Food Chemistry 115 (2009) 1164–1169

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Evaluation of cost-effective methods in the pesticide residue analysis of non-fatty baby foods

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ARTICLE INFO

Article history:
Received 23 June 2008
Received in revised form 27 October 2008
Accepted 24 December 2008

Keywords:
Baby food
Pesticide residues
Gas chromatography

ABSTRACT

Three non-fatty ready-to-eat baby food matrices (fruits: juice, purée and cocktail plus rice flour/starch and sugar) were fortified with 0.01, 0.05, 0.1 and 0.2 mg/kg of dimethoate, chlorpyrifos, methidathion, phosalone and diazinon. Simple methods including extraction by ethyl acetate [EtAc] and acetone partition and determination by gas chromatography with Nitrogen–Phosphorus Detector (GC–NPD) were used. Acceptable pesticides recoveries (70–110%), low detection and quantification limits (0.001 to >0.1 mg/kg and 0.005 to 0.04 mg/kg, respectively) and repeatabilities (%RSDs), in 0.01 mg/kg, within 2.9–13.9% were observed. However, analytes recoveries were affected ($p < 0.05$) by both the baby food formulation and the extraction method used. Specifically, fruits purée and cocktail EtAc extracts gave excessively over-(dimethoate recoveries of 119.7–153.5%) or underestimation (phosalone and especially diazinon recoveries of 19.3–79.2%) in contrast to fruits juice (e.g., 61.3–87.9%). Also, EtAc extracts showed higher amount of lipophilic compounds and provided lower recoveries for non-polar analytes than those of acetone partition. Consequently, the examined methods may be successfully applied in non-fatty baby foods with the matrix-matched standards determination, following improvements of certain parameters in relation to the clean-up of samples.

1. Introduction

The presence of pesticide residues is regarded as a potential chemical hazard both in the final products and in the raw materials of ready-to-eat baby foods, such as fruits and vegetables. Effective and reliable monitoring in the pesticide analysis of this category by conventional cost-effective multiresidue extraction methods (MRMs) may be applied followed by "optional" clean-up step and inexpensive laboratory equipment. The most widely used MRMs involve extraction by EtAc and acetone and quantification by gas chromatography (GC) with selective detectors (Dorea, Tadeo, & Sanchez-Brunete, 1996; Greve, 1988; Luke, Froberg, & Masumoto, 1975; Van Zoonen, 1996). However, the behaviour of such methods has been evaluated in fruit and vegetable pesticides' determination, whereas limited information is available in the analysis of baby foods contrary to the performance of modern expensive techniques, such as acetonitrile extraction plus dispersive solid phase extraction (dSPE) combined with gas chromatography-tandem quadrupole mass spectrometry (GC–MS/MS) (Leandro, Fussell, & Keely, 2005) and stir bar sorptive extraction (SBSE) combined with GC–MS (Sandra, Tienbond, & David, 2003).

The objectives of this study were: (i) to evaluate the recoveries of five representative organophosphates (OPs) fortified in three ready-to-eat baby foods at four indicative levels [0.01 (Maximum Residue Level, MRL, by European Commission for the majority of pesticide residues, potentially occurring in baby foods), 0.05, 0.1 and 0.2 mg/kg], by two simple, rapid and inexpensive methods (thoroughly applied in primary plant products) with matrix-matched standards quantification and (ii) to evaluate some (further) critical parameters for possible methods validation. The results proposed efficient MRMs for pesticides monitoring in non-fatty baby foods.

2. Materials and methods

2.1. Materials and reagents

All solvents used were of pesticide grade. Particularly, ethyl acetate, acetone, dichloromethane and petroleum ether (Riedel de Haën, Hanover, Germany) were the extraction solvents (Fig. 1), whilst 2,2,4-trimethyl pentane and toluene (Riedel de Haën, Hanover, Germany) were used in the final step of analytical procedures and as solvents in standard solutions (Fig. 1b). Pesticide standards (dimethoate, chlorpyrifos, methidathion, phosalone and diazinon) of more than 98% purity were obtained from Chem Service (West

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doi:10.1016/j.foodchem.2008.12.106

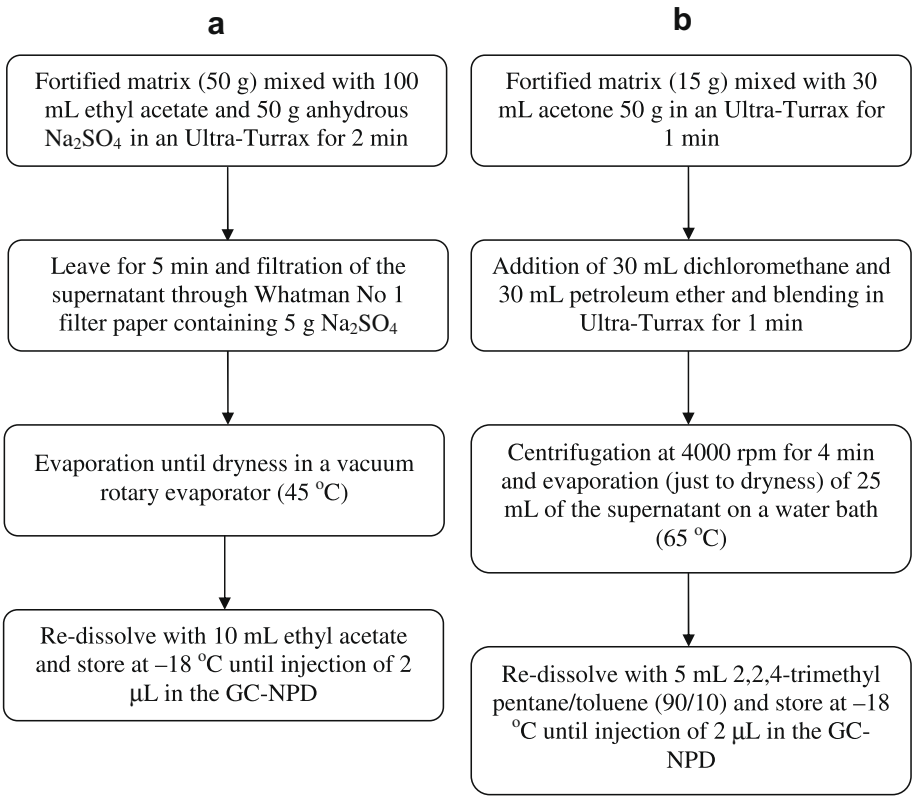

Fig. 1. Analytical description of procedures used to prepare the fortified samples for analyses, by (a) ethyl acetate and (b) acetone partition method.

Chester, PA, USA). Stock solutions of 100 mg/L, stored at −18 °C, were prepared both in ethyl acetate and 2,2,4-trimethyl pentane/toluene (90/10), and working standard mixtures were obtained with appropriate dilutions before use. Anhydrous sodium sulphate for residue analysis was of pesticide grade (Riedel de Haën, Hanover, Germany).

2.2. Matrices

Seven non-fatty, fruit-based, ready-to-eat baby food matrices were obtained from a local market. Of them, three matrices were used for determination of recovery, limits of detection and quantification, as well as for estimation of repeatability of the proposed methods. Specifically, *fruits juice* containing water, apple juice (83%) and peach pulp (17%), *fruits purée* of apple (56%), pear (16%), apricot (12%), banana (9%) and apple/orange juice (5%), and *cocktail* of banana (41%), apple (39%), water, sugar (21.7%), orange juice (2%), rice flour/starch and lemon juice were of 0.0, <0.1 and 0.1 g/100 mL fat content, respectively. These commodities were firstly checked by the used MRMs to ensure the absence of pesticide residues (Dorea et al., 1996; Greve, 1988; Van Zoonen, 1996; Pugliese et al., 2004).

For robustness monitoring of the MRMs, four additional baby foods (apple and pear, pear and pineapple purée, apple and banana juice, and apple, banana, orange cocktail plus rice flour and sugar) of similar composition to those analysed were used.

2.3. Fortification and analytical procedures

Appropriate quantities of each analyte from the individual pesticide stock standard solutions were added to matrix quantities proposed by the extraction methods (Fig. 1). Specifically, 5, 25, 50 and 100 μl of the stock solutions were added to 50 g of the examined commodities for ethyl acetate method (Greve, 1988), whilst 1.5, 7.5, 15 and 30 μl were added to 15 g matrix of acetone partition method (Van Zoonen, 1996). The latter resulted in every matrix being fortified with 0.01, 0.05, 0.1 and 0.2 mg/kg of pesticides. The level of 0.01 mg/kg is the established MRL by European Commission for the majority of pesticide residues (including ours) potentially occurring in baby foods. Then, samples were prepared in triplicate for each of both extraction methods described in Fig. 1 (Greve, 1988; Van Zoonen, 1996). For acetone partition, the final volume of 5 mL is proposed for GC-NPD analysis of fruits, vegetables and potatoes (Van Zoonen, 1996). For ethyl acetate extraction, the volume of 10 mL has been utilised in the Standard (Greve, 1988) and in some studies (i.e., Pugliese et al., 2004) because of higher residue concentration after the extraction/evaporation step (Fig. 1).

2.4. Solvent and matrix-matched standards preparation

The solvent/matrix standards derived by the stock solutions of each individual pesticide (100 mg/L) as follows: (a) solvent standards were prepared with appropriate dilutions from each solution to reach concentrations ranging from 0.01–0.5 mg/kg (b) matrix-matched standards were prepared by adding appropriate pesticide quantities in blank extracts.

2.5. Gas chromatographic determination

A gas chromatograph (Hewlett-Packard 5890, Series II) equipped with an NPD (Agilent, Wilmington, DE, USA), a hot split/splitless injector and an autosampler was used. The separation of analytical sample components was performed with an Rtx®-5 ms 30 m × 0.32 mm id × 0.25 μm film thickness (Restek, Bellefonte, PA, USA) capillary column, whilst there was used a

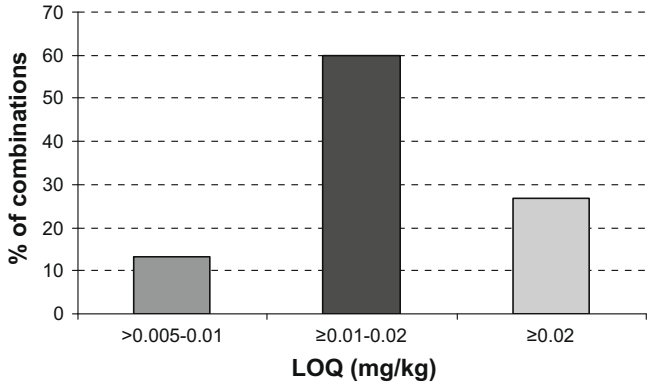

Fig. 2. Overall LOQ (mg/kg) profiles of the target analytes obtained by the examined matrix – extraction method combinations. Results represent merged data of both methods.

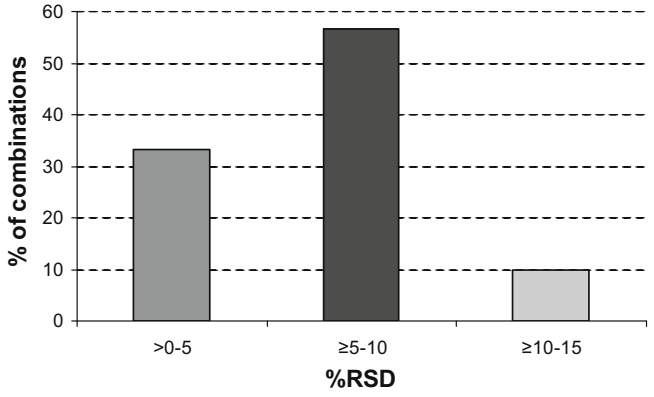

Fig. 3. Overall repeatability (%RSD) profiles of the examined analyte – matrix combinations at 0.01 mg/kg (or LOQ) obtained by ethyl acetate and acetone partition method (merged data).

non-polar deactivated pre-column (Rtx® 5 m × 0.32 mm id) (Restek, Bellefonte, PA, USA). The injection port temperature was 260 °C and the detector temperature 300 °C. The oven temperature was programmed as follows: initial temperature 70 °C for 1 min, raised at 15 °C/min to 130 °C for 0 min, raised at 5 °C/min to 230 °C and finally raised at 20 °C/min to 250 °C with a residence time of 18 min. Helium carrier gas at flow rate of 8 mL/min was used, based on previous studies performed in our lab (Georgakopoulos, Foteinopoulou, Athanasopoulos, Drosinos, & Skandamis, 2007; Stavropoulos, Athanasopoulos, & Kyriakidis, 2001). Triplicate extracts (2 μl) were injected and quantification of the insecticides was performed by automatic integration of the peak areas. Quantification/recovery of the pesticides in the fortified samples was carried out by comparing the detector responses for each of the three independent samples with those measured in matrix calibration standards, of similar concentration with the fortified, injected both before and after each sample.

2.6. Evaluation of validation parameters

The linearity of NPD response was studied in both solvent and matrix standards solutions in the range of 0.01–0.5 mg/kg (seven different concentrations prepared in triplicate). The LOD was estimated as the analyte concentration resulted in signal (S)-to-noise (N) ratio of 3 (S/N = 3) (Huber, 1998) and verified by the analysis of the pesticides mixture fortified at 0.01 mg/kg (six independent replicates) as three times the standard deviation (SD) (LOD = 3 × SD). The LOQ was defined as the analyte concentration resulting in S/N of 10 (Huber, 1998) and verified by the afore-mentioned procedure applied for LOD. LOQ equals to "mean + 10 × SD". The value of "mean" is the average of concentration levels determined in the six independent replicates by the analysis procedures. Finally, six independent matrix extracts, fortified with 0.01 mg/kg (or LOQ where this level was not quantified), were used for determination of repeatability (%RSD).

Table 1
Percentage recoveries ± Relative standard deviation (RSD) of the examined analytes derived from the application of ethyl acetate and acetone partition method in different matrices fortified at different levels (mg/kg) (n = 3).

Matrix	Recovery ± RSD (%) amongst the different extraction methods and fortification levels							
	Ethyl acetate				Acetone partition			
	0.2 mg/kg	0.1 mg/kg	0.05 mg/kg	0.01 mg/kg	0.2 mg/kg	0.1 mg/kg	0.05 mg/kg	0.01 mg/kg
Dimethoate								
Fruits juice	79.0 ± 1.8ax	84.0 ± 2.0ax	67.3 ± 0.2ax	74.3 ± 3.4a	119.8 ± 7.4by	114.2 ± 1.4ay	136.9 ± 6.3by	Non detectable
Fruits purée	142.6 ± 0.8by	131.2 ± 1.0by	127.0 ± 3.2by	119.7 ± 1.7bx	115.5 ± 6.6bx	108.6 ± 4.1ax	157.8 ± 13.3bx	115.8 ± 10.0bx
Fruits cocktail	145.6 ± 2.6by	152.4 ± 1.3cy	143.8 ± 1.8cy	153.5 ± 5.1cy	98.0 ± 4.8ax	104.6 ± 5.3ax	71.8 ± 3.2ax	86.9 ± 6.6ax
Chlorpyrifos								
Fruits juice	83.0 ± 0.6bx	82.2 ± 1.5cx	74.0 ± 0.3ax	70.9 ± 1.6ax	92.2 ± 6.1ay	89.1 ± 2.6by	101.7 ± 4.6ay	94.3 ± 2.6by
Fruits purée	88.4 ± 0.3bx	72.7 ± 0.3ax	84.9 ± 7.6bx	64.7 ± 7.7ax	84.0 ± 6.1ax	77.8 ± 4.7ax	101.0 ± 6.1ay	77.7 ± 3.9ay
Fruits cocktail	74.6 ± 0.4ax	77.9 ± 0.9bx	71.1 ± 2.3ax	79.1 ± 2.4bx	95.5 ± 2.6ay	107.4 ± 2.3cy	108.3 ± 2.1ay	73.3 ± 11.0ax
Methidathion								
Fruits juice	80.0 ± 2.5bx	88.9 ± 2.4cx	71.4 ± 1.3ax	78.6 ± 1.2ax	100.7 ± 3.9aby	98.9 ± 1.0by	110.9 ± 4.5ay	82.3 ± 3.1ax
Fruits purée	95.0 ± 0.7cx	76.5 ± 0.6ax	81.1 ± 6.3bx	76.2 ± 4.3ax	108.7 ± 6.9by	74.1 ± 10.8ax	95.5 ± 6.2ay	85.5 ± 1.8ay
Fruits cocktail	72.2 ± 1.4ax	84.4 ± 2.9bx	70.3 ± 0.9ax	79.5 ± 3.2ax	91.2 ± 1.3ay	111.6 ± 1.7cy	108.5 ± 7.8ay	102.4 ± 4.2by
Phosalone								
Fruits juice	73.5 ± 3.5bx	71.5 ± 1.1cx	61.3 ± 3.2bx	69.4 ± 2.0b	102.1 ± 3.8ay	85.7 ± 3.0by	117.0 ± 1.6ay	Non detectable
Fruits purée	73.1 ± 1.2bx	57.2 ± 1.3bx	51.6 ± 1.8abx	55.8 ± 5.6a	100.8 ± 2.9ay	70.1 ± 8.0ay	121.6 ± 6.4ay	Non detectable
Fruits cocktail	46.3 ± 2.4ax	49.8 ± 2.9ax	46.4 ± 4.7ax	60.1 ± 4.8a	103.2 ± 5.3ay	110.9 ± 3.9cy	118.7 ± 7.8ay	Non detectable
Diazinon								
Fruits juice	79.6 ± 0.5cx	74.9 ± 1.2cx	87.9 ± 0.9cx	62.0 ± 1.7ax	90.9 ± 3.7ay	97.0 ± 0.6by	123.3 ± 1.7by	68.8 ± 8.2ay
Fruits purée	25.4 ± 0.9bx	21.3 ± 0.5bx	26.3 ± 0.8bx	79.2 ± 5.0bx	98.1 ± 6.9ay	82.5 ± 6.1ay	129.9 ± 2.1cy	73.8 ± 2.0abx
Fruits cocktail	22.1 ± 0.8ax	19.3 ± 1.0ax	21.4 ± 0.8ax	66.3 ± 1.0ax	95.4 ± 3.0ay	111.6 ± 3.5cy	99.8 ± 3.4ay	78.2 ± 4.3ay

a, b, c: means within a matrix column for a specific extraction method and fortification level lacking a common letter are different ($p < 0.05$); x, y, z: means within an extraction method for a specific matrix and fortification level lacking a common letter are different ($p < 0.05$)

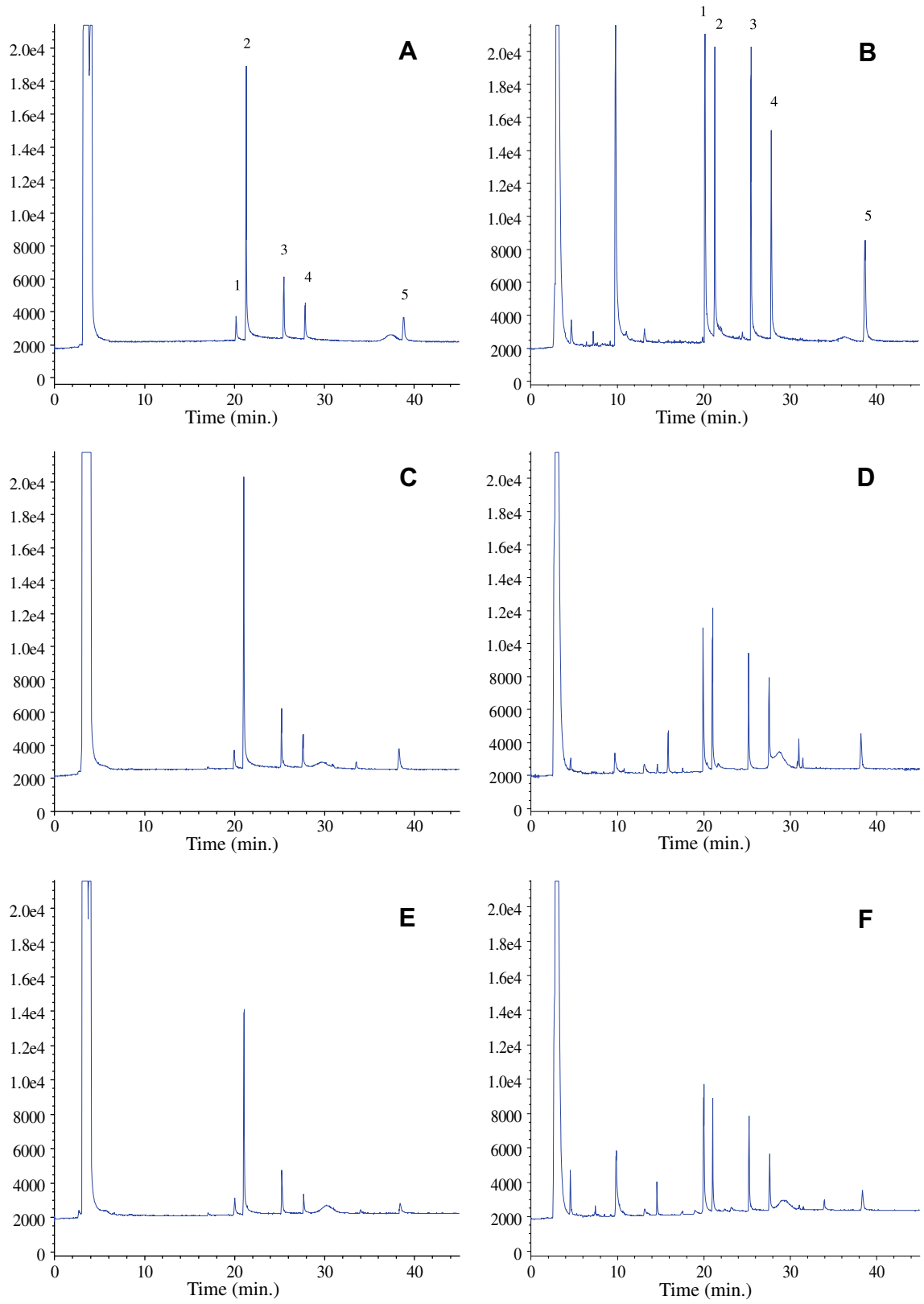

Fig. 4. GC-NPD chromatograms of the fortified with a pesticides mixture of 0.1 mg/kg) fruits juice (A, B), fruits purée (C, D) and fruits cocktail (E, F), prepared by acetone partition method (A, C, E) and ethyl acetate method (B, D, F). Peaks identification: 1. dimethoate, 2. diazinon, 3. chlorpyrifos, 4. methidathion, 5. phosalone.

2.7. Statistical analysis

Three-way analysis of variance (ANOVA) was applied in order to examine the fixed effects of three experimental factors (matrix, extraction method, analyte) in the recovery of pesticides using the general linear models of SPSS. Then, least squared means of recoveries in triplicate trials were calculated and significant differences were estimated by post-hoc tests using Tuc-

key's method. A significant level of 0.05 was used for all statistical analysis.

3. Results and discussion

The performance of an analytical method is assessed by several criteria, with the recovery portion of the examined analyte(s) being the most important one. Furthermore, the detector response to the target analytes represents an essential requirement for the linearity estimation and recovery calculation. All NPD responses corresponded to linear equations ($R^2 > 0.99$). Observed LODs were in the range of 0.001–>0.1 mg/kg. Specifically, most of them were within 0.001–0.004 mg/kg, with the lowest values deriving from the EtAc extracts possibly due to the higher concentration following the dilution step. LOQs were in the range 0.005–0.04 mg/kg, with the majority being equal or close to the lowest examined fortification level (0.01 mg/kg). Fig. 2 demonstrates the quantification ability of both methods applied (data of both methods are merged) at such low levels, since >70% of LOQs were lower, equal or close to 0.01 mg/kg. Regarding the repeatability of the methods examined, RSDs of the six matrix extracts (fortified with 0.01 mg/kg or LOQ) tests were in the range 2.9–13.9%. It is notable that the 90% of the provided %RSDs was <10% at the LOQ, whilst in the remaining cases there was no value >15% (Fig. 3). Therefore, the examined methods seem to allow accurate determination of representative OPs in baby foods in as low levels as the established MRLs.

The present MRMs generally gave acceptable pesticides recoveries. It is notable that almost all chlorpyrifos (64.7–108.3%) and methidathion (70.3–111.6%) recoveries were acceptable for method validation purposes (Table 1). This is much different from other studies, in which recovery determination was based in solvents standards and extensive matrix co-extracts effects were revealed. Indeed, the use of matrix-matched standard solutions has been shown to result in accurate quantification of pesticides in various food commodities. For example, excessively high OP recoveries (>120–240%) were reduced to 81–97% with matrix standard calibration for potato extracts (Lehotay & Eller, 1995). Extremely high recoveries (>200–1000%) for lots of analytes in honey extracts were also corrected to the acceptable range with controlled spiked blank extracts (Jiménez, Bernal, del Nozal, Toribio, & Martín, 1998). Thus, there is elimination of the co-extracts effects in the final results even if no clean-up step (to remove the additional components) was applied in the analysis of more complicated matrices than primary plant products. However, some

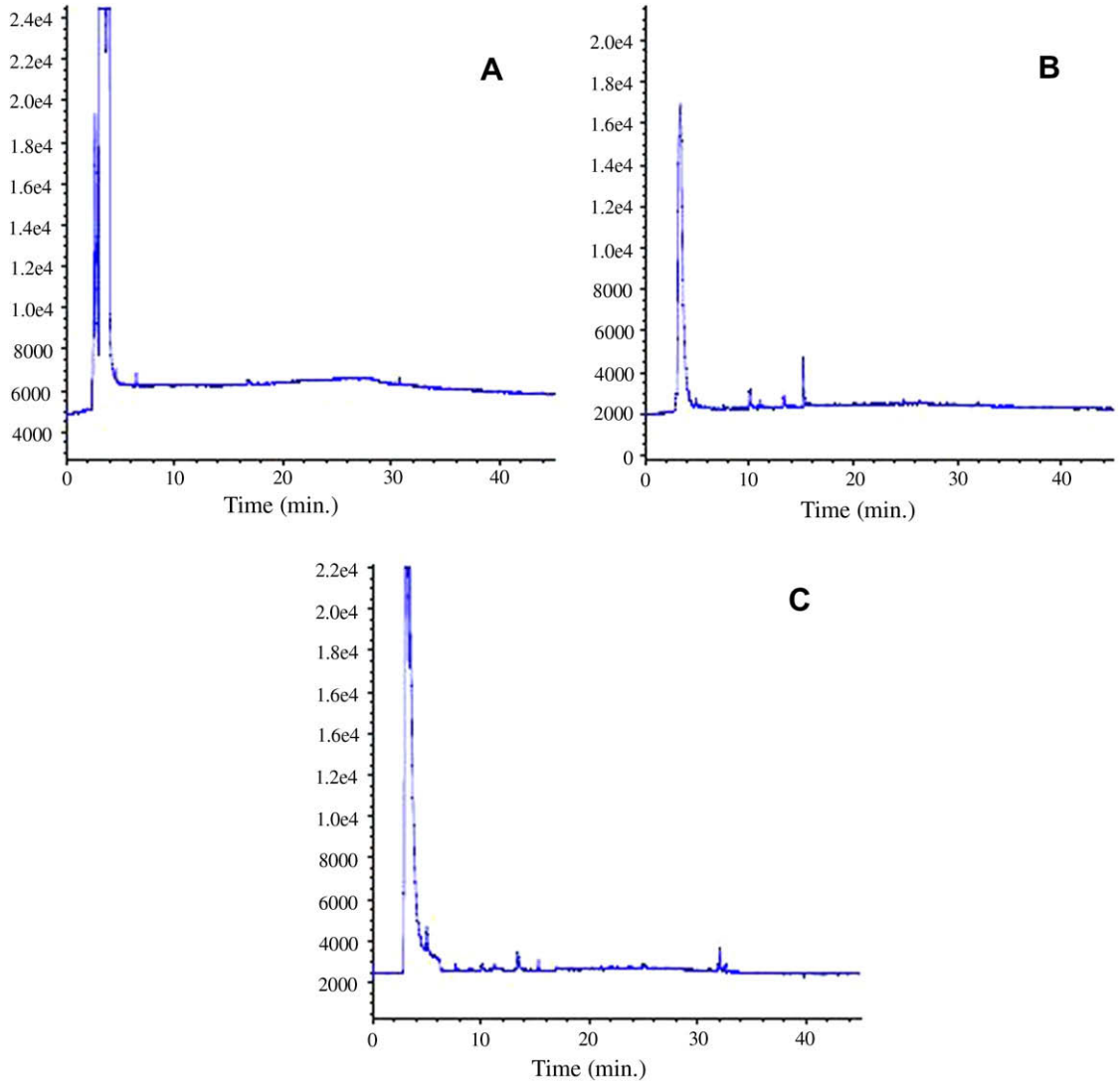

Fig. 5. Representative GC-NPD chromatograms of blank extract commercial baby foods: (A) acetone partition apple and banana juice extract, (B) ethyl acetate pear and pineapple purée extract and (C) ethyl acetate fruits cocktail extract.

extensively underestimated determinations of specific analytes (phosalone and especially diazinon) in EtAc procedure can be attributed to their partial adsorption on matrices and/or incomplete extraction in analytical samples preparation. This is in agreement with recent findings dealing with the application of external (single point) method of quantification (Ostroukhova & Zenkevich, 2006).

The examined matrices resulted in different pesticides recoveries depending on the analyte ($p < 0.05$). Specifically, fruit juice extracts presented the most recoveries within 70–110%. On the contrary, the other two matrix extracts resulted in significant over- or underestimation in some recoveries of specific analytes (Table 1). These phenomena may partially be attributed to the ineffective removal of co-extracts (Fig. 4) by the extraction method(s). The higher co-extracts amount was observed in the chromatograms of EtAc fruit purée and cocktail analytical samples (Fig. 4D and F). Therefore, the composition of the examined products is able to positively or negatively influence the detector response, or cause inaccurate determination by interfering with elution of analytes. Hajšlová and Zrostlíková (2003) suggested that the more complex is the examined matrix, the more difficult is the efficient removing of matrix components from the crude extracts and their effects are higher. It is also notable that citrus juice has been effectively analysed without any clean-up step with EtAc extraction. On the contrary, citrus peel required additional clean-up by a Florisil column to remove the co-extracts and provide accurate determination (Dorea et al., 1996). The application of matrix solid phase dispersion (MSPD) in tomatoes demonstrated that Florisil clean-up layer was more effective in removing interfering compounds than without additional clean-up (Menezes Filho, Navickiene, & Dorea, 2006).

Provided the fact that these methods are able to determine these five representative analytes of different polarity/physicochemical properties, the proposed MRMs were applied to the analysis of ten organophosphorus insecticides, routinely tested in our laboratory, in four commercial baby foods (apple and pear, pear and pineapple purée, apple and banana juice, and apple, banana, orange cocktail plus rice flour and sugar). All chromatograms gave no detectable peaks of the examined analytes (Fig. 5) (based on the retention times of the relevant standards) with large amounts of co-extracts in the EtAc pear and pineapple purée and fruits cocktail at different retention times (Fig. 5B and C). In conclusion, the presented data indicate that these MRMs could be alternative methodologies for the identification/quantification of pesticide residues in non-fatty baby foods where modern extraction techniques and equipment are unavailable. In order to further examine the analytical performance of the applied MRMs in such high chemical risk products, a higher number of similar composition matrices fortified with more analytes by further clean-up steps, is strongly recommended and may lead to the establishment of simple analysis methods. Therefore, the majority of the non-fatty baby food commodities (i.e., fruit juices) could be adequately analysed by the ethyl acetate extraction, whilst some of them (i.e., purées) may require an extra purification step with Florisil sorbents.

Acknowledgement

The work is funded by General Secretary of Research and Development, key action "PEP ATTIKIS" and thematic area Agricultural Science and Food Safety.

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