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RESEARCH PAPER

Simultaneous determination of organophosphorus pesticides and phthalates in baby food samples by ultrasound–vortex-assisted liquid–liquid microextraction and GC–IT/MS

Ivan Notardonato[1] · Elisabetta Salimei[1] · Mario Vincenzo Russo[1] · Pasquale Avino[1,2]

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Abstract

Baby foods are either a soft, liquid paste or an easily chewed food since babies lack developed muscles and teeth to chew effectively. Babies typically move to consuming baby food once nursing or formula is not sufficient for the child's appetite. Some commercial baby foods have been criticized for their contents. This article focuses on the simultaneous determination of organophosphorus pesticides and phthalates by means of a method based on ultrasound–vortex-assisted liquid–liquid microextraction coupled with gas chromatography–ion trap mass spectrometry (GC–IT/MS). The protocol developed allowed the determination of six phthalates [dimethyl phthalate, diethyl phthalate, dibutyl phthalate, isobutyl cyclohexyl phthalate, benzyl butyl phthalate, bis(2-ethylhexyl) phthalate] and 19 organophosphorus pesticides. Freeze-dried product samples (0.1-0.2 g) were dissolved in 10 mL of warm distilled water along with 5 μL of an internal standard (anthracene at 10 mg mL^{-1} in acetone): the choice of extraction solvent was studied, with the most suitable being *n*-heptane, which is used for phthalate determination in similar matrices. The solution, held for 5 min in a vortex mixer and for 6 min in a 100-W ultrasonic bath to favor solvent dispersion and consequently analyte extraction, was centrifuged at 4000 rpm for 30 min. Then 1 μL was injected into the GC–IT/MS system (SE-54 capillary column; length 30 m, inner diameter 250 μm, film thickness 0.25 μm). All analytical parameters investigated are discussed in depth. The method was applied to real commercial freeze-dried samples: significant contaminant concentrations were not found.

Keywords Ultrasound–vortex-assisted liquid–liquid microextraction · Residue · Phthalates · Organophosphorus pesticides Gas chromatography–ion trap mass spectrometry · Baby food

Introduction

Pesticides (mainly herbicides and insecticides) are largely used to kill/repel unwanted agricultural pests, but they are also responsible for many human diseases. The widespread use of pesticides has had several benefits but has also caused many problems (e.g., they are mobile in the environment, especially through water, air, and soil; they poison humans by means of mechanisms such bioaccumulation and/or biomagnification). A recent study reviewed in depth the literature on the human health effects: the result was the implication of several environmental "factors" associated with the development of autism spectrum disorders [1]. These factors are hazardous compounds such as pesticides, phthalates, polychlorinated biphenyls, solvents, air pollutants, glyphosate, and heavy metals, especially aluminum used in vaccines as an adjuvant. Another interesting review was published by Annamalai and Namasivayam [2] in 2015. They focused on the major endocrine-disrupting chemicals present in the atmosphere: attention was devoted to phthalates, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, pesticides, brominated flame retardants, dioxins, alkylphenols, and perfluorinated

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✉ Pasquale Avino
avino@unimol.it

[1] Department of Agricultural, Environmental and Food Sciences, University of Molise, via De Sanctis, 86100 Campobasso, Italy

[2] DIT, INAIL, via R. Ferruzzi 38/40, 00143 Rome, Italy

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chemicals and on their relative sources, routes, half-lives, mechanisms, concentrations in air, and bioaccumulation.

For many years, scientists' attention has been focused on two classes of compounds: phthalates and pesticides. This is essentially due to the analytical difficulties (e.g., analysis at ultratrace levels with high accuracy, simultaneous determination) and the high toxicity of such compounds. The second issue is relevant because it is the origin of the importance of phthalates and pesticides in human health. Phthalate risk assessment is a considerable target worldwide [3–9]. Phthalates are characterized by low acute toxicity (median lethal dose of 1–30 g kg^{-1} body weight), with relevant differences between the sexes. They are not mutagenic and/or genotoxic, whereas their carcinogenicity is an open issue [10]: for instance, the carcinogenicity of diethyl phthalate (DEP) is questionable, there is no proof for the carcinogenicity of diisononyl phthalate, tumor activity could be due to dibutyl phthalate (DBP), and bis(2-ethylhexyl) phthalate (DEHP) is responsible for carcinomas [11]. One of the main sources for human exposure to these compounds is the diet: the population assimilates small quantities (residues) from different foods, such as meat, fruit, and vegetables. The European Food Safety Authority fixed daily intakes for DBP, benzyl butyl phthalate (BBP), DEHP, dinonyl phthalate, and didecyl phthalate (0.01, 0.5, 0.05, 0.15, and 0.15 mg kg^{-1} weight per day, respectively) [12]. Similarly, pesticides are subject to strict legislation worldwide [13]: In the USA, there is routine control of pesticides to be used. The European Union defined the maximum residue limit; that is, the highest level of a pesticide residue that is legally tolerated in or on food or feed when the pesticide is applied correctly (good agricultural practice; see the definition at https://ec.europa.eu/food/plant/pesticides/max_residue_levels_en). In Japan the situation is similar. The maximum residue limit definition means that the food is safe for the consumer if it contains pesticides at levels below that limit. For both contaminants (i.e., phthalates and pesticides) the legislation provides a maximum assumption level for adults, whereas there is no information for the very important subpopulation of newborns and infants, who are exposed to these compounds in freeze-dried and soft baby foods through the diet. Newborns and infants are very sensitive, and their metabolism (e.g., activation, detoxification, and excretion of xenobiotics) is not so well developed as that of adults: they should not be considered little adults, and the relative exposure limits should be different [14–16]. A report from 1993 stated that "neurologic and behavioral effects may result from low-level chronic exposure to some organophosphate and organochlorine pesticides" and "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" [17]. Twenty-five years later the issue is still very important and has not been solved: some articles (source Scopus database, between 1968 and 2017) dealt with the simultaneous determination of organochlorine pesticides and organophosphorus pesticides (OPPs) and phthalates in different matrices [18–31], whereas only two articles reported the simultaneous determination of OPPs and phthalates in similar baby foods; that is foods (and beverages) for preschool-age children [20, 32]. Although the foods are quite different as are the metabolisms of the two populations, the analytical procedure developed in the two studies [20, 32]—that is, extraction with dichloromethane, fractionation by gel permeation chromatography, and analysis by gas chromatography (GC)–mass spectrometry (MS)—allowed a limit of detection (LOD) of 0.04 ng g^{-1} to be obtained, whereas the percentage recoveries were not reported.

Here we propose a sensitive, simple, reproducible, and inexpensive analytical protocol for investigation of OPPs and phthalates at very low levels in baby food samples, including freeze-dried and soft ones. The proposed analytical protocol is based on a (modified) dispersive liquid–liquid microextraction (DLLME) procedure with no dispersant solvent. The compounds, all being apolar, were chosen considering that possible contamination could come from the chemical substances used during intensive cultivation/breeding (OPPs) and during the packaging process or could be released from the packaging (phthalates). All steps of the proposed approach will be evaluated and discussed with the aim of achieving the best analytical conditions.

Experimental

Materials

Phthalate standards (dimethyl phthalate, DEP, DBP, BBP, isobutyl cyclohexyl phthalate, BBP, DEHP) were purchased from Sigma-Aldrich (Milan, Italy), whereas pesticide standards (methacrifos, pirofos, phorate, seraphos, diazinon, etrimphos, dichlofenthion, chlorpyrifos-methyl, pirimiphos-methyl, malathion, chlorpyrifos, parathion-ethyl, pirimiphos-ethyl, bromophos, chlorfenvinphos, bromophos-ethyl, stiriphos, diethion, coumaphos) were obtained from Società Italiana Chimici (Rome, Italy): standard solutions of each OPP and phthalate were prepared at 0.1 mg mL^{-1} by our dissolving the pesticides in absolute ethanol/acetone, followed by dilution, to prepare a final mixture solution for spiking. Five microliters of bromopropylate, used as an internal standard (5 ng μL^{-1}), was added to each sample before the whole analytical procedure was started. Isooctane, *n*-heptane, benzene, toluene, cyclohexane, ethyl ether, and sodium chloride (ACS reagent grade) were obtained from Carlo Erba (Milan, Italy).

Ultrasound–vortex-assisted DLLME procedure

The extraction method is based on a variant of the DLLME method. About 5 g of soft baby food sample was freeze-dried

for 4 h at -53 °C and 0.017 mbar in an LIO5P freeze-drier (Cinquepascal, Trezzano sul Naviglio, Italy). Afterward, 0.1–0.2 g of each freeze-dried sample was transferred into a 10-mL screw-cap glass tube with a conical bottom, and 10 mL of distilled water at pH 4.0–4.2 was added along with 5 μL of the internal standard and 250 μL of *n*-heptane as the extraction solvent. The dispersion was performed by means of 5 min of vortex mixing by mechanical rotation followed by 6 min in an ultrasonic bath. The solution became cloudy. To promote the separation, 0.1 g of NaCl (10 g L^{-1}) was added. Finally, the solution was centrifuged at 4000 rpm for 30 min: 1 μL of the final solution was injected into the GC–ion trap (IT)/MS system to determine the six phthalates and 19 OPPs.

GC–IT/MS analysis

A Trace GC Ultra gas chromatograph (Thermo Finnigan, Bremen, Germany) equipped with a programmed temperature vaporizer injector and connected to a PolarisQ IT mass spectrometer (Thermo Finnigan) and an Xcalibur data system (Thermo Fischer Scientific, Waltham. MA,USA) was used for GC–MS analysis in total ion current and selected ion monitoring modes.

A homemade fused-silica capillary column with a chemically bonded phase (SE-54; 5% phenyl–95% dimethylpolysiloxane; length 30 m, inner diameter 250 μm, film thickness 0.25 μm)was used for economic reasons. In previous studies [33–36] it was demonstrated that the chromatographic parameters are quite similar between this column and commercial ones.

Helium was used as the carrier gas at constant flow rate of 1 mL min^{-1} and as the dumping gas in the IT at 0.3 mL min^{-1}. The programmed temperature vaporizer injection was performed in splitless mode; 10 s after injection the vaporizer was heated from 100 to 280 °C at 800 °C min^{-1} and cooled after 5 min; the splitless valve was opened 120 s after the injection. The column was kept at 90 °C for 60 s, and then the temperature was changed from 90 to 290 °C at 10 °C min^{-1}. The transfer line and the ion source were held at 270 and 250 °C, respectively. The chromatogram was captured in positive electron impact mode (70 eV) in the range between 55 and 380 amu.

All samples were quantified in triplicate: the concentrations were obtained from calibration graphs of the ratio of the area for the OPP/phthalate to the area for the internal standard versus the concentration of each OPP/phthalate (pg μL^{-1}).

Results and discussion

Optimization of the extraction protocol

Starting from the scope of proposing an analytical method for the simultaneous determination of OPPs and phthalates, particular attention should be paid to the matrix. Baby foods are present on the (Italian) market in two different commercial forms: soft baby food and freeze-dried baby food. The first is soft and gelatinous, and the second is a powder. The different compositions were reflected during processing of the samples, particularly during the extraction step: the gelatinous sample does not allow a good emulsion to be obtained for the protocol, unlike the powder sample, which is ready for the analytical procedure. Particularly, in the first case, an extremely gelatinous form is obtained, and it prevents further steps of the procedure from being performed. We decided to add another step (i.e., a lyophilization process; performed at -52 °C and 0.080 mbar for 4 h in a freeze-drier system) to obtain, also in this case, a powder sample for analysis. The whole procedure becomes longer, but the result is a clear solution to be subjected to the proposed analytical procedure.

With use of a standard mixture solution formed from 19 OPPs (each at 50 ng g^{-1}) and six phthalates (each at 50 ng g^{-1}), all the analytical parameters were investigated, such as the extraction solvent, the influence of pH on the extraction process, the salt effect, the method reproducibility, and the recoveries at different concentrations.

The first step of the proposed analytical protocol was to find the best extraction solvent, followed by identification of the pH for optimization of the procedure. For this aim, six solvents (250 μL)—*n*-hexane, *n*-heptane, isooctane, benzene, toluene and diethyl ether—were considered, whereas the use of chlorinated solvents was rejected. The solvents were chosen for their density being less than that of water (0.6548 g cm^{-3}, 0.6795 g cm^{-3}, 0.692 g cm^{-3}, 0.8765 g cm^{-1}, 0.87 g cm^{-3}, and 0.7134 g cm^{-3}, respectively) to avoid elimination of the supernatant.

Basically, DLLME is based on the addition of a dispersant solvent to improve the contact between two immiscible solvents and that has the characteristic of being soluble in both immiscible solvents: so the interface plays an important role in the extraction process, and its development is facilitated by the addition of just the dispersant solvent. In this case, this solvent is not added, but the same performance should be obtained by the combined action of a vortex and ultrasound.

The results are reported in Fig. 1. The two aromatic solvents (i.e., benzene and toluene) result in poor recoveries ranging between 69% and 83% and between 40% and 70%, respectively, whereas diethyl ether does not recover any OPP or phthalate, meaning that neither aromatic hydrocarbons nor polar solvents give good analytical conditions. On the other hand, all the linear or branched hydrocarbons (i.e. *n*-hexane, *n*-heptane, and isooctane) gave good OPP and phthalate recoveries: 91–110%, 91–110%, and 88–110%, respectively. Looking at these first results, we decided to continue the optimization of the procedure by testing *n*-hexane, *n*-heptane, and isooctane.

Vortex mixing and ultrasonication are two important steps of the whole analytical procedure: both operations are necessary to

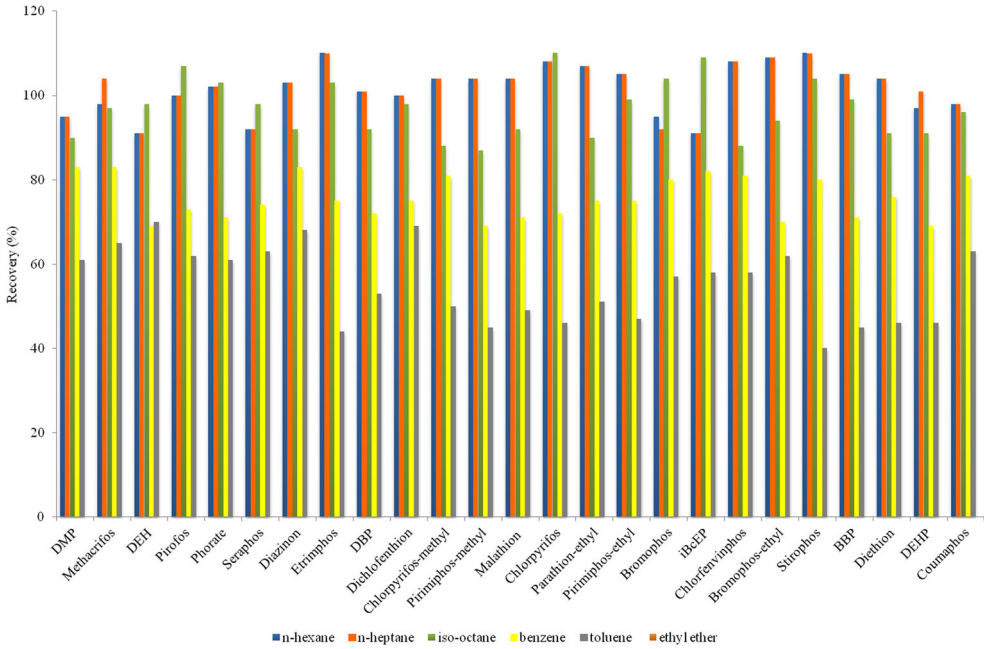

Fig. 1 Recoveries according to the different solvents tested in this study (each organophosphorus pesticide and phthalate at 50 ng g^{-1}). The relative standard deviations average less than 9%. BBP benzyl butyl phthalate, DBP dibutyl phthalate, DEHP bis(2-ethylhexyl) phthalate, DEP diethyl phthalate, DMP dimethyl phthalate, iBcEP butyl cyclohexyl phthalate

optimize the extraction instead of using the dispersant solvent. Different mixing times and solution temperatures were investigated in both cases. Tables 1 and 2 show the cumulative recoveries obtained during vortex mixing and ultrasonication: 250 μL of *n*-heptane, 5 min of vortex mixing, and 6 min of ultrasonication at 20/25 °C (room temperature) resulted in good recoveries being obtained for all the compounds.

The data related to the centrifugation step are reported in Table 3. Looking at the data, we see the combination of 20 min and 5000 rpm is better than the others but the relative standard deviations (RSDs) are greater; on the other hand, the coupling of 10 min and 1000 rpm does not result in mixing of the solutions. The best condition is 30 min at 4000 rpm for both solvents. After these experiments, we focused our attention on *n*-heptane as the extraction solvent even though *n*-hexane and isooctane have similar performances (but with higher RSDs for the branched hydrocarbon).

Following these considerations, some experiments were also performed to identify the optimum pH. Preliminarily, the pH was studied in the range from 3.5 to 8.2 (Fig. 2): at pH 4.1 the recoveries are quite good (91–110%), whereas they drastically decrease at lower pH (49–77% at pH 3.5) or higher pH (79–91% at pH 5.1 and 71–81% at pH 6.2) and they become very high at pH 7.4 (more than 169%) and pH 8.2 (more than 169%). Afterward, to better narrow the pH range, some experiments were performed at five different pH values in the range between 3.8 and 4.5 (i.e., 3.8, 4.0, 4.1, 4.2, and 4.5) (data not shown): the best pH values were around 4.1 (recoveries ranged between 90% and 104% at pH 4.0, between 95% and 110% at pH 4.1, and between 96% and

Table 1 Cumulative organophosphorus pesticide and phthalate recoveries (%) related to different mixing times and solution temperatures obtained with three hydrocarbons for vortex mixing

	Mixing time				
	3 min	4 min	5 min	6 min	10 min
n-Hexane	75–98	96–105	91–106	90–108	92–101
n-Heptane	82–94	91–106	92–108	91–108	90–110
Isooctane	80–94	93–104	91–109	89–104	91–104
	Solution temperature				
	10 °C	15 °C	20 °C	25 °C	30 °C
n-Hexane	81–95	96–105	94–91	97–109	84–98
n-Heptane	85–101	90–101	91–107	95–104	91–95
Isooctane	79–99	93–104	92–109	91–102	81–96

The conditions were as follows: each organophosphorus pesticide and phthalate at 50 ng g^{-1}, and 250 μL of extraction solvent.

Table 2 Cumulative organophosphorus pesticide and phthalate recoveries (%) related to different mixing times and solution temperatures obtained with three hydrocarbons for sonication

	Mixing time				
	3 min	4 min	5 min	6 min	10 min
n-Hexane	35–56	84–105	91–109	90–109	89–109
n-Heptane	49–51	89–101	91–110	92–110	89–105
Isooctane	44–79	73–84	87–110	89–102	88–106
	Solution temperature				
	10 °C	15 °C	20 °C	25 °C	30 °C
n-Hexane	80–97	95–106	94–91	93–109	81–98
n-Heptane	80–94	92–104	92–106	91–105	84–105
Isooctane	79–99	79–106	92–109	91–109	81–99

The conditions were as follows: each organophosphorus pesticide and phthalate at 50 ng g^{-1}, and 250 μL of extraction solvent.

Table 3 Cumulative organophosphorus pesticide and phthalate recoveries (%) related to different rotation times obtained with three hydrocarbons

Rotation time (min)	Compound	Rotation speed (rpm)				
		1000	2000	3000	4000	5000
10	*n*-Hexane	–	66–85	74–91	79–92	84–98
	n-Heptane	–	70–81	70–83	75–94	91–95
	Isooctane	–	73–84	81–89	79–92	81–96
20	*n*-Hexane	–	71–83	78–91	84–99	90–109
	n-Heptane	74–85	74–86	84–93	81–101	92–110
	Isooctane	69–85	73–91	79–90	83–103	89–108
30	*n*-Hexane	74–89	81–93	84–98	91–109	91–108
	n-Heptane	84–96	83–91	85–93	91–110	92–110
	Isooctane	81–88	73–91	79–94	87–110	87–111
40	*n*-Hexane	88–109	89–105	89–109	91–109	90–109
	n-Heptane	85–104	91–108	91–110	91–110	91–107
	Isooctane	88–102	84–104	87–110	87–110	89–109

The conditions were as follows: each organophosphorus pesticide and phthalate at 50 ng g^{-1}, 250 μL of extraction solvent, vortex mixing for 5 min, ultrasonication for 6 min, and 25 °C.

110% at pH 4.2), whereas at pH 4.5 the OPP/phthalate recoveries were very low (77–96%).

The salt effect is another parameter investigated, particularly the effect of salting out; that is, the addition of salt to a solution to reduce the solubility of the electrolyte and break the emulsion. Different amounts (5, 10, 15, and 20 g L^{-1}) of four salts (i.e., sodium chloride, potassium chloride, calcium chloride, and sodium sulfate) were tested. Figure 3 shows the cumulative recoveries of the OPPs and phthalates for the different amounts of the salts, whereas Fig. 4 reports the data for the addition of NaCl; to avoid adding too much salt, we decided to use NaCl at 10 g L^{-1}, and this addition allows the emulsion to be broken and the total analyte extraction.

The entire procedure (best analytical conditions of 250 μL of *n*-heptane as the extraction solvent, vortex mixing for 5 min, ultrasonication for 6 min, 25 °C, pH 4.1, centrifugation for 30 min at 4000 rpm, and NaCl at 10 g L^{-1}) was applied to investigate the analytical parameters for determining OPPs and phthalates in baby foods, such as commercial freeze-dried products (chicken, rabbit, and turkey) and four soft baby foods (chicken, rabbit, sea bream, and plaice).

Protocol validation

Table 4 shows the analytical parameters for the OPPs and phthalates investigated in this study: together with the CAS

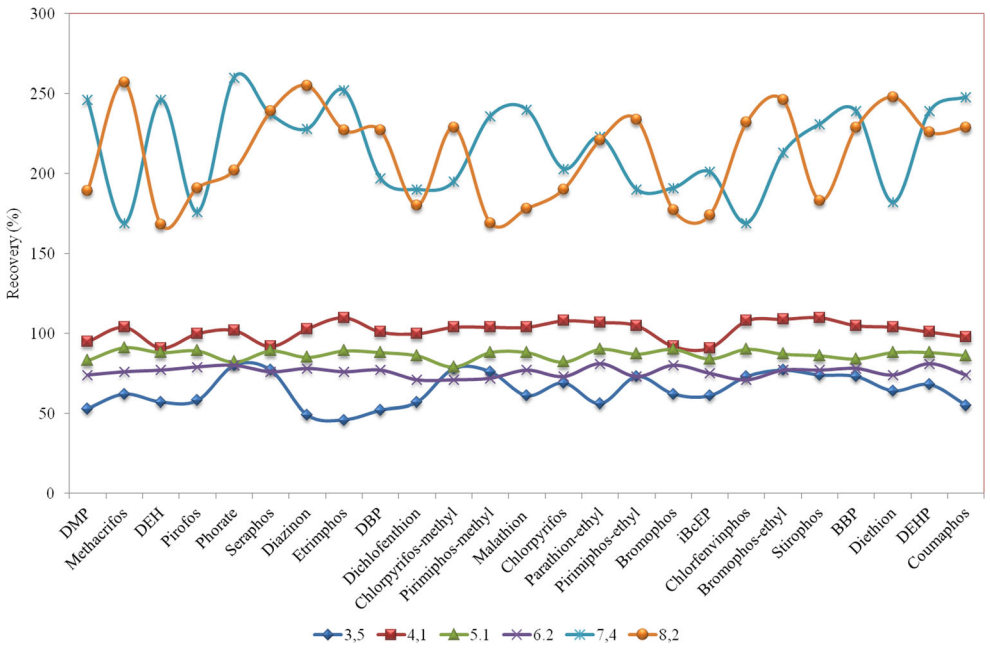

Fig. 2 Effect of pH on the organophosphorus pesticide and phthalate recoveries (each organophosphorus pesticide and phthalate at 50 ng g^{-1}, 250 μL of *n*-heptane as extraction solvent, vortex mixing for 5 min, ultrasonication for 6 min, 25 °C, centrifugation for 30 min at 4000 rpm). The relative standard deviations average less than 12%. BBP benzyl butyl phthalate, DBP dibutyl phthalate, DEHP bis(2-ethylhexyl) phthalate, DEP diethyl phthalate, DMP dimethyl phthalate, iBcEP isobutyl cyclohexyl phthalate

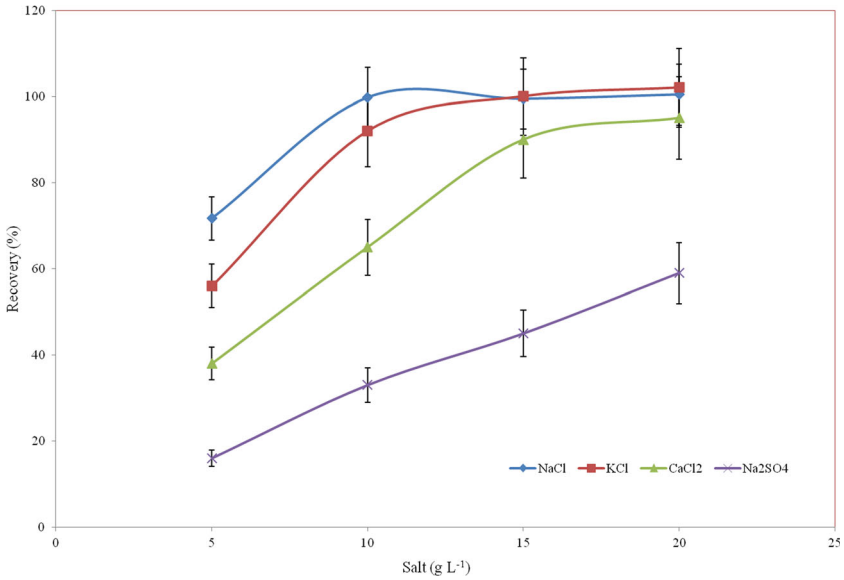

Fig. 3 Cumulative recoveries of organophosphorus pesticides and phthalates for different amounts of sodium chloride, potassium chloride, calcium chloride, and sodium sulfate (each organophosphorus pesticide and phthalate at 50 ng g^{-1}, 250 μL of *n*-heptane as extraction solvent, vortex mixing for 5 min, ultrasonication for 6 min, 25 °C, pH 4.1, centrifugation for 30 min at 4000 rpm)

Registry Number and the selected ion monitoring *m/z* values (abundance 100%), the linear equations, correlation coefficients (R^2), LODs, and limits of quantification (LOQs) are given for each compound. Even though there are some compounds with ions with the same *m/z* values, there was no overlapping in the chromatograms because of the different retention times of the various compounds. Figure 5 shows a typical chromatogram of the standard mixture solution (each OPP and phthalate at 50 ng g^{-1}) subjected to the whole procedure: as can be seen, no peak overlapping is present nor do interferences affect the qualitative and quantitative analysis, and the peaks are well shaped and clear. Good linearity (i.e., the relationship between signal and concentration in the range from 10 to 5000 ng g^{-1}) is testified both by the good coefficients of determination ($R^2 > 0.9446$) for all the compounds and by the RSD (*y*-mean in the linear equations), averaging 2.5%. For the linearity, a seven-point calibration curve was plotted in the concentration range from 10 to 5000 ng g^{-1} (i.e., 10, 50, 100, 200, 500, 1000, and 5000 ng g^{-1}). The LODs of the compounds analyzed, with use of the typical fragment ion for each of them, are between 0.2 and 4.7 ng g^{-1}, with an RSD of 14% or less, whereas the LOQs are between 2.3 and 8.5 ng g^{-1}, with an RSD of 11% or less. These values were determined according to Knoll's definition [37]; that is, an analyte concentration that produces a chromatographic peak equal to three times (LOD) or seven times (LOQ) the standard deviation of the baseline noise. The LODs and LOQs, directly determined in the matrices investigated, are significant for analyzing OPPs and phthalates in these foods. Even though the levels may be high compared with those in previous studies of

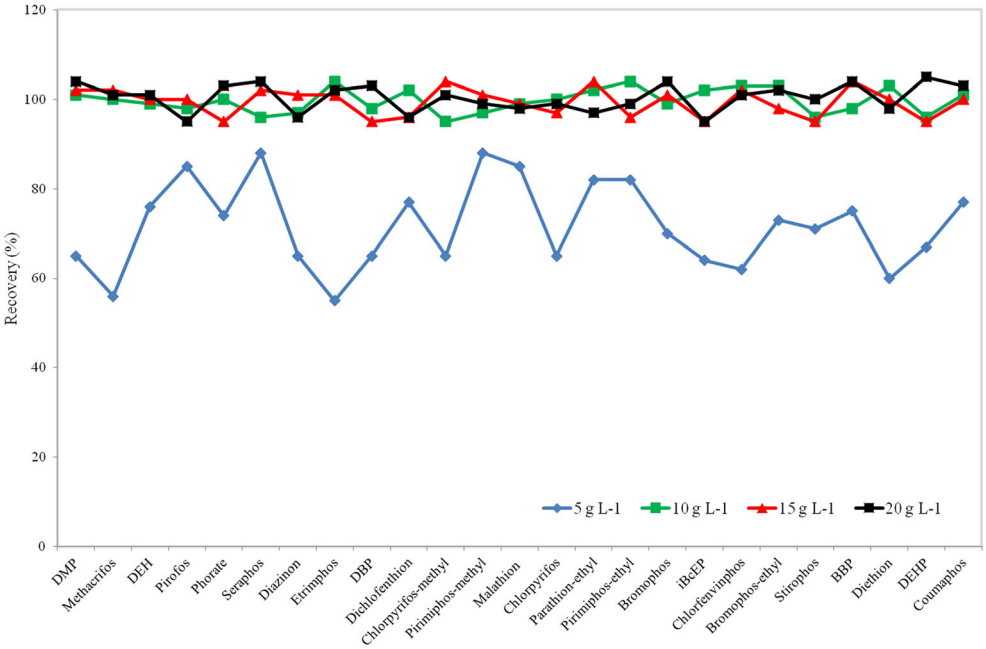

Fig. 4 Salting-out effect on the organophosphorus pesticide and phthalate recoveries (each organophosphorus pesticide and phthalate at 50 ng g^{-1}, 250 μL of *n*-heptane as extraction solvent, vortex mixing for 5 min, ultrasonication for 6 min, 25 °C, pH 4.1, centrifugation for 30 min at 4000 rpm). BBP benzyl butyl phthalate, DBP dibutyl phthalate, DEHP bis(2-ethylhexyl) phthalate, DEP diethyl phthalate, DMP dimethyl phthalate, iBcEP butyl cyclohexyl phthalate

Table 4 The 19 organophosphorus pesticides and six phthalates along with the CAS Registry Number, retention time (t_R), selected ion monitoring (SIM) m/z of the typical fragment ion (abundance 100%), regression equation, correlation coefficient (R^2) in the range from 10 to 5000 ng g^{-1}, limit of detection (LOD), limit of quantification (LOQ), and intraday and interday pericision (as the relative standard deviation, RSD) of each compound investigated in this study

Compound	CAS Registry Number	t_R (min)	SIM m/z	Regression equation	R^2	LOD (ng g^{-1})	LOQ (ng g^{-1})	Intraday precision (RSD, %)	Interday precision (RSD, %)
DMP	131-11-3	9.32	194	$y = 8.1613x - 0.1052$	0.9446	2.9	4.9	3.3	4.1
Methacrifos	62610-77-9	9.70	125	$y = 2.8754x + 0.3564$	0.9931	1.4	7.6	7.6	8.4
DEP	84-66-2	10.84	177	$y = 6.6719x - 0.0413$	0.9605	1.0	6.3	8.6	7.4
Pirofos	3689-24-5	11.80	294	$y = 3.7842x + 0.7468$	0.9937	0.2	3.4	4.2	6.8
Phorate	298-02-2	12.00	231	$y = 4.1481x + 0.7103$	0.9987	2.6	8.3	6.5	7.2
Seraphos	31218-83-4	12.94	138	$y = 2.7956x - 0.6495$	0.9862	3.6	7.2	5.8	6.6
Diazinon	333-41-5	13.07	137	$y = 2.5971x + 0.4563$	0.9896	2.4	4.9	8.7	7.7
Etrimphos	38260-54-7	13.43	153	$y = 3.7695x - 0.4223$	0.9822	4.3	6.6	5.2	8.0
DBP	84-74-2	13.72	205	$y = 6.9572\ x + 0.2675$	0.9822	0.4	2.3	3.9	3.5
Dichlofenthion	97-17-6	13.95	279	$y = 2.7448x + 0.6215$	0.9974	0.7	5.4	2.8	4.7
Chlorpyrifos-methyl	5598-13-0	14.10	286	$y = 3.5985x + 1.4344$	0.9869	0.3	2.5	4.0	4.9
Pirimiphos-methyl	29232-93-7	14.66	290	$y = 2.2594x - 0.5323$	0.9965	1.5	3.5	5.4	7.0
Malathion	121-75-5	14.90	173	$y = 7.8131x + 0.9256$	0.9980	0.7	4.2	8.5	9.7
Chlorpyrifos	2921-88-2	15.04	197	$y = 4.0076x + 0.2159$	0.9949	0.2	3.7	4.8	5.6
Parathion-ethyl	56-38-2	15.18	109	$y = 4.8467x + 0.5194$	0.9984	0.4	2.9	4.6	7.5
Pirimiphos-ethyl	5221-49-8	15.44	153	$y = 3.5926x + 0.7042$	0.9929	4.7	6.9	8.6	7.9
Bromophos	2104-96-3	15.50	331	$y = 3.4692x - 0.6043$	0.9987	1.1	7.0	7.1	6.9
iBcEP	84-64-0	15.83	223	$y = 5.8947x + 0.2949$	0.9568	4.4	7.5	9.8	9.0
Chlorfenvinphos	470-90-6	15.92	323	$y = 3.7359x - 0.1970$	0.9992	4.2	6.9	2.8	5.3
Bromophos-ethyl	4824-78-6	16.31	359	$y = 3.8716x - 0.5729$	0.9884	3.5	4.3	4.6	5.5
Stirophos	22248-79-9	16.45	331	$y = 2.7587x + 0.3994$	0.9931	3.9	7.0	4.3	8.3
BBP	85-68-7	17.43	206	$y = 7.8947x - 0.2949$	0.9895	0.6	7.5	4.7	6.6
Diethion	563-12-2	17.80	231	$y = 2.7830x + 0.9321$	0.9878	1.9	3.9	9.6	9.3
DEHP	117-81-7	18.98	167	$y = 5.0296x + 1.4133$	0.9938	1.2	6.3	2.8	5.6
Coumaphos	56-72-4	21.66	210	$y = 3.5694x + 0.2834$	0.9957	4.7	8.5	3.3	5.7

The analytical conditions were as follows: 250 μL of *n*-heptane as extraction solvent, vortex mixing for 5 min, ultrasonication for 6 min, 25 °C, pH 4.1, centrifugation for 30 min at 4000 rpm, NaCl at 10 g L^{-1}.

BBP benzyl butyl phthalate, *DBP* dibutyl phthalate, *DEHP* bis(2-ethylhexyl) phthalate, *DEP* diethyl phthalate, *DMP* dimethyl phthalate, *iBcEP* butyl cyclohexyl phthalate

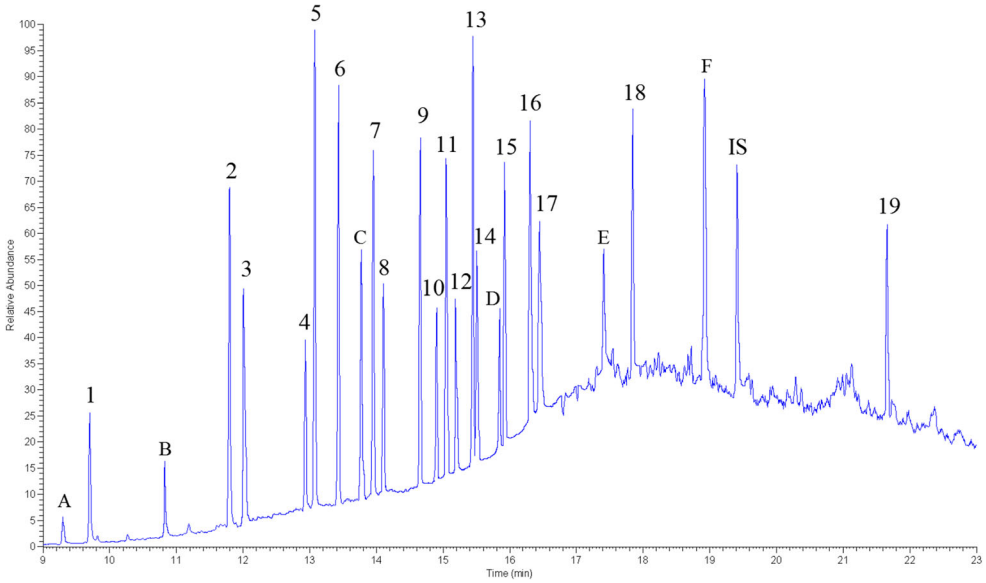

Fig. 5 Gas chromatography–ion trap mass spectrometry chromatograms of standard organophosphorus pesticide and phthalate mixture solution (each organophosphorus pesticide and phthalate at 50 ng g^{-1}; 250 μL of *n*-heptane as extraction solvent, vortex mixing for 5 min, ultrasonication for 6 min, 25 °C, pH 4.1, centrifugation for 30 min at 4000 rpm; NaCl at 10 g L^{-1}). For the experimental conditions, see the text. 1 methacrifos, 2 pirofos, 3 phorate, 4 seraphos, 5 diazinon, 6 etrimphos, 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, 19 coumaphos, A dimethyl phthalate, B diethyl phthalate, C dibutyl phthalate, D butyl cyclohexyl phthalate, E benzyl butyl phthalate, F bis(2-ethylhexyl) phthalate, IS internal standard

Table 5 Recoveries obtained after the spiking of two different baby food samples with a solution of the 19 organophosphorus pesticides and six phthalates at 20 ng g^{-1} (low fortification) and 500 ng g^{-1} (high fortification)

Compound	Recovery (%)			
	Soft		Freeze-dried	
	20 ng g^{-1}	500 ng g^{-1}	20 ng g^{-1}	500 ng g^{-1}
DMP	110 (5)	92 (4)	103 (4)	101 (2)
Methacrifos	110 (9)	106 (6)	98 (7)	92 (4)
DEP	101 (6)	105 (3)	93 (6)	108 (4)
Pirofos	108 (8)	96 (5)	96 (7)	94 (4)
Phorate	103 (8)	93 (6)	108 (8)	99 (3)
Seraphos	95 (7)	104 (4)	95 (8)	106 (6)
Diazinon	105 (7)	104 (3)	106 (6)	99 (3)
Etrimphos	102 (9)	96 (5)	106 (7)	92 (4)
DBP	98 (3)	102 (1)	110 (8)	106 (5)
Dichlofenthion	94 (6)	108 (2)	105 (6)	108 (4)
Chlorpyrifos-methyl	108 (8)	101 (4)	108 (6)	105 (6)
Pirimiphos-methyl	98 (8)	92 (4)	107 (7)	109 (6)
Malathion	93 (9)	94 (5)	94 (7)	107 (3)
Chlorpyrifos	94 (6)	104 (3)	110 (8)	109 (6)
Parathion-ethyl	103 (9)	109 (6)	107 (8)	95 (4)
Pirimiphos-ethyl	110 (7)	105 (4)	96 (5)	98 (2)
Bromophos	105 (7)	98 (5)	106 (5)	97 (3)
iBcEP	98 (8)	92 (3)	109 (7)	105 (4)
Chlorfenvinphos	94 (7)	109 (5)	109 (8)	94 (5)
Bromophos-ethyl	91 (6)	110 (4)	92 (5)	94 (3)
Stirophos	91 (9)	93 (6)	93 (8)	99 (3)
BBP	94 (8)	108 (5)	94 (7)	105 (5)
Diethion	90 (9)	94 (6)	104 (8)	109 (4)
DEHP	109 (3)	108 (3)	92 (6)	109 (6)
Coumaphos	91 (3)	97 (1)	104 (7)	104 (3)

The analytical conditions were as follows 250 μL of *n*-heptane as extraction solvent, vortex mixing for 5 min, ultrasonication for 6 min, 25 °C, pH 4.1, and NaCl at 10 g L^{-1}. The relative standard deviation is given in parentheses.

BBP benzyl butyl phthalate, *DBP* dibutyl phthalate, *DEHP* bis(2-ethylhexyl) phthalate, *DEP* diethyl phthalate, *DMP* dimethyl phthalate, *iBcEP* isobutyl cyclohexyl phthalate

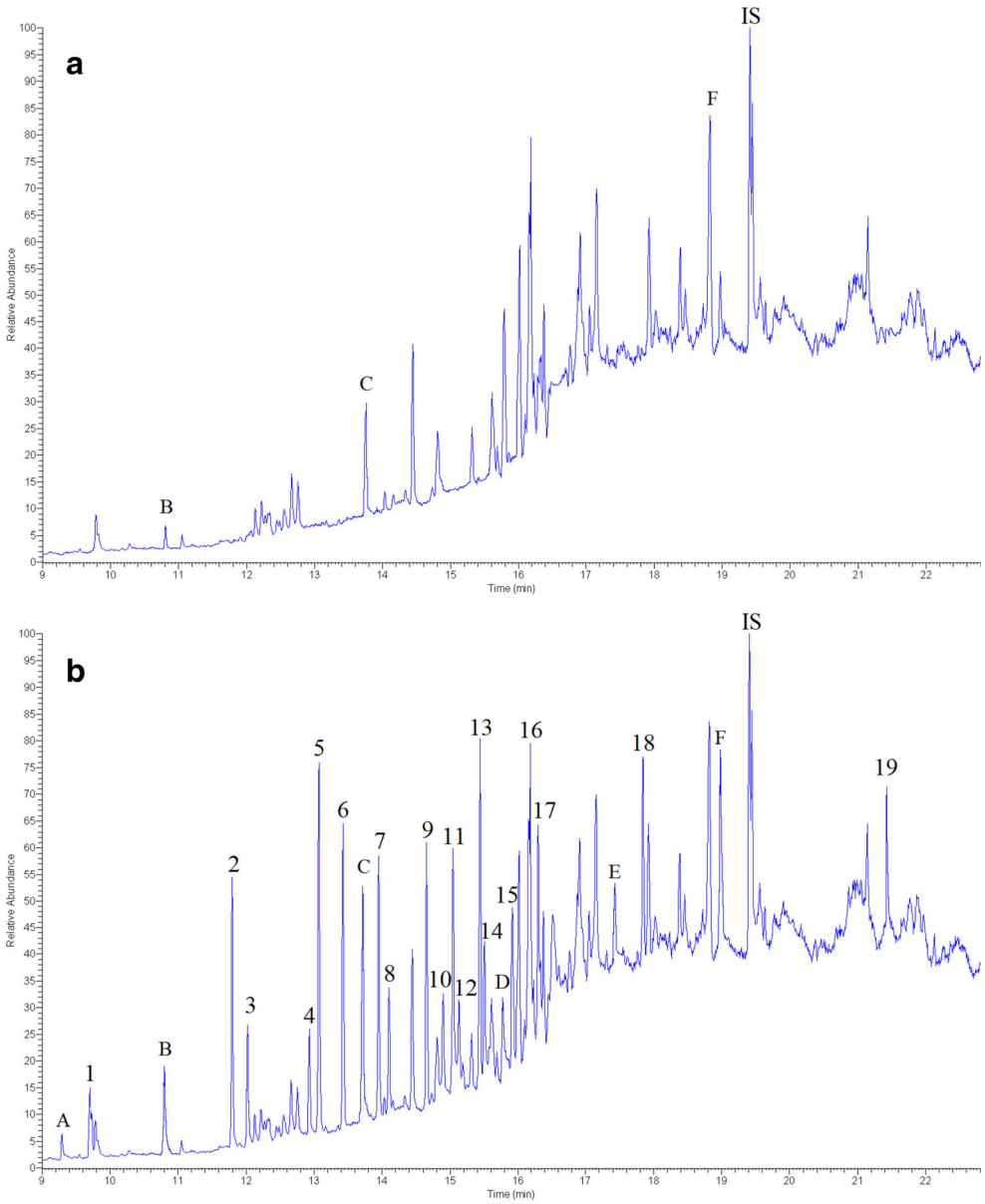

Fig. 6 Gas chromatography–ion trap mass spectrometry chromatograms of **a** freeze-dried sample (turkey) and **b** the same sample spiked with standard organophosphorus pesticide and phthalate mixture solution (250 μL of *n*-heptane as extraction solvent, vortex mixing for 5 min, ultrasonication for 6 min, 25 °C, pH 4.1, centrifugation for 30 min at 4000 rpm, NaCl at 10 g L^{-1}). For the experimental conditions, see the text. 1 methacrifos, 2 pirofos, 3 phorate, 4 seraphos, 5 diazinon, 6 etrimphos, 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, 19 coumaphos, A dimethyl phthalate, B diethyl phthalate, C dibutyl phthalate, D isobutyl cyclohexyl phthalate, E benzyl butyl phthalate, F bis(2-ethylhexyl) phthalate, IS internal standard

similar compounds [14, 38–47], it should be considered that, for the first time in the literature, the proposed analytical protocol deals with the simultaneous determination of OPPs and phthalates in baby foods, avoiding invasive extraction procedures.

The precision and accuracy are also reported in Table 4. Two different solutions were injected six times on 1 day (intraday precision or accuracy) and on two different days, respectively (interday precision or accuracy). The RSDs with respect to the retention times were between 0.42% and 0.65% for intraday precision and between 0.58% and 0.82% for interday precision; on the other hand, in the case of the corrected peak area ratio, the RSDs for intraday precision ranged from 2.8% to 9.8% and for interday precision ranged from 3.5% to 9.7%, meaning the method is precise.

Finally, to complete the robustness assessment of the proposed analytical protocol, the recoveries were studied in depth in both matrices (soft and freeze-dried baby foods) and at different concentrations after their spiking with standard mixture solutions (i.e., 20 ng g^{-1} and 500 ng g^{-1}). It should be underlined that the recoveries were calculated for addition of the spiking solution and the internal standard before the analytical procedure was started in both cases, and they were added before the soft baby food was subjected to the lyophilization process. In this way all the protocol can be monitored and the recoveries are representative of the entire procedure. Table 5 lists the percentage recoveries of the 19 OPPs and six phthalates in soft and freeze-dried baby foods and at two different concentrations (20 ng g^{-1} and 500 ng g^{-1}) in freeze-dried products. The recoveries range between 90% and 110% for

Table 6 Comparison between the analytical parameters found in this study and those reported in other studies

Compounds	Matrix	Recovery (%)	LOD/LOQ (ng g^{-1})	Reference
Phthalates, pesticides	Alcoholic beverages, wine	–	Parts per billion levels/–	[18]
Phthalates, chlorinated pesticides	pork, beef, fish, chicken, egg, rice	102–116	–/–	[19]
PAHs, OCPs, PCBs, 2 OPPs, 3 phenols, 2 phthalates	Indoor and outdoor air, play area soil, floor dust, daily liquid/solid food	–	0.04–0.5/–	[20]
OPPs, OCPs, phthalates	Bergamot essential oils	94–106	2–40[a]/–	[21]
OPPs, OCPs, phthalates	Citrus essential oils	–	2–95[a]/–	[22]
Phthalates	Oily food	–	100–1000/–	[23]
Pesticides, PAHs, PCBs, phthalates	Fruit juices, fruits, milk	74–104	0.01–12.7/–	[24]
OPPs, phthalates, bisphenol A	Urine	–	0.01–2.0[b]/–	[25]
Mycotoxins, phthalates, PAHs, metals	Oil and flour	–	–/–	[26]
PAHs, phthalates, OCPs, PCBs	Vegetables	–	–	[27]
OCPs, phthalates	Soil	72–106	0.05–1.0/–	[28]
Bisphenol A, parabens, phthalates, OPPs	Urine	–	0.01–0.84[b]/–	[29]
Phthalates, OCPs	Agricultural soils, vegetables	77–108	0.10–0.45/–	[30]
OPPs, OCPs, phthalates	Raw tea, infusion tea	66–101	4–18/10–58	[31]
PAHs, phthalates, PCBs, OCPs, OPPs	Indoor and outdoor air, food, beverages, dust, soil	–	0.04–0.5/–	[32]
OPPs, phthalates	Turkey, rabbit, chicken, sea bream, plaice	90–110	0.2–4.7/2.3–8.5	This study

LOD limit of detection, *LOQ* limit of quantification, *OCP* organochlorine pesticide, *OPP* organophosphorus pesticide, *PAH* polycyclic aromatic hydrocarbon, *PCB* polychlorinated biphenyl

[a] Picograms per gram

[b] Micrograms per liter

the soft samples and between 92% and 110% for the freeze-dried samples, with RSDs of 9% or less and 8% or less, respectively. Once again, these values take into account all the analytical procedure (i.e., lyophilization, OPP extraction, and GC–IT/MS analysis steps), avoiding any further physical-chemical treatment of the sample. The recoveries are not based on the matrix, and the errors (as the RSD) are higher in the soft samples than in the freeze-dried ones because of the further preliminary process (i.e., lyophilization step) to which the soft baby foods were subjected before the entire analytical procedure was applied.

Figure 6 shows the chromatograms obtained from analysis of a freeze-dried (turkey) sample (Fig. 6a) and the same sample spiked with the standard OPP and phthalate solution (Fig. 6b): the chromatograms confirmed the determination of all the compounds.

Comparison with similar studies

We compared the recoveries and LODs/LOQs with those obtained by other authors [18–32] (Table 6). From Table 6 it can be seen that only a few articles investigated the all analytical parameters (i.e., recoveries, LODs, and LOQs), and the articles mainly focused on LOD determination. Further, in the various studies the recoveries range between 66% and 108%, except for two studies where the LODs are very high and studies where the LODs are not reported. Among the different studies, two are similar to this study: they are dedicated to the investigation of some persistent organic pollutants (POPs) in food/beverage matrices served to preschool children [20, 32]. Among the different POPs, two OPPs (diazinon and chlorpyrifos) and two phthalates (DBP and BBP) were determined: LODs of 0.04–0.5 ng g^{-1} were found for all the compounds, whereas no information was given for the recoveries or other analytical parameters. The proposed analytical protocol allowed the determination of 19 OPPs and six phthalates in commercial soft and freeze-dried baby foods at parts per billion (nanograms per gram) levels with good precision and accuracy as demonstrated by all the experimentation.

Application to real samples

Seven commercial baby food samples (three freeze-dried baby food samples, i.e., chicken, rabbit, and turkey, and four soft baby food samples, i.e., chicken, rabbit, sea bream, and plaice) were analyzed by means of the ultrasound–vortex-assisted DLLME–GC–IT/MS procedure: no OPP was detected or quantified (levels below the LOD) in any sample, whereas DEP, DBP, and DEHP were quantified in almost all the samples at levels ranging between 1 and 40 ng g^{-1} (Table 7). DEP

Table 7 Levels of phthalates found in the seven commercial (soft and freeze-dried) baby foods analyzed by means of the proposed analytical protocol

Compound	Commercial baby food			
Soft				
	Chicken	Rabbit	Turkey	
DEP	2.3±0.2	< LOD	1.4±0.2	
DBP	3.8±0.3	1.4±0.2	1.1±0.1	
DEHP	39.9±1.5	12.1±0.7	6.8±0.5	
Freeze-dried				
	Chicken	Rabbit	Sea bream	Plaice
DEP	< LOD	6.3±0.5	1.5±0.1	< LOD
DBP	12.0±1.1	17.2±1.4	< LOD	6.5±0.4
DEHP	25.0±2.3	34.6±1.8	16.0±1,1	16.4±0.8

In all the samples the organophosphorus pesticides are below the limits of detection.

LOD limit of detection, *DBP* dibutyl phthalate, *DEHP* bis(2-ethylhexyl) phthalate, *DEP* diethyl phthalate

and DBP in a few samples were determined at levels below their LOQs but above the LODs, demonstrating the levels of such compounds in the matrices and the importance of a very sensitive and accurate analytical method.

Conclusions

The analysis of hazardous compounds in food matrices is a very important task, particularly in matrices such as baby foods, where even low contamination could be very harmful for the population involved (i.e., newborns and infants). The protocol described in this article was used to investigate the levels of 19 OPPs and six phthalates in commercial soft and freeze-dried baby foods by means of a rapid and effective analytical procedure. By ultrasound–vortex-assisted DLLME–GC–IT/MS analysis, LODs between 0.2 and 4.7 ng g^{-1}, LOQs between 2.3 and 8.5 ng g^{-1}, and recoveries between 90% and 110% were obtained: this is the first analytical protocol for simultaneous analysis of OPPs and phthalates in such matrices. The application of this analytical procedure to baby foods available on the Italian market evidenced no OPPs at such levels and the presence of three phthalates at levels below 40 ng g^{-1}. This confirms that pesticide residues are absent from or, at least, present at very low levels in baby foods, whereas phthalates are found coming essentially from the packaging. In any case, on the one hand the results are important and do not suggest any concerns, but on the other hand it is necessary to keep attention high because very few studies have investigated the "synergistic effects" of exposure to several pesticides in small amounts. The main question is: What does it mean to feed an infant with baby food contaminated at low levels? This interesting question needs to be answered sooner or later, and a very reliable analytical protocol (such this one) will help to answer it.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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