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Analytical Method Validation for Determining Organophosphorus Pesticides in Baby Foods by a Modified Liquid–Liquid Microextraction Method and Gas Chromatography–Ion Trap/Mass Spectrometry Analysis

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Abstract

Baby food is made specifically for babies between the ages of 4/6 months up to 2 years, and it comes in multiple varieties and tastes. This paper proposes a protocol for a rapid and reliable determination of 19 organophosphorus pesticides (OPs) by means of a method based on ultrasound-vortex-assisted liquid–liquid microextraction coupled with a gas chromatography–ion trap mass spectrometry (UVALLME–GC-IT/MS). The method development starts from the extraction solvent evaluation, i.e., *n*-heptane; the solution, held for 5 min on the vortex mixer and for 6 min in an ultrasonic bath to 100 W for favoring the solvent dispersion and the determinant extraction, is centrifuged at 4000 rpm for 30 min: 1 μL of the organic extract is injected into the GC-IT/MS. All the analytical parameters investigated are deeply discussed; LODs/LOQs (0.2–1.3 and 0.5–2.9 ng/g) and recoveries (81–109%) are compared with other papers dealing the determination of OPs in baby food matrix. The whole method has been applied to real commercial freeze-dried and soft baby food samples: the results do not show any significant contaminant values.

Keywords LLME · Vortex · GC-IT/MS · Organophosphorous pesticides · Baby food

Introduction

Pesticides are widely used worldwide with the main goal of improving the crop yields and the quantity of fresh fruits and vegetables in the diet (National Research Council 1993). They include more than 1000 compounds employed for checking and eradicating pests. Severe regulations guarantee the use of such substances in a safe and effective way. In fact, pesticides may simultaneously be harmful both for the environment (e.g., accumulating in ecosystems) and for the human health. In this second task, they can give adverse effects, including cancer, acute and chronic injury to the nervous system, lung damage, reproductive dysfunction, and possibly dysfunction of the endocrine and immune systems (Russo et al. 2002; Eskenazi et al.

2007). One of the main pesticide sources is the diet. Indirectly, the consumer assimilates small pesticide quantities (residues) from different foods such as meat, fruit, and vegetables. For these reasons, each nation has promoted a very strict legislation for these compounds (Codex Alimentarius Commission 2009). The U.S. Government has instituted regulatory controls on pesticide use; the European Union has harmonized and simplified the Maximum Residue Limits (MRL) for each pesticide; the Japan Food Chemical Research Foundation has made available a database of MRLs, searchable by pesticide and crop, also providing links to legislation on the Positive List system. MRLs of pesticides in foods have been set up by many international bodies (European Commission 2005). This issue is important for adults, but it is even more important for infants and children. These two categories (not considered as "little adults") show a rapid metabolic rate compared with that of mature with the consequence of dissimilarities in the skill of making activation, detoxification, and excretion of xenobiotic species (Fenske et al. 2002; D'Souza 2011; Meeker 2012). Furthermore, the newborns are even more different, anatomically and physiologically, than adults. This sub-population exhibits highest sensitivity to pesticides: "emerging data suggest that neurologic and behavioral effects may result from low-

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level chronic exposure to some organophosphate and organochlorine pesticides" as well as "exposure to neurotoxic compounds at levels believed to be safe for adults could result in permanent loss of brain function if it occurred during the prenatal or early childhood period of brain development" (National Research Council 1993).

Starting from these human health considerations, and despite their worldwide use, some papers reporting protocols or data on organophosphorous pesticides (OPs) in milk-based infant formulas or in baby food are present in literature as confirmed by D'Souza (2011). A multi-residue pesticide determination basically covers a wide range of residues; the first step is to make a homogenized sample followed by extraction of pesticides from sample matrix, removal of co-extracted water from the sample matrix, cleanup by solid phase extraction (SPE), and finally determination by GC or LC. Then most common organic solvents used for pesticide extraction are acetone, acetonitrile, and ethyl acetate. Erney (1995) investigated 29 OPs in whole/chocolate/skim milk and infant formula by solid phase extraction (SPE) with gas chromatography and flame photometric detection (GC–FPD) whereas Mezcua et al. (2007) published an automated analytical method for determining 12 organochlorine and OPs in milk-based infant formulas. On the other hand, interesting discussions on the sample preparation steps and matrix interference removal have been reported by Georgakopoulos et al. (2009, 2011).

The protocol, based on extraction with pressurized liquid extraction (PLE) and determination by GC tandem mass spectrometry (GC-MS/MS), allows to reach limits of detection (LODs) ranging between 0.01 and 2.6 μg/kg with recoveries between 70 and 110%. Another interesting approach was performed by Melgar et al. (2010) for analyzing seven OPs by means of a SPE into acetone and GC with a nitrogen–phosphorus detector (NPD), reaching LODs of 8–65 μg/kg. This topic is really interesting, so different international organizations recommend the evaluation of residues in baby foods (European Commission 2013).

This paper aims to set up a simple, cost-effective, sensitive, and reproducible analytical methodology for determining OPs at trace levels in commercial freeze-dried and soft baby food samples. All the steps of this novel method were evaluated and largely discussed in order to achieve high levels of sensitivity, reproducibility, and selectivity.

Materials and Methods

Chemicals

Pesticide standards (methacrifos, pirofos, phorate, seraphos, diazinon, etrimphos, dichlofenthion, chlorpyrifos-methyl, pirimiphos-methyl, malathion, chlorpyriphos, parathion-ethyl, pirimiphos-ethyl, bromophos, chlorfenvinphos, bromophos-

ethyl, stiriphos, diethion, coumaphos) were obtained from Società Italiana Chimici S.p.A. (Rome, Italy): the standard solutions (1 g/L) were prepared by dissolving the pesticides in acetone. These solutions were further diluted with *n*-hexane to prepare final solutions for spiking. Isoctane, heptane, benzene, toluene, cyclohexane, ethyl ether, and sodium chloride (ACS reagent grade) were obtained from Carlo Erba (Milan, Italy). Bromopropylate (Società Italiana Chimici S.p.A.) in acetone has been used as Internal Standard (IS): 5 ng/μL is added to each sample before starting the analytical procedure with the aim of following each analytical step and minimizing the possible errors. The IS was added to each soft baby food sample prior to freeze drying.

Ultrasound-Vortex-Assisted Dispersive Liquid–Liquid Microextraction (USVADLLME) Procedure

The extraction method is based on a variant of the DLLME extraction method. About 5 g of soft baby food sample, with the addition of 5 μL of IS, was freeze-dried for 4 h at temperatures of − 53 °C and at a pressure of 0.017 mbar in a freeze-drier LIO5P (5Pascal, Trezzano sul Naviglio, Italy); the loss weight was about 85% for the fruit samples (pear and plum) and about 80% for the meat/fish samples (chicken, rabbit, sea bream, and plaice). After, 0.1 g of each freeze-dried sample (i.e., each soft baby food subjected to lyophilization process and each commercial freeze-dried product) was transferred into a 10-mL screw cap glass tube with a conical bottom and 10 mL of buffer solution at pH 4.0 was added (buffer solutions at different pH were tested—different experiments were performing by means of the addition of the buffer solution to the freeze-dried sample): this solution is required for 1 min on the vortex. Then 250 μL of heptane (extraction solvent) was added.

The dispersion was performed by means of 5 min of vortex by mechanical rotation followed by means of 6 min of ultrasound bath. The solution becomes cloudy. For promoting the separation of this solution, 0.1 g of NaCl (10 g/L) was added. Finally, the solution was centrifuged at 4000 rpm for 30 min: the obtained micro-drop is transferred into a vial. The vortex and ultrasound parameters (e.g., vortex/ultrasound time) were deeply investigated in a previous paper (Russo et al. 2015): these steps were tested and confirmed in this study (data are not reported for not weighting the manuscript). After sodium sulfate addition for eliminating water residual, 1 μL of the final solution was injected in GC-IT/MS for determining the 19 OPs.

GC-IT/MS Determination and Quantification

A gas chromatograph model Trace GC Ultra Finnigan (ThermFinnigan, Bremen, Germany) equipped with a programmed temperature vaporizer (PTV) injector and

connected to an ion trap mass spectrometer model PolarisQ (ThermFinnigan), and data system "Xcalibur" (ThermoFisher Scientific, Waltham. MA) were used for GC-MS determination in the total ion current (TIC) and selected ion monitoring (SIM) modes.

A fused-silica capillary column with chemically bonded phase (SE-54, 5% phenyl–95% dimethylpolysiloxane) was prepared in our laboratory with the following characteristics: 30 m × 250 μm i.d.; N (theoretical plate number) 125,000 for *n*-dodecane at 90 °C; K′ (capacity factor) 6.9; d_f (film thickness) 0.24 μm; u_{opt} (optimum linear velocity of carrier gas, hydrogen) 38.0 cm/s; UTE% (utilization of theoretical efficiency) 92% (Cartoni et al. 1986, 1991; Russo et al. 1996, 2014a). The fused-silica capillary column used is very similar to commercial ones showing very good chromatographic efficiency and being more convenient from an economic point of view.

Helium was used as carrier gas at constant flow rate of 1 mL min^{-1} and as dumping gas in the ion trap at 0.3 mL/min. The PTV injector was performed in splitless mode, 10 s after injection the vaporizer was heated from 100 to 280 °C at 800 °C/min and cooled after 5.00 min; the splitless valve was opened after 120 s from the injection. The column was kept at 90 °C for 60 s and then the temperature was programmed from 90 to 290 °C at 10 °C/min. The transfer line and the ion source were held at 270 and 250 °C, respectively. The chromatogram was captured in positive electronic impact (70 eV) in a range between 55 and 380 amu.

The compound concentrations were obtained by calibration graphs of the ratio Area$_{(OP)}$/Area$_{(IS,bromopropylate)}$ plotted versus each OP concentration (pg/μL). All the samples were quantified in triplicate.

Results and Discussion

Role of the Matrix

In the analytical protocol development for investigating OPs, the two matrices investigated, i.e., baby and freeze-dried foods, play a very important role. The first is soft, almost gelatinous; the second, i.e., freeze-dried product, popular baby foodstuff in Italy, looks like a powder. Processing this last sample, the emulsion is easily obtained: the extraction analytical protocol can be directly applied to freeze-dried food. Instead, for the baby foods, the situation is more complicated. The considerable difference between the two matrices (gelatinous and powder) does not allow obtaining a clear emulsion when baby foods are analyzed: the solution appears as an extremely gelatinous emulsion and this occurrence inhibits the procedure. For this reason, the baby foods were subjected to a lyophilization step before the extraction procedure: in this way, a clear solution is obtained. The authors consider that the

surfactants added for stabilizing the food could be responsible for it. The lyophilization step was performed at − 52 °C and 0.080 mbar for 4 h in a freeze-drier system. Each solution was further processed according to the analytical methodology developed.

Analytical Methodology Evaluation

An important issue has been devoted to finding the best analytical conditions for the OP extraction and the GC-IT/MS determination. For evaluating the reliability of the whole analytical method, freeze-dried samples were spiked with a standard solution of five OPs (i.e., phorate, malathion, chlorpyrifos, parathion, coumaphos, 40 ng/g each). These five OPs were chosen because they were considered representative of the different analyzed compounds. By this solution, all the analytical experimental variables have been investigated, such as the extraction solvent, the pH influence on the extraction process, the salt effect, the method reproducibility, and the recoveries at different concentrations.

First, the best extraction solvent, an important step in the development of novel extraction procedures, was studied. The extraction solvent should be immiscible with water, should have high extraction efficiency for the target determinant, and should be distinguished with a high signal-to-noise ratio. Six different solvents such as *n*-heptane, *iso*-octane, cyclohexane, benzene, toluene, and diethyl ether were used for evaluating the extraction process. Table 1 shows the results: the two aromatic solvents, benzene and toluene, allow poor recoveries with very high RSD, ranging between 49 and 86% and 34–71%, respectively, as well as the cycloalkane (recoveries between 35 and 68%), whereas the ether does not recover any OP.

n-Heptane and *iso*-octane are solvents able to give good OP recoveries, 92–102% and 72–89%, respectively: *n*-heptane was chosen as extraction solvent. The main consequence is that the method provides efficient extractions if a linear, or branched at least, apolar hydrocarbon is used as a solvent, whereas it does not give any significant result with aromatic hydrocarbons, cyclic hydrocarbons, and solvents with a certain polarity.

After, the pH of the buffer solution, which is added to the freeze-dried sample for obtaining the dispersion, has been deeply studied (European Commission 2013). This step is really important because it represents the starting point of the procedure: a poor sample solubilization affects the procedure, e.g., it does not allow achieving good recoveries. Preliminarily, different pH levels of such solution, from acid to alkaline range, were studied for identifying the optimal zone where the extraction process could occur (Table 2).

The best recoveries are obtained at pH 4.1, whereas very high percentage recoveries are achieved at alkaline pH (probably, it is due to low IS extraction, and, consequently, the ratio

Table 1 Recovery (%) of the extraction solvent (250 µL) on the recovery (%) of the five selected OPs (40 ng/g)

Compound	Recovery (%) of the extraction solvent					
	Cyclohexane	n-Heptane	iso-Octane	Benzene	Toluene	Diethyl-ether
Phorate	67.8 (30.8)	98.5 (2.9)	87.3 (6.7)	78.3 (2.7)	68.1 (27.9)	− ()
Malathion	58.7 (11.5)	92.3 (2.1)	85.9 (7.3)	69.5 (7.2)	70.9 (26.9)	− ()
Chlorpyrifos	59.9 (30.9)	102.4 (3.3)	89.1 (9.5)	86.1 (2.8)	70.9 (25.2)	− ()
Parathion	61.5 (35.2)	96.3 (4.4)	83.0 (7.0)	77.4 (4.8)	59.0 (24.1)	− ()
Coumaphos	35.5 (42.5)	100.2 (5.7)	72.1 (8.0)	49.1 (9.0)	34.5 (49.3)	− ()

In brackets are reported the RSDs (five replicates)

is very high). Further experiments were performed for the identification of the best pH. Different extractions were performed at five different pH levels ranging between 3.8 and 4.5 (Table 2): the method shows good recoveries for pH ranging between 4.0 and 4.2.

Another investigated parameter was the salt effect and, particularly, the effect of the salting-out, i.e., the addition of sodium chloride to the solution for reducing the solubility of the electrolyte. The recoveries of OPs obtained by adding both 10 and 15 g/L of NaCl are comparable (Table 3), whereas the recoveries were very bad with higher amounts (with 20 g/L, recoveries are over 129%, due both to a co-extraction of matrix interference and to a poor IS extraction). It is advisable to use a concentration of 10 g/L for avoiding the addition of much salt. On the other hand, adding a lower NaCl amount (5 g/L), the emulsion is not well broken and the determinants are not totally extracted; an excessive NaCl amount will negatively affect the IS extraction and consequently alter the percentage recovery values.

It is well known that the efficiency of microextraction methods can be affected by the composition of the sample matrix (Yiantzi et al., 2010). For this aim, the entire procedure has been applied for investigating the recoveries of the five OPs in the different matrices analyzed in this study: two commercial freeze-dried products (turkey and rabbit) and six soft baby foods (chicken, rabbit, sea bream, plaice, pear, plum). In both cases, the recoveries were studied at two different concentrations of spiking, i.e., 10 and 50 ng/g, with the instruction

of freeze-drying the soft baby foods before processing. The spiking occurred before the freeze-drying: the results, reported in Table 4, are good, meaning that the whole analytical procedure could be applied to all 19 OPs.

GC-IT/MS Performance

Figure 1a shows the chromatogram of the mix standard solution of 19 OPs (10 ng/mL of each OP): the peaks are well separated and with good efficiency. Figure 1b and c shows the chromatograms obtained analyzing a freeze-dried (rabbit) sample (a) and the same sample spiked with the mix standard OP solution (10 ng mL^{-1} of each OP) (b).

Table 5 shows the retention times, the molecular weights, the typical fragment ions (abundance 100%), the limits of detection (LOD), and the limits of quantification (LOQ) of the 19 OPs (and IS as well) studied.

The typical fragment ions depend on the type of molecule. No compound shows ions with the same m/z value (a deep discussion on the SIM ion selection could be found in a previous paper, Russo et al. 2002). Table 5 also reports the calibration data: the relationship between signal and concentration is quite good for almost all the compounds, the correlation coefficients range between 0.9822 and 0.9997 in the range 5–1000 ng/g. For the linearity, a seven-point calibration curve was plotted in the concentration range above reported, i.e., 5, 10, 50, 100, 200, 500, and 1000 ng/g. The LODs are included between 0.2 and 1.3 ng/g with a RSD ≤ 8.7%

Table 2 Effect of the pH level on the recovery (%) of the five selected OPs (40 ng/g; 250 µL of n-heptane as extraction solvent)

Compound	Recovery (%) of the pH									
	3.1	3.8	4.0	4.1	4.2	4.5	5.1	6.1	7.2	8.5
Phorate	41.7	89.9	97.8	101.7	100.7	93.2	81.7	71.7	111.7	131.7
Malathion	62.8	76.1	83.1	92.3	81.3	79.5	92.0	81.7	108.7	146.1
Chlorpyrifos	83.4	98.4	108.0	109.3	103.4	98.7	85.1	75.3	109.7	124.2
Parathion	75.9	89.2	97.8	101.2	94.5	83.9	83.5	76.8	111.9	132.7
Coumaphos	52.5	63.8	59.5	62.1	61.7	73.8	44.6	71.2	102.7	119.7

Table 3 Salting-out effect on the recovery (%) of the five selected OPs (40 ng/g; 250 μL of *n*-heptane as extraction solvent; pH 4.1)

Compound	Salting-out effect			
	5 g L^{-1}	10 g L^{-1}	15 g L^{-1}	20 g L^{-1}
Phorate	102.2	101.4	106.9	140.4
Malathion	41.3	83.5	82.4	128.9
Chlorpyrifos	92.2	107.1	104.0	135.1
Parathion	78.0	95.8	94.4	134.4
Coumaphos	29.8	63.7	68.5	53.4

whereas the LOQs were between 0.5 and 2.9 ng/g with a RSD ≤ 9.2%. These values were determining according to Knoll's definition (Knoll 1985), i.e., the smallest measured quantities of the target determinants that produce chromatographic peaks equal to three (LOD) or seven (LOQ) times the standard deviation of the baseline noise (using three or seven times the standard deviation to assess S/N required to make a measurement defined as "different from zero"). These LODs and LOQs are meaningful for determining OPs in such matrices.

The entire analytical procedure has been applied to the determination of 19 OPs in soft and freeze-dried baby foods for evaluating the matrix effect: Table 6 shows the percentage OP recoveries at different spiking concentrations (10 and 50 ng/g) in two different matrices (rabbit and pear).

The recoveries range between 85 and 109% for the freeze-dried matrix and 81–106% for the soft baby with the relative standard deviations (RSDs) below 10 and 12, respectively. It should be underlined that these values take in account the whole analytical procedure (i.e., lyophilization, OP extraction, and GC-IT/MS determination steps) avoiding any further physical–chemical treatment of the sample. The recoveries seem to be independent of the matrix, even if those obtained from processing the soft sample are lower than those obtained from freeze-dried ones. In this last case, the reason could be

due to the further process (freeze-drying process) to which soft food is the subject before applying the entire analytical procedure.

Comparison with Similar Studies

An important issue of this paper was to develop a rapid, easy, and sensitive methodology for analyzing OPs in such matrices. For this aim, the authors compared the results obtained with those reported in literature for similar papers. Actually, as just reported, there are few papers dealing with such determination in baby food, so the literature was extended to papers reporting such determinations in foods for newborns or, at least, for (primary) school children. Table 7 shows a comparison among the main analytical parameters, i.e., recoveries, LODs, and LOQs, determined in this study and those found by other authors in similar baby food matrices. The comparison between different analytical methods including the detection apparatus confirms the goodness of this approach previously used with other compounds in same matrices (Russo et al. 2012, 2014b, 2014c, 2016; Cinelli et al. 2014a, 2014b). In particular, the analytical methodology used by other authors should be considered.

Many studies are performed by QuEchERS followed by high technology detection systems (e.g., MS/MS(QqQ), LC-MS, QT-OFMS). In few cases, LODs/LOQs are better than those found in this paper, but the instrumental implications are very expensive (PLE-GC-MS/MS in Erney 1995, with very poor recoveries, 50–83%, and QuEchERS-nano-LC-MS in Mirabelli et al. 2016), whereas this determination is based on the application of a very easy and inexpensive analytical protocol. This issue should be considered in a laboratory where routine analyses are performed. Finally, it should be considered that, although the LOQs obtained from this study are not better than those from other studies, the improvement of sensitivity resulted from the lyophilization step.

Table 4 Recoveries (%) of the five OPs in the different matrices analyzed in this study (10/50 ng/g; 250 μL of *n*-heptane as extraction solvent; pH 4.1; NaCl 10 g/L)

Compound	Freeze-dried			Soft baby food						
	Rabbit (a)	Turkey (a)	Turkey (b)	Chick (a)	Rabbit (a)	Sea bream (b)	Plaice (b)	Pear (a)	Pear (b)	Plum (a)
Phorate	108.1	107.7	102.1	93.4	94.6	91.5	93.6	98.2	96.9	94.3
Malathion	88.0	81.1	88.7	81.5	83.2	79.8	79.3	77.8	87.4	86.9
Chlorpyrifos	103.7	106.7	98.0	99.5	105.0	104.9	102.4	101.9	101.7	107.6
Parathion	92.3	96.4	96.9	96.4	87.2	98.9	98.7	94.2	96.5	98.2
Coumaphos	87.2	85.8	90.3	91.6	88.2	83.7	92.5	87.2	92.9	86.4

(a) 10 ng/g, *(b)* 50 ng/g

Fig. 1 GC-IT/MS
chromatograms of **a** standard OP
solution (10 ng mL⁻¹ of each OP),
b freeze-dried sample (rabbit),
and **c** the same sample spiked
with standard OP solution (ana-
lytical condition—250 µL of *n*-
heptane as extraction solvent;
pH 4.1; NaCl 10 g L⁻¹). Peak
legend: 1, methacrifos; 2, pirofos;
3, phorate; 4, seraphos; 5, diazi-
non; 6, etrimfhos; 7,
dichlofenthion; 8, chlorpyrifos-
methyl; 9, pirimiphos-methyl; 10,
malathion; 11, chlorpyrifos; 12,
parathion-ethyl; 13, pirimiphos-
ethyl; 14, bromophos; 15,
chlorfenvinphos; 16, bromophos-
ethyl; 17, stirophos; 18, diethion;
IS, internal standard
(bromopropylate); 19, couma-
phos. For the experimental con-
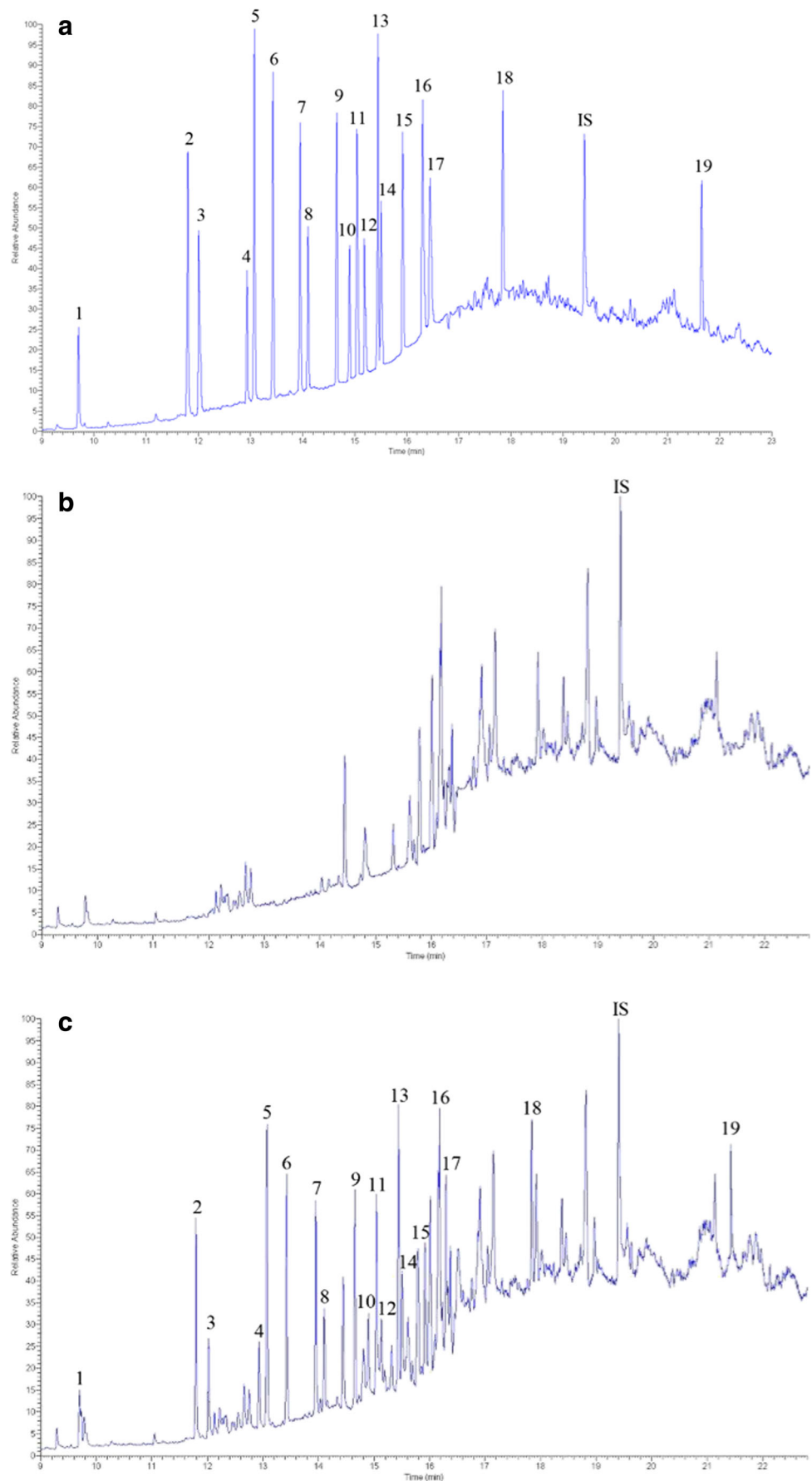ditions, see the text

Table 5 List of 19 OPs along with CAS number, formula, molecular weight, retention time, SIM of the typical fragment ion (abundance 100%), correlation coefficient in the range 5–1000 ng/g, limit of detection (LOD) (ng/g), and limit of quantification (LOQ) (ng/g) of each compound investigated in this study

Number	Compound	CAS	MW	Formula	R_t	SIM	Typical fragment	R^2	LOD	LOQ
1	Methacrifos	62610-77-9	240	$C_7H_{13}O_5PS$	9.70	125	$C_2H_6O_2PS$	0.9931	0.2 (6.1)	0.5 (7.3)
2	Pirofos	3689-24-5	322	$C_8H_{20}O_5P_2S_2$	11.80	294	$C_6H_{16}O_5P_2S_2$	0.9937	0.4 (5.8)	0.8 (6.6)
3	Phorate	298-02-2	260	$C_7H_{17}O_2PS_3$	12.00	231	$C_5H_{12}O_2PS_3$	0.9987	0.3 (6.7)	0.7 (8.0)
4	Seraphos	31218-83-4	281	$C_{10}H_{20}NO_4PS$	12.94	138	C_3H_9NOPS	0.9862	0.5 (5.0)	0.9 (6.4)
5	Diazinon	333-41-5	304	$C_{12}H_{21}N_2O_3PS$	13.07	137	$C_8H_{12}N_2O$	0.9896	0.2 (5.9)	0.7 (6.5)
6	Etrimfhos	38260-54-7	292	$C_{10}H_{17}N_2O_4PS$	13.43	153	$C_8H_{12}N_2O_2$	0.9822	0.3 (7.0)	0.8 (4.9)
7	Dichlofenthion	97-17-6	314	$C_{10}H_{13}Cl_2O_3PS$	13.95	279	$C_{10}H_{13}ClO_3PS$	0.9974	0.6 (8.5)	0.9 (6.2)
8	Chlorpyrifos-methyl	5598-13-0	321	$C_7H_7Cl_3NO_3PS$	14.10	286	$C_7H_7Cl_2NO_3PS$	0.9869	0.3 (6.0)	0.7 (5.9)
9	Pirimiphos-methyl	29232-93-7	305	$C_{11}H_{20}N_3O_3PS$	14.66	290	$C_{10}H_{17}N_3O_3PS$	0.9965	0.4 (4.2)	0.7 (7.2)
10	Malathion	121-75-5	330	$C_{10}H_{19}O_6PS_2$	14.90	173	$C_8H_{13}O_4$	0.9980	1.3 (5.6)	2.9 (8.9)
11	Chlorpyrifos	2921-88-2	349	$C_9H_{11}Cl_3NO_3PS$	15.04	197	$C_5H_2Cl_3NO$	0.9949	0.7 (7.3)	1.6 (9.2)
12	Parathion-ethyl	56-38-2	291	$C_{10}H_{14}NO_5PS$	15.18	109	C_2H_6OPS	0.9984	0.9 (6.4)	2.1 (4.7)
13	Pirimiphos-ethyl	5221-49-8	305	$C_{11}H_{20}N_3O_3PS$	15.44	153	$C_7H_{11}N_3O$	0.9929	0.5 (6.5)	1.0 (6.2)
14	Bromophos	2104-96-3	364	$C_8H_8BrCl_2O_3PS$	15.50	331	$C_8H_8BrClO_3PS$	0.9987	0.7 (5.0)	1.2 (5.8)
15	Chlorfenvinphos	470-90-6	358	$C_{12}H_{14}Cl_3O_4P$	15.92	323	$C_{12}H_{14}Cl_2O_4P$	0.9992	0.8 (8.7)	1.4 (7.3)
16	Bromophos-ethyl	4824-78-6	392	$C_{10}H_{12}BrCl_2O_3PS$	16.31	359	$C_{10}H_{12}BrClO_3PS$	0.9884	0.6 (6.7)	0.9 (6.6)
17	Stirophos	22248-79-9	364	$C_{10}H_9Cl_4O_4P$	16.45	331	$C_{10}H_9Cl_3O_4P$	0.9931	0.4 (8.0)	0.8 (5.1)
18	Diethion	563-12-2	384	$C_9H_{22}O_4P_2S_4$	17.80	231	$C_5H_{12}O_2PS_3$	0.9878	0.5 (5.4)	0.9 (7.2)
IS	Bromopropylate	18181-80-1	428	$C_{17}H_{16}Br_2O_3$	19.66	341	$C_{17}H_{16}Br_2O_3$	0.9905		
19	Coumaphos	56-72-4	362	$C_{14}H_{16}ClO_5PS$	21.66	210	$C_{10}H_7ClO_3$	0.9957	0.9 (7.2)	1.4 (6.0)

In brackets are reported the relative standard deviation, RSD, for five replicates (analytical condition—250 μL of n-heptane as extraction solvent; pH 4.1; NaCl 10 g/L)

Table 6 Recoveries (%) obtained spiking two different samples with 10 ng/g (low fortification) and 50 ng/g (high fortification) of the 19 OPs solution (analytical condition—250 μL of n-heptane as extraction solvent; pH 4.1; NaCl 10 g/L)

Compound	Freeze-dried Rabbit		Soft baby food Pear	
	10 ng/g	50 ng/g	10 ng/g	50 ng/g
Methacrifos	89.0 (7.3)	97.8 (5.1)	93.4 (8.2)	99.4 (5.9)
Pirofos	106.6 (6.2)	102.4 (2.5)	91.9 (9.2)	96.9 (4.8)
Phorate	94.6 (6.3)	98.3 (4.7)	90.1 (8.3)	95.4 (2.9)
Seraphos	109.3 (9.5)	93.9 (4.8)	84.0 (6.4)	93.4 (3.5)
Diazinon	91.1 (7.3)	100.7 (2.9)	84.0 (5.8)	96.1 (3.1)
Etrimfhos	104.1 (9.5)	101.1 (5.7)	87.3 (6.1)	101.5 (4.1)
Dichlofenthion	85.4 (7.2)	98.9 (4.9)	90.8 (8.2)	101.0 (6.2)
Chlorpyrifos-methyl	107.4 (8.4)	102.8 (6.1)	92.5 (4.9)	94.3 (1.9)
Pirimiphos-methyl	106.2 (6.9)	103.5 (5.2)	90.6 (6.2)	100.4 (2.5)
Malathion	96.4 (8.1)	99.1 (6.2)	81.1 (5.3)	90.2 (4.2)
Chlorpyrifos	105.0 (6.2)	101.0 (3.9)	85.7 (7.1)	106.8 (3.4)
Parathion-ethyl	92.5 (8.2)	95.2 (5.7)	89.2 (6.9)	96.5 (4.2)
Pirimiphos-ethyl	104.4 (6.1)	98.4 (5.4)	85.6 (4.9)	102.6 (2.6)
Bromophos	108.6 (10.3)	101.0 (4.7)	87.3 (12.2)	94.9 (7.2)
Chlorfenvinphos	107.1 (8.2)	96.9 (6.1)	81.0 (7.8)	93.3 (4.4)
Bromophos-ethyl	106.0 (7.1)	100.6 (5.9)	89.4 (6.7)	95.3 (3.7)
Stirophos	96.3 (8.9)	99.6 (4.7)	92.6 (7.4)	97.3 (5.2)
Diethion	106.3 (6.7)	102.8 (5.1)	91.2 (6.9)	94.8 (4.1)
Coumaphos	95.2 (7.2)	101.2 (4.8)	86.3 (7.2)	98.2 (5.4)

In brackets are reported the RSDs

Table 7 Comparison between the methodology proposed in this study and the ones reported in literature (recovery expressed as %; LODs and LOQs expressed as ng/g except "a" as pg/mL; −, data not available)

Analytical methodology	Baby food matrix	Recovery	LOD/LOQ	Reference
Acetonitrile-GC-FPD	Fruits, vegetables, beverages, processed foods (children)	49–250	−/5–20	(Fenske et al. 2002)
PLE-GC-MS/MS	Milk-based infant formula	50–83	0.005–0.070/0.01–0.2	(Mezcua et al. 2007)
SPE-GC-NPD	Infant formula	62–98	−/8–65	(Melgar et al. 2010)
GC-TSD	Baby milk		90/−	(Mukherjee and Gopal 1996)
GC-MS	Milk, soy	−	−/2–1000	(Gelardi and Mountford 1993)
GC-FPD	Baby rice, milk	−	−/10–30	(Authority of Ireland 2004)
QuEChERS-GC-ECD	Cereal-based	70–110	−/< 3	(Anagnostopoulos et al. 2010)
SPE-GC-MS	Apple baby	46–123	−/0.07–18.8	(Hercegová et al. 2005)
QuEChERS-GC-MS/MS	Apple baby	70–110	−/0.07–1.2	(Hercegová et al. 2006)
QuEChERS-GC-MS	Cloudy juices, purees	70–101	−/5–10	(Vukovic et al. 2012)
HF-LPME-GC-NPD	Cereal-, wheat-based	89–106	0.32–3.20/0.96–10.7	(González-Curbelo et al. 2013)
QuEChERS-GC-MS/MS(QqQ)	Chicken broth, fruit	80–110	(reported low ppb)	(Shimadzu 2013)
QuEChERS-GC-MS	Fruit-based	83–113	5–10/10–100	(Petrarca et al., 2016)
QuEChERS-LC-MS/MS	Cereals, boiled potatoes, fruit, milk	69–127	1.5–3/5–10	(Yang et al. 2014)
SPE-GC-MS/MS(QqQ)	Rabbit, chicken, beef, plaice, salmon, cheese	−	−/3–8	(Amendola et al. 2015)
QuEChERS-GC-MS/MS	Generic baby food	−	0.2–3.7/−	(Cojocariu et al. 2015)
QuEChERS-nano-LC-MS	Organic apple puree	82–108	17–111/50–336[a]	(Mirabelli et al. 2016)
QuEChERS-UHPLC-QTOFMS	Cereal-, meat-, fruit-, vegetable-based, powdered milk-based	−	−/< 10	(Pérez-Ortega et al. 2017)
UVALLME-GC-IT/MS	Turkey, rabbit, chicken, sea bream, plaice, pear, plum	81–109	0.2–1.3/0.5–2.9	This study

In all the papers using QuEChERS, acetonitrile is used as extraction solvent

[a] *QuEChERS* quick, easy, cheap, effective, robust, and safe; *TSD* thermionic specific detector; *PLE* pressurized liquid extraction; *MS/MS(QqQ)* triple quadrupole mass spectrometer; *HF-LPME* hollow-fiber liquid-phase microextraction; *UVALLLME* ultrasound-vortex-assisted liquid–liquid microextraction

Application to Real Samples

As part of this study, 16 samples (2 commercial freeze-dried and 6 soft baby foods as above described; 2 different Italian brands for each type) have been analyzed by means of the UVALLME–GC-IT/MS procedure: no OP has been detected or quantified in any sample.

Conclusions

The determination of hazard compounds in very sensitive matrices is a really important task, particularly in matrices like baby foods where even a small contamination could be very harmful for the relative sub-population (i.e., newborns and infants). The protocol set up in this paper would likely help in a rapid and effective determination of organophosphorous compounds in commercial freeze-dried and soft baby foods. Using a modified LLME extraction followed by a GC-IT/MS determination, LODs/LOQs between 0.2–1.3 and 0.5–2.9 ng/

g, respectively, and recoveries ranging between 81 and 109% were achieved. If these data are compared with similar studies in literature, this protocol could be almost considered a very interesting method for detecting OPs in baby foods worldwide, also taking into account the very low physical–chemical sample manipulation and the inexpensive costs of the entire procedure. The application of this analytical procedure to baby foods available in the Italian market (two brands) has evidenced no presence of OPs at such levels. In any case, for avoiding misunderstanding or considering the topic ended, it should be important to note some considerations: (a) pesticide traces are worldwide found in baby food in very small amounts, (b) very few studies have been performed on the "synergistic effects" of exposure to several pesticides in small amounts, and (c) OPs may act differently in combination than they do alone. The authors' question is: what does it mean to eat a celery stalk with traces of some pesticides (Ault et al. 1979), or to feed baby food to an infant laced at low levels with five? In authors' opinion, this is an interesting question to be deciphered and could be a future target in this field: a

systematic analytical approach for determining different classes of pesticides by means of an easy and effective analytical protocol (for instance, this proposed protocol applied to other pesticides) will help to solve it.

Compliance with Ethical Standards

Conflict of Interest Ivan Notardonato declares that he has no conflict of interest. Mario Vincenzo Russo declares that he has no conflict of interest. Matteo Vitali declares that he has no conflict of interest. Carmela Protano declares that he has no conflict of interest. Pasquale Avino declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human or animal subjects and so the ethical approval is not necessary and not required.

Informed Consent Not applicable. This article does not contain any studies with human or animal subjects.

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