acetate solvent.

Negligible matrix effect was obtained for procymidone pesticide for both studied matrices (equal to or lower than ±20%). On the other hand, values higher than 20%, indicating a strong matrix enhancement, was observed for the other compounds for both matrices. A strong matrix effect (ME) is observed when |ME| exceeds 50%, while a moderate matrix effect is indicated by values ranging from 20% to 50% for |ME|. If |ME| is less than or equal to 20%, it is classified as having no matrix effect.t (Ferrer et al., 2011).

The compounds exhibited limits of quantification (LOQs) spanning from 2 to 10 μg kg^{-1} for meat and vegetable baby food, and 2 μg kg^{-1} for fish and vegetable baby food. Additionally, the LOQ values for pesticides remained below the maximum residue limit (MRL) permitted for pesticide traces in baby foods. These attained levels were satisfactory in ensuring the surveillance of pesticides at the MRL set by the European Commission for baby foods (10 μg kg^{-1}) (European Commission, 2006).

The effectiveness of the extraction method was evaluated by intra- and inter-day conditions measured by the mean recovery (%). For enhancing interaction between the analytes and the matrix, a representative "blank" baby food sample was fortified with the mix of all standard solutions at three different concentrations (2, 10, and 100 μg kg^{-1}) prior to the initiation of the extraction procedure (1 h). Acceptable mean recoveries were attained for both representative baby food matrices, demonstrating recovery rates within the range of 87% to 120%. Recoveries were within the ranges fixed by the SANTE/2021/11312 guidance (70–120%) (SANTE, 2021).

The RSD values for the intra-day study were in the range 1 to 17 % for meat and vegetables baby food and between 4 to 18 % for fish and vegetables baby food. During the assessment of inter-day precision, the relative standard deviation (RSD) values fell within the range of 7% to 20% for meat and vegetable baby food, and between 6% to 16% for fish and vegetable baby food. These values obtained are in accordance with the established by SANTE/2021/11312 guidelines, where RSD values ≤ 20% are acceptable (SANTE, 2021).

The recovery was utilized to evaluate the extraction efficiency of the validated method before described. For this, two blank baby food samples were also analyzed (fruit and vegetable), guaranteeing that the matrices chosen are representative of all distinct matrices. The procedure outlined in Section 2.4 was used to extract the baby foods after spiking them with working standard solutions at levels of 10 and 100 μg kg^{-1}. In both analyzed samples, great recoveries were obtained for all analysed pesticides (between 77-113%) for both matrices **(Fig.**

2). These results show the feasibility of the method to analysis of pesticides residues in baby food.

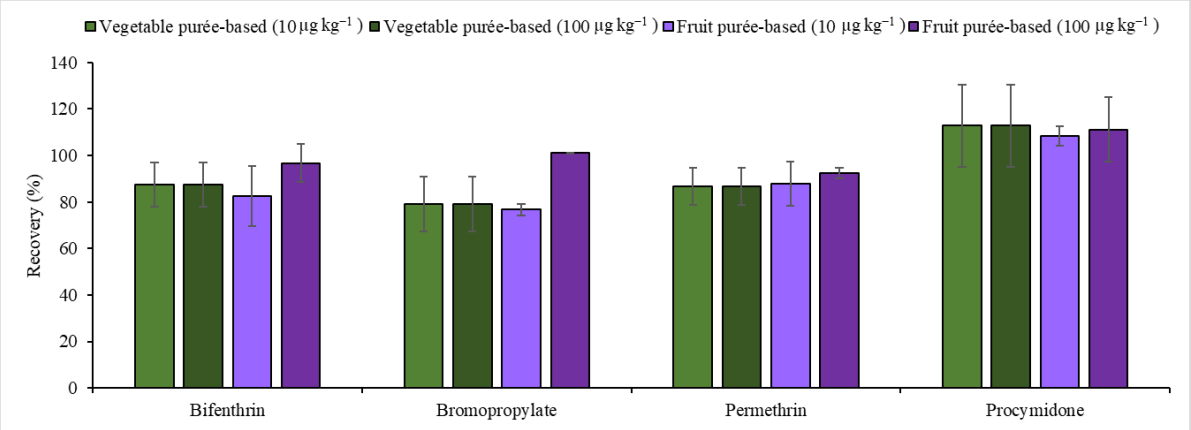

Fig. 2. Evaluation of the recovery (%) for pesticides in two spiked matrices at 10 and 100 µg kg^{-1} levels: (a) pea and broccoli purée-based baby food and (b) banana, apple, peach, orange, and apricot based baby food (*n = 3*).

3.2.1. Method for PAHs

The performance characteristics of the method were assessed using homemade baby food labeled as "blank," in which the analyzed substances were not found. The representative baby food (commercial) composed of fish and vegetables, meat and vegetables, fruits, and vegetables were used in trueness (recovery), precision studies and to obtain matrix-matched calibration curves used for quantification purposes. Analytical selectivity was demonstrated on the basis of the ability of the method to accurately detect the target compounds even when co-extracted matrix components were present. (Petrarca & Godoy, 2018). It is highly important because, if the analytical method is not free from the interferences, all other performance parameters are less reliable (Raposo & Ibelli-Bianco, 2020). No interfering peaks were detected in the retention times of the PAHs, ensuring a clear and definitive identification of the analyzed compounds.

By the peak area ratio of analyte/IS the calibration curves were constructed against the concentration levels. The determination coefficients (R^2) consistently exceeded 0.99 for all PAHs, confirming the robustness and reliability of the analytical method. High analytical sensitivity was achieved with LODs of 0.05 µg kg^{-1}. The LOQs were set to 0.1 µg kg^{-1} for the different compounds and blank samples used.

Under repeatability and reproducibility of the method, the relative standard deviations (RSD) ranged from 1-20% and 5-20%, respectively.

The same samples were also used to determine the recovery of PAHs. The mean recovery was excellent, varying between 71-120%. The results can be observed in **Table 3**. In accordance with the performance criteria set forth in Commission Regulation (EU) No. 836/2011, methods of analysis for BaA, BaP, BbFA, and CHR in foodstuffs are considered acceptable when the recoveries fall within the range of 50% to 120% (European Commission, 2011).

Regarding the matrix effect, several factors may have contributed, taking into account the nature of the analyte, the type of matrix, and the applied sample preparation. It was observed a significant difference between the solvent and matrix-matched calibrations. An enhancement of the chromatographic signal was observed for matrix-matched calibrations probably due to an improvement of the volatilization conditions into the GC injection. This has been previously reported by other authors (Petrarca & Godoy, 2018).

3.3 *Analysis of commercial samples of baby foods*

Both validated methods were applied to real baby food samples, commercialized in Brazil. Regarding the analysis of GC-amenable pesticides, the summary of the results can be found in **Table 4.** Three different pesticide residues were quantified or detected in 24% of analyzed samples at a concentration ranging below LOQ to 4.1 μg kg^{-1}. The pesticide permethrin although it was detected at low concentrations (< LOQ), it was found in 22% of the analyzed samples. For fruit-based baby food, in 3 of the samples permethrin was detected, being apple the main ingredient of the formulation. This insecticide is not authorized to be used in apples (ANVISA, 2021). The fungicide procymidone was also found in an apple purée-based baby food sample. However, its use is authorized for this crop (ANVISA, 2021).

The bean soup baby food sample, containing beans, chicken, pasta, squash, carrot, and kale, exhibited the highest pesticide levels, with the detection of bifenthrin at a concentration of 2.6 μg kg^{-1}. This elevated pesticide content was especially noticeable in baby food samples incorporating meat and vegetables. In baby food composed mainly of maize flour, chicken, and yam, bifenthrin was detected at levels of 4.1 μg kg^{-1}. In **Fig. 3**, it can be observed the XIC of bifenthrin in bean soup. The pyrethroid pesticide bifenthrin was also detected in vegetable purée and meat-vegetable based purée baby food marketed in Romania at a level of up to 57 μg kg^{-1} (Dobrinas et al., 2011)

Table 3. Method performance characteristics for the determination of PAHs in baby foods.

Fish and vegetables based baby food					
Compound	**Linearity, R^2 (range of 0.1–1 µg kg^{-1})**	**Recovery (%)**		**Precision (RSD %) Intra-day (n=3) (Inter-day (n=6))**	
		0.1 µg kg^{-1}	**1.0 µg kg^{-1}**	**0.1 µg kg^{-1}**	**1.0 µg kg^{-1}**
Acenaphthylene	0.996	86	85	17 (19)	5 (14)
Acenaphthene	0.999	93	84	16 (19)	4 (7)
Fluorene	0.997	105	90	8 (13)	2 (13)
Phenanthrene	0.998	109	91	4 (9)	4 (8)
Anthracene	0.997	71	89	5 (12)	6 (15)
Fluoranthene	0.993	93	92	16 (17)	8 (11)
Pyrene	0.993	117	92	16 (19)	4 (6)
Benz[*a*]anthracene + Chrysene	0.999	100	95	3 (10)	7 (13)
Benzo[*k*]fluoranthene + Benzo[*b*]fluoranthene	0.994	115	92	10 (16)	5 (8)
Benzo[*a*]pyrene	0.991	110	83	10 (19)	7 (13)
Dibenz[*a.h*]anthracene	0.998	119	84	8 (14)	5 (10)
Indeno[*1.2.3-cd*]pyrene	0.998	110	79	9 (18)	13 (17)
Meat and vegetables based baby food					
Compound	**Linearity, R^2 (range of 0.1–1 µg kg^{-1})**	**Recovery (%)**		**Precision (RSD %) Intra-day (n=3) (Inter-day (n=6)**	
		0.1 µg kg^{-1}	**1.0 µg kg^{-1}**	**0.1 µg kg^{-1}**	**1.0 µg kg^{-1}**
Acenaphthylene	0.996	91	96	10 (13)	11 (12)
Acenaphthene	0.995	72	83	7 (15)	7 (12)
Fluorene	0.998	117	105	8 (12)	5 (8)
Phenanthrene	0.992	116	110	11 (14)	11 (13)
Anthracene	0.997	102	103	7 (8)	11 (14)
Fluoranthene	0.997	71	117	10 (16)	6 (13)
Pyrene	0.998	119	120	3 (5)	8 (14)
Benz[*a*]anthracene + Chrysene	0.997	94	78	2 (8)	4 (10)
Benzo[*k*]fluoranthene + Benzo[*b*]fluoranthene	0.996	91	89	10 (12)	13 (16)
Benzo[*a*]pyrene	0.994	92	79	16 (18)	11 (13)
Dibenz[*a.h*]anthracene	0.996	119	102	6 (11)	13 (18)
Indeno[*1.2.3-cd*]pyrene	0.994	120	105	10 (13)	17 (19)

Fruit based baby food					
Compound	**Linearity, R^2 (range of 0.1–1 µg kg^{-1})**	**Recovery (%)**		**Precision (RSD %) Intra-day (n=3) (Inter-day (n=6)**	
		0.1 µg kg^{-1}	**1.0 µg kg^{-1}**	**0.1 µg kg^{-1}**	**1.0 µg kg^{-1}**
Acenaphthylene	0.993	101	77	19 (20)	2 (7)
Acenaphthene	0.997	78	79	7 (12)	5 (12)
Fluorene	0.99	100	117	12 (17)	9 (15)
Phenanthrene	0.996	99	105	3 (6)	4 (8)
Anthracene	0.992	100	104	17 (20)	9 (16)
Fluoranthene	0.991	85	81	7 (15)	2 (10)
Pyrene	0.992	112	95	12 (19)	10 (13)
Benz[*a*]anthracene + Chrysene	0.992	105	97	4 (9)	4 (7)
Benzo[*k*]fluoranthene + Benzo[*b*]fluoranthene	0.992	81	91	16 (18)	5 (14)
Benzo[*a*]pyrene	0.992	84	86	10 (14)	3 (8)
Dibenz[*a.h*]anthracene	0.996	75	87	18 (20)	6 (12)
Indeno[*1.2.3-cd*]pyrene	0.997	75	87	12 (15)	3 (5)
Vegetable based baby food					
Compound	**Linearity, R^2 (range of 0.1–1 µg kg^{-1})**	**Recovery (%)**		**Precision (RSD %) Intra-day (n=3) (Inter-day (n=6)**	
		0.1 µg kg^{-1}	**1.0 µg kg^{-1}**	**0.1 µg kg^{-1}**	**1.0 µg kg^{-1}**
Acenaphthylene	0.996	95	90	10 (13)	2 (8)
Acenaphthene	0.995	113	92	16 (20)	3 (7)
Fluorene	0.992	72	72	5 (7)	9 (13)
Phenanthrene	0.996	89	100	16 (17)	3 (6)
Anthracene	0.998	117	93	17 (20)	3 (9)
Fluoranthene	0.991	90	103	4 (15)	1 (8)
Pyrene	0.997	115	107	6 (9)	7 (13)
Benz[*a*]anthracene + Chrysene	0.997	107	96	1 (5)	3 (9)
Benzo[*k*]fluoranthene + Benzo[*b*]fluoranthene	0.995	100	110	3 (10)	10 (16)
Benzo[*a*]pyrene	0.996	94	86	13 (18)	4 (12)
Dibenz[*a.h*]anthracene	0.996	117	103	9 (17)	2 (6)
Indeno[*1.2.3-cd*]pyrene	0.998	107	102	15 (19)	4 (13)

Table 4. Concentration (μg kg^{-1}) of pesticides detected in different baby food samples.

Sample	Composition[a]	Permethrin	Procymidone	Bifenthrin
	Fruit-based			
1	Apple and plum (plastic bag)	<LOQ	-	-
2	Apple purée (plastic bag)	<LOQ	-	-
3	Apple purée (brand B)	<LOQ	<LOQ	-
4	Pear, banana, and blueberry (plastic bag)	<LOQ	-	-
	Meat and vegetable based			
1	Bean soup	-	-	2.6
2	Creamed corn, chicken, and yam	<LOQ	-	4.1
3	Pasta, meat, and vegetables *	<LOQ	-	-
4	Pasta, meat, and vegetables *	<LOQ	-	-
5	Rice, bean, meat, and vegetables	<LOQ	-	-
6	Spaghetti Bolognese	<LOQ	-	<LOQ
7	Squash, black bean, and chicken breast	<LOQ	-	<LOQ
8	Sweet potato, black bean and chicken	<LOQ	-	<LOQ

<LOQ: Compound detected below LOQ but not quantified; -: Compound not detected; LOQ: 2.0 μg kg^{-1} (considering validation parameter for fish and vegetablesbased baby food); *Different concentrations of each ingredient (same brand); [a] Principal ingredients.

Petrarca et al. (2016) detected the fungicide procymidone in a sample composed of a mixture of banana, strawberry, apple, starch, papaya, rice flour and, yogurt at at a level of 68.8 μg kg^{-1}, largely exceeding the MRL authorized for the EC for pesticides in baby food. As it can be seen in **Table 4**, the same fungicide was detected below of LOQ (2.0 μg kg^{-1}) in apple purée -based baby food.

In Brazil, the fungicide procymidone is permitted for application in apple and various other crops, with a maximum residue limit (MRL) of 2000 μg kg^{-1} as specified by ANVISA (ANVISA, 2021). Procymidone, was also detected in fruit-based baby foods in the Czech Republic (Štěpán et al., 2005).

The investigation focused on the presence of specific PAHs, but none were found in any of the samples. This is in contrast to prior studies where various PAHs had been detected. For instance, in Italy, the average of total PAHs was 11.82 μg kg^{-1} in meat/fish-based baby foods (Santonicola et al., 2017). In general, meat/fish-based baby foods, which were low in fat, presented a lower PAH concentration than those were high in fat (Santonicola et al., 2017). In Iran, the results complied with European regulations, where the sum of PAH4 has been founded at a concentration lower than 1 μg kg^{-1} (Badibostan et al., 2019; Moazzen et al., 2022). Though, BaP has been detected in 80% of baby food samples (Badibostan et al., 2019). BaP has also been detected in infant foods marketed in Poland (Ciecierska & Obiedziński, 2010) and Romanian (Soceanu et al., 2016), with concentrations of less than 0.25 μg kg^{-1} and 0.26 μg kg^{-1}, respectively.

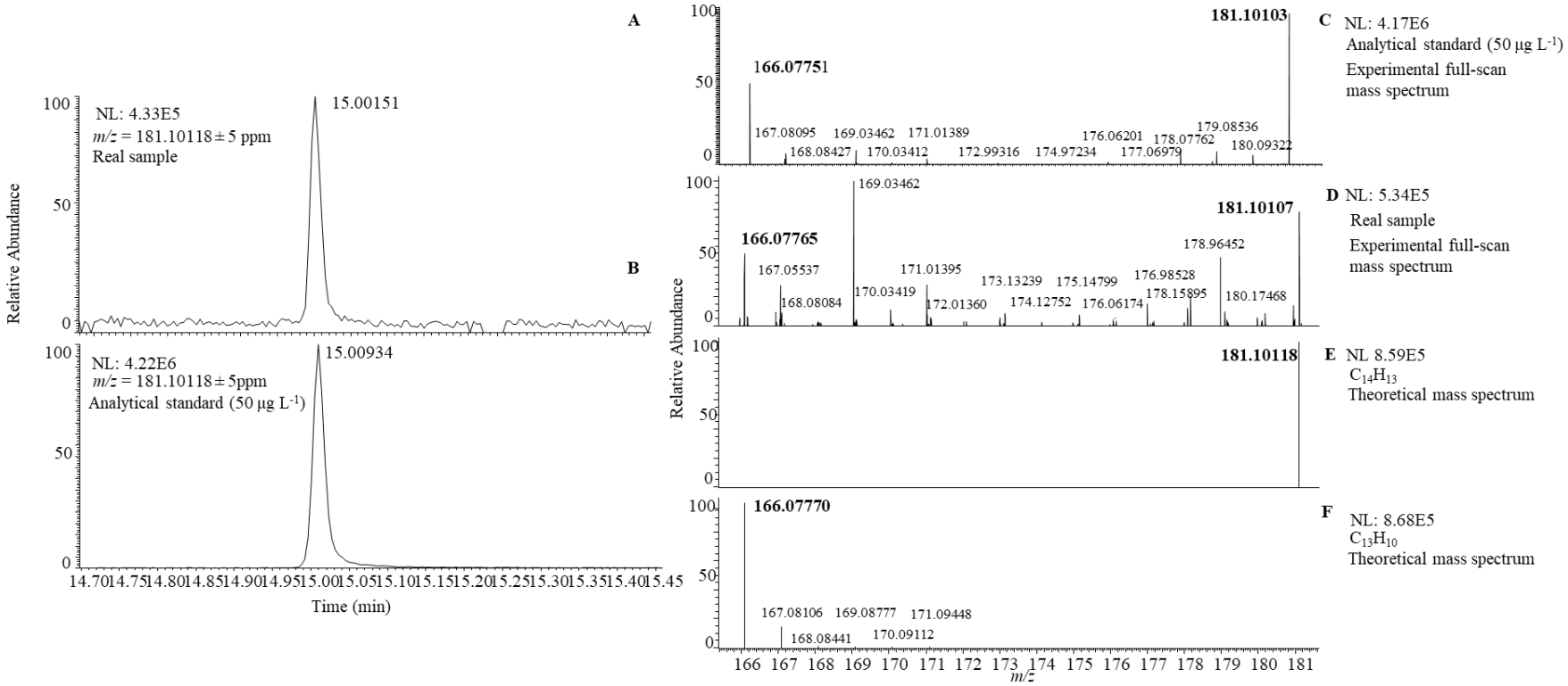

Fig. 3. GC-Q-Orbitrap-MS extracted ion chromatograms of bifenthrin in (a) bean soup -based baby food (2.6 µg kg^{-1}) and (b) analytical standard (50 µg L^{-1}); Experimental full-scan mass spectrum of (c) analytical standard (50 µg L^{-1}) and (d) bean soup -based baby food (2.6 µg kg^{-1}) ; Theoretical spectrum of (e) quantifier ion (*m/z* 181.10118) and (f) qualifier ion (*m/z* 166.07770) for bifenthrin.

4. Conclusions

This study focused on utilizing modified QuEChERS extraction combined with dispersive solid-phase extraction (d-SPE) followed by gas chromatography–high resolution mass spectrometry analysis achieved suitable performance characteristics for determining 14 PAHs and 4 pesticides in baby food. It resulted as a simple and inexpensive way to obtain a lower detection limit for PAHs and pesticides from different chemical classes. The limits of quantifications are according to the MRLs established in the regulation of the European Union for both studied contaminants. The baby food samples acquired from local markets contained low levels of pesticides of different groups. This demonstrates the need for greater oversight of protecting food intended for infants to avoid feeding baby food contaminated even at low concentrations.

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DISCUSSÃO GERAL

O preparo de amostra costuma ser a etapa mais crítica no desenvolvimento de métodos para análise de contaminantes devido à complexidade de diversas matrizes e os baixos níveis em que estes compostos podem estar presentes. A análise de contaminantes em alimentos infantis representam um grande desafio cromatográfico uma vez que são consideradas matrizes complexas os quais são fontes de potenciais interferentes. Assim, a obtenção de um método de preparo de amostra simplificado e fácil, com geração mínima de resíduos e menor número de etapas para reduzir o tempo e as fontes de erro são imprescindíveis durante o desenvolvimento de metodologias.

A técnica de preparo de amostra QuEChERS (*quick, easy, cheap, effective, rugged and safe*) com posterior extração em fase sólida dispersiva (d-SPE - dispersive solid-phase extraction), utilizando amina primária e secundária (PSA – *primary secondary amine*), octadecilsilano (C18 - octadecylsilane) e o sorvente Z-Sep+ (C18 e sílica revestida com dióxido de zircônio) demonstrou ser eficiente na determinação de 21 agroquímicos e 4 aflatoxinas em alimentos infantis. O método QuEChERS apresenta um preparo simples de amostras, reduzindo o número de procedimentos e minimizando tempo e fontes de erro, além de exigir menos análises separadas quando comparado ao método WAHSPE (*water, acetonitrile, and n-heptane as solvents in combination with solid-phase extraction*). O método multi-resíduos alcançou alta sensibilidade de modo a atender o limite máximo de resíduos (LMR) de 10 $\mu g\ kg^{-1}$ estabelecido pela Comunidade Europeia para a presença de agroquímicos em alimentos infantis e 1 $\mu g\ kg^{-1}$ para aflatoxinas em alimentos para bebês estabelecido pela ANVISA. Os resultados obtidos contribuíram com os primeiros dados de avaliação da presença de agroquímicos em alimentos infantis contendo carnes e vegetais no país. Além disso, o uso de *high resolution mass spectrometers* (HRMS) permitiu complementar a análise através de uma abordagem não direcionada (*non-targeted*) para a detecção de outros 2424 compostos como outros agroquímicos e micotoxinas, hormônios, medicamentos veterinários e seus metabólitos utilizando cromatografia a líquido.

Foi elaborado um procedimento de amostragem que se caracteriza por sua simplicidade, eficiência econômica e rapidez. Esse método foi criado com o objetivo de realizar a análise simultânea de furfural, 5-hidroximetilfurfural e 4-hidroxi-2,5-dimetil-3(2H)-furanona em alimentos destinados à alimentação infantil. Essa pesquisa representou um importante marco ao fornecer as primeiras informações sobre a presença de compostos furânicos em produtos alimentares à base de vegetais e/ou carnes disponíveis no mercado brasileiro para

bebês e crianças. Para isso, os alimentos infantis foram extraídos com acetonitrila, seguido de partição com n-hexano. O extrato foi então diluído com água deionizada para um grande volume para minimizar as interferências da matriz. Esta etapa de diluição utilizada no preparo da amostra eliminou a necessidade de procedimentos adicionais de limpeza, tornando o método mais simples e barato. Com esta metodologia, conseguimos alcançar alta sensibilidade analítica e seletividade ao empregar cromatografia líquida com um detector de matriz de diodos.

Frente à alta toxicidade da acrilamida, classificada como "provavelmente cancerígena para humanos" no grupo 2A de acordo com a Agência Internacional de Pesquisa em Câncer (IARC), este composto também foi analisado utilizando uma abordagem simples de preparo de amostra, juntamente com cromatografia a líquido acoplada a espectrometria de massas em tandem, que pode facilmente ser utilizado em análises rotineiras. O preparo de amostra envolveu um processo de extração usando uma mistura de acetonitrila:água:ácido fórmico (69:30:1, v/v/v) em combinação com d-SPE usando alumina. Este processo elimina a necessidade de preparo de amostras com custos elevados e técnicas demoradas como o uso de cartuchos de extração em fase sólida (SPE – *solid phase extraction*), que são normalmente utilizados na análise de acrilamida em amostras de alimentos.

Ainda de acordo com a IARC, outros compostos como os hidrocarbonetos policíclicos aromáticos (HPAs) também são considerados perigosos. O benzo[a]pireno foi classificado como grupo 1 (cancerígeno para humanos). Além disso, dibenz[a,h]antraceno foi classificado como provavelmente cancerígeno (grupo 2A), e benz[a]antraceno, benzo[b]fluoranteno, benzo[k]fluoranteno, criseno e indeno[1,2,3-cd]pireno como possíveis carcinogênicos para humanos (grupo 2B).

A Comunidade Europeia estabeleceu um limite máximo de 1 µg kg–1 para o composto benzo[a]pireno em alimentos infantis, seja de forma individual ou em combinação com criseno, benzo[a]antraceno e benzo[b]fluoranteno, devido à sua significativa importância toxicológica. Logo, é importante que o método de preparo de amostra utilizado apresente características adequadas de desempenho, incluindo alta seletividade e sensibilidade analítica. A extração com QuEChERS demonstrou ter várias vantagens na análise de HPAs frente a extrações tradicionais baseada em metodologia de três estágios, incluindo saponificação, extração líquido-líquido (LLE - *liquid–liquid extraction*) e limpeza por cromatografia em coluna e cartuchos de SPE. QuEChERS combinado com d-SPE utilizando PSA, C18, e florisil, seguido por análise com HRMS – acoplado com cromatografia gasosa alcançou características de desempenho adequadas para determinar 14 HPAs em alimentos infantis comercializados no

Brasil. Os resultados alcançados apresentam os primeiros conjuntos de informações a nível nacional referentes à detecção de substâncias como a acrilamida, derivados furânicos e resíduos de agroquímicos em produtos alimentícios para crianças que contenham carne e/ou vegetais e que estão disponíveis no mercado brasileiro, portanto, é notório a importância dos dados obtidos neste trabalho. Além disso, nos alimentos infantis a base de carne e/ou vegetais, os contaminantes analisados se apresentaram em uma maior concentração e/ou em um maior números de amostras quando comparados aos alimentos infantis a base de frutas. Portanto, os resultados desse trabalho alertam para o monitoramento de contaminantes em diferentes alimentos destinados ao público infantil.

CONCLUSÃO GERAL

Alimentos infantis comercializados no Brasil (popularmente conhecidos como "papinhas") foram submetidos a análises instrumentais utilizando técnicas cromatográficas para avaliar a presença de diferentes resíduos e contaminantes.

Para isso, novos métodos de preparo de amostra foram desenvolvidos/otimizados e então aplicados em diferentes amostras compostas majoritariamente por purê de frutas e/ou compostas por carnes e vegetais. O principal objetivo foi encontrar um método que conciliasse o mesmo preparo de amostras para essas duas distintas matrizes, e que atendessem a critérios como ser de fácil aplicação, barato e ter um menor consumo de reagentes/ solventes.

Ao longo trabalho, informações relevantes sobre a composição destas amostras foram observadas, entre elas a presença de agroquímicos, mesmo que em baixas concentrações, em uma grande quantidade de amostras analisadas. Além disso, de modo geral, as amostras compostas por carnes e vegetais apresentaram uma maior concentração para diversos contaminantes quando comparadas as amostras à base de frutas.

Fica evidente que ainda temos um longo caminho a percorrer no que diz respeito a análise de resíduos e contaminantes em alimentos infantis quando comparados a outros países. Além da falta de legislações específicas, são escassos os dados disponíveis na literatura sobre a presença desses contaminantes nesse tipo de alimentos. De modo geral, este trabalho acrescentou à literatura dados muito importantes a respeito da composição desses alimentos, alguns inclusive inéditos e que podem servir de alerta para as autoridades regulatórias do país.

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Anexo III

JFCA-D-23-02153 - Confirming your submission to Journal of Food Composition and Analysis

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