Available online at www.sciencedirect.com
SCIENCE DIRECT®
Analytica Chimica Acta 495 (2003) 123–132
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

Correlation study of enzyme-linked immunosorbent assay and high-performance liquid chromatography/tandem mass spectrometry for the determination of *N*-methylcarbamate insecticides in baby food

Barbora Mickova[a], Jitka Zrostlikova[b], Jana Hajslova[b], Pavel Rauch[a,*], María José Moreno[c], Antonio Abad[c], Angel Montoya[c]

[a] *Department of Biochemistry and Microbiology, Faculty of Food and Biochemical Technology, University of Chemical Technology, Technická 3, 16628 Prague 6, Czech Republic*
[b] *Department of Food Chemistry and Analysis, Faculty of Food and Biochemical Technology, University of Chemical Technology, Technická 3, 16628 Prague 6, Czech Republic*
[c] *Centro de Investigación e Innovación en Bioingeniería, Universidad Politécnica de Valencia, Camino de Vera, s/n. 46022 Valencia, Spain*

Received 21 January 2003; received in revised form 22 July 2003; accepted 11 August 2003

Abstract

In this work, a correlation study of monoclonal antibody-based enzyme-linked immunosorbent assays (ELISAs) and a liquid chromatography–electrospray mass spectrometric (HPLC/ESI/MS/MS) method for the determination of *N*-methylcarbamate insecticides carbofuran, carbaryl and methiocarb in fruit baby food is presented. The comparison of performance characteristics of the two methods was carried out by simultaneous analysis of apple–strawberry baby food (GPC purified) extracts spiked with *N*-methylcarbamates at six different concentration levels. Results obtained by ELISA correlated well with those obtained by LC/MS/MS, both in terms of trueness and precision. Recoveries, i.e. the ratio of the determined concentration to the known spiked concentration, were in the 60–100% range for ELISA and in the 73–104% range by LC/MS/MS with the RSDs from seven replicate analyses 3.6–23.3 and 1.7–8.2%, respectively. The influence of sample pre-treatment on the analytical performance of immunoassay method was also assessed. Using ELISA recoveries close to 90% were obtained even in crude non-purified baby food extracts. The limits of detection (LODs) by ELISA were 0.3, 0.04 and 0.02 $\mu g/kg^{-1}$ for carbofuran, carbaryl and methiocarb, respectively, whereas using LC/MS/MS 1 μg/kg was the detection limit for all three insecticides. The results clearly indicate that the developed ELISA is suitable for the fast, quantitative and reliable determination of carbaryl, carbofuran and methiocarb in baby food even for the analysis of crude non-purified extracts.

Keywords: Correlation study; *N*-methylcarbamate insecticides; Pesticides residues; Baby food; ELISA; HPLC/MS/MS

1. Introduction

N-methylcarbamate insecticides such as carbaryl, carbofuran and methiocarb were introduced worldwide as substituents of persistent organochlorine compounds, due to their broad spectrum of activity and

* Corresponding author. Tel.: +420-22-4353076; fax: +420-22-4353010.
E-mail address: pavel.rauch@vscht.cz (P. Rauch).

0003-2670/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2003.08.022

their low bioaccumulation potential. However, being inhibitors of acetylcholinesterase, they are regarded as toxic for the environment and for humans. As regards the potential risk arising from the use of pesticides, a vulnerable consumer's group are children. As shown by human exposure studies [1], dietary ingestion may be an important pathway to become exposed to pesticides, which is reflected in very low maximum residue limits set for baby food by the EU ($10\,\mu g\,kg^{-1}$).

The thermal instability of most *N*-methylcarbamates makes their sensitive and accurate determination by traditional gas chromatographic methods difficult. Their decomposition to degradation products—corresponding phenols—can occur during the GC separation or in the injection port of a gas chromatograph. Therefore, the preferred analytical technique for these pesticides is high-performance liquid chromatography employing ultraviolet (UV), diode array (DAD), fluorescence (FLD) or mass spectrometric (MS) detection [2–5]. The last mentioned method provides very low detection limits and superior specificity of detection, unless derivatisation of the analytes is needed.

Nowadays, with the increasing development of immunoassay methods for pesticide screening, it is also possible to quantify *N*-methylcarbamates at sub-ppb levels in food and environmental samples by these techniques [6,7]. Enzyme-linked immunosorbent assays (ELISA) have been found to be a simple and cost-effective alternative to instrumental analysis especially when large series of samples are to be analysed. Recently, immunoassays based on polyclonal antibodies have been developed and applied for the analysis of carbofuran and carbaryl in water and soil [8–10], in fruits and vegetables [11,12], in animal feed grains [13] and in meat and liver [14]. For carbofuran the analyses of non-fatty and fatty baby food have been reported. This study included the off-line coupling of supercritical fluid extraction (SFE) with ELISA [1,15,16].

Recently, indirect competitive ELISA format based on monoclonal antibodies for the determination of carbofuran, carbaryl and methiocarb has been developed by Montoya and coworkers [17–20] and the applications to the analysis of various fruits, vegetables [21], fruit juices [22] and water [23] have been reported.

In the study presented here, this monoclonal antibody-based ELISA format was applied for the determination of above pesticides in non-fatty baby food. As already mentioned, relatively strict regulation limits are set for pesticides in baby food commodities. Therefore, availability of rapid, sensitive and cheap analytical methods is highly desirable in this case. In the paper presented here, the performance of ELISA method is compared with liquid chromatography–electrospray mass spectrometric (HPLC/ESI/MS/MS) method. Apple–strawberry baby food was selected as a target matrix since both apples and strawberries represent important sources of baby food contamination by *N*-methylcarbamate insecticides.

2. Experimental

2.1. Chemicals and immunoreagents

For immunoassays, monoclonal antibodies (MAbs) specific for carbaryl, carbofuran and methiocarb, as well as the corresponding OVA–hapten conjugates, were prepared in the Laboratorio Integrado de Bioingeniería, Universidad Politécnica de Valencia, Spain. LIB–CNH45 MAb and OVA–2NAH conjugate were used for carbaryl analysis [23], LIB–BFNB67 MAb and OVA–BFNH conjugate were used for carbofuran analysis [19], and LIB–MXNB31 MAb and OVA–DPNH conjugate were used for methiocarb analysis [20]. Bovine serum albumin (BSA), Tween 20 and *o*-phenylenediamine (OPD) for ELISA colour development were obtained from Sigma Chemical Company (St. Louis, USA). Horseradish peroxidase (HRP)-labelled swine anti-mouse IgG (SwAM, HRP/SwIgG = 1.81, concentration $8.9\,mg\,ml^{-1}$) was obtained from Seva Pharma, Czech Republic.

Certified standards of carbaryl, carbofuran and methiocarb (purity 95–99%) were obtained from Dr. Ehrenstoffer, Germany. Individual stock standard solutions (concentration range $3.00–3.50\,mg\,ml^{-1}$) were prepared by dissolving 30–35 mg of neat pesticide standard in 10 ml of methanol. The mixed standard stock solution of all analytes (ca. $50\,\mu g\,ml^{-1}$ in methanol) was made by diluting these individual pesticide solutions. Stock solutions were stored at $+5\,^{\circ}C$ and used every day to prepare fresh mixed working standard solutions for ELISA and HPLC determinations. Pesticide residue grade solvents were

obtained from Scharlau, Italy (ethyl acetate) and from Merck, Germany (cyclohexane, methanol). Deionised water for mixing of a mobile phase was produced in Milli-Q apparatus (Millipore, Germany). Anhydrous sodium sulphate (Penta Chrudim, Czech Republic) was activated for 5 h at 450 °C.

Apple–strawberry baby food from a retail market was used for the preparation of samples.

2.2. Apparatus and instrumentation

Extractions of target analytes were performed using tissumiser Turrax (IKA Werke, Germany). All solvent reductions were performed on a Büchi rotary evaporator (Büchi, Switzerland). An automated high-performance gel permeation chromatography (HPGPC) system (Gilson, France) equipped with a PL gel (600 mm × 7.5 mm, 0.005 μm) column (Polymer Labs, UK) was used for clean-up of extracts.

Ninety-six-well ELISA polystyrene microplates Costar (catalog no. 9018) were from Corning, USA. ELISA plates were washed with Labsystem Multiwash, USA, and absorbances were read in Labsystem Multiscan MCC/340, Finland. Data were processed using Microsoft Excel software (Microsoft, USA).

HPLC separation was carried out using a HP1100 liquid chromatograph (Hewlett-Packard, USA). HPLC column Discovery C_{18} (15 cm × 3 mm, 5 μm) (Supelco, USA) with mobile phase methanol–water was used and the following linear gradient conditions: 0 min, 50% methanol; 7 min, 80% methanol; 7.2–13 min, 100% methanol. Analysis time was 20 min including the 7 min post-run, when column was conditioned at starting mobile phase composition. Flow rate was 0.5 ml min^{-1}, column temperature was 25 °C and the injection volume was 20 μl.

MS/MS analysis was performed by LCQ Deca ion trap instrument from Finnigan, USA. Electrospray ionisation (ESI) was applied in all experiments. The following experimental conditions were used: capillary temperature, 230 °C; flow rates of sheath gas and auxiliary gas, 1.5 and 3 l min^{-1}, respectively; spray voltage, 6 kV; capillary voltage, 11 V for carbofuran and carbaryl, 18 V for methiocarb. For MS/MS analysis time segments were set up and one analyte was scanned in each segment. All three *N*-methylcarbamates were monitored in positive ion mode with following parent → daughter masses used: carbofuran 222 → 165; carbaryl 202 → 145; methiocarb 226 → 169. Acquired data were processed by XCalibur software (Finnigan, USA).

2.3. Preparation of samples

2.3.1. Blank purified extract

The blank apple–strawberry baby food (25 g), in which the absence of *N*-methylcarbamate residues had been verified by LC/MS analysis, was mixed with 100 ml ethyl acetate and 75 g sodium sulfate and homogenised for 2 min by a Turrax tissumiser. The suspension was filtered under vacuum; the volume of filtrate was reduced by evaporation to 12.5 ml and made-up with cyclohexane in 25 ml volumetric flask. The crude extract was purified by high-performance gel permeation chromatography (HPGPC) under the following conditions—mobile phase: cyclohexane–ethyl acetate (1:1, v/v); flow: 1 ml min^{-1}; injection volume: 2 ml. The eluate within the range 14.5–30 ml was collected. After solvent evaporation in a rotary evaporator and removal of its traces under a mild stream of nitrogen, the residue was redissolved in 2 ml of a methanol–water (1:4, v/v) mixture and passed through a Millipore membrane filter. A volume of 14 ml of purified blank extract was obtained by pooling of seven redissolved fractions after HPGPC. This step was performed to ensure that the matrix composition was exactly the same for all the samples and matrix-matched standards for LC/MS/MS calibration.

2.3.2. Samples for correlation study

Baby food extracts spiked with *N*-methylcarbamate at 1, 5, 15, 50, 250 and 500 ng ml^{-1} were prepared as unknown samples (for details, see Section 2.5.2). An amount of 150 μl of the pesticide standard at the appropriate concentration was added to 2.85 ml of purified blank extract.

2.3.3. Non-purified baby food samples

Non-purified baby food samples were prepared by evaporation of aliquot part of crude extract of blank and redissolving the residue in methanol–water (1:4, v/v). Spiked samples at 10 and 250 ng ml^{-1} were prepared by adding 150 μl of the pesticide standard at the appropriate concentration level to 2.85 ml of this non-purified blank sample.

2.3.4. Matrix-matched standards for HPLC/MS/MS calibration

Matrix-matched standards for HPLC/MS/MS calibration were prepared from the same purified blank extract as the test samples (see above). Calibration solutions at concentrations of 1, 4, 10, 20, 100, 200 and 500 ng ml^{-1} were prepared by adding 100 μl of the appropriate standard solution to 1.9 ml purified sample extract.

2.4. ELISA determinations

Immunochemical determinations of *N*-methylcarbamates in samples were carried out by using three specific monoclonal immunoassays performed in the indirect competitive ELISA format on polystyrene microplates, with photometric detection at 492 nm. Samples were quantitatively analysed for a single pesticide in each plate, irrespective of the presence of the two other analytes. The ELISA conditions were exactly the same for all immunoassays and in fact they were performed simultaneously. Each ELISA plate included its own six-point calibration curve to determine *N*-methylcarbamate concentrations. The central section of the curve with a nearly linear response was accepted as the assay working range. Specific details about immunoreagent concentrations and sample dilutions applied in ELISA are summarised in Table 1, while the used assay working ranges are shown in Table 4. Working standard solutions for each immunoassay were prepared from the standard stock solution by serial dilution in phosphate-buffered saline (PBS, 0.14 M NaCl, 1.5 mM KH_2PO_4, 8.0 mM Na_2HPO_4, 2.7 mM KCl, pH 7.4).

The immunoassays were accomplished as follows: polystyrene microplates were coated with the ovalbumin–pesticide conjugate solution in 0.05 M carbonate–hydrogen carbonate buffer, pH 9.6 (100 μl per well) and left to incubate overnight at laboratory temperature. Microplates were washed four times with PBS containing Tween 20 (0.05%, v/v). Then the aliquots (50 μl per well) of pesticide standards or sample extracts, diluted in PBS, and the aliquots (50 μl per well) of specific monoclonal antibodies, diluted in PBS containing 0.1% BSA (bovine serum albumin), were pipetted to the microplate wells coated with the conjugates. Microplates were incubated for 2 h at laboratory temperature and washed as described above. Subsequently, the second antibody conjugated with peroxidase (SwAM) was added (100 μl per well), left to interact for 1 h at laboratory temperature, and washed as described above. Then, the peroxidase substrate in reaction buffer (4.6 mM OPD in 0.1 M phosphate—citrate buffer, pH 5.0, containing 0.1% (v/v) of 30% H_2O_2) was added to each well (100 μl per well). After 10 min incubation at laboratory temperature, the enzyme reaction was stopped by adding 50 μl per well of 2.5 M sulphuric acid, and the absorbance at 492 nm was measured directly in the wells with the microplate reader. Absorbance values from standards were mathematically fitted to a four-parameter logistic equation. The analyte concentration in samples was determined by interpolation of their mean absorbance values on the resulting standard curve.

Table 1
Concentrations of immunoreagents and sample dilutions applied in the ELISAs for carbaryl, carbofuran and methiocarb

	Carbaryl	Carbofuran	Methiocarb
Concentration of antibody (μg ml^{-1})	LIB–CNH45: 0.06	LIB–BFNB67: 0.03	LIB–MXNB31: 0.06
Concentration of conjugate (μg ml^{-1})	OVA–2NAH: 0.25	OVA–BFNH: 0.06	OVA–DPNH: 0.5
Sample dilution (ng ml^{-1})			
1	1/20	1	1/5
5	1/25	2/5	1/25
15	1/30	1/15	1/30
50	1/100	1/50	1/100
250	1/500	1/250	1/500
500	1/1000	1/500	1/1000

2.5. Validation study

2.5.1. Determination of overall recovery

Baby food sample was spiked with a mixture of carbaryl, carbofuran and methiocarb at 50 $\mu g\,kg^{-1}$ of each pesticide (1 ml of the standard mixture at 1.25 $\mu g\,ml^{-1}$ was added into 25 g of sample). The extraction and clean-up of spiked samples were carried out according to the method described above. The recovery was determined in five replicates and the repeatability of recovery determination was calculated. HPLC/MS/MS method using matrix-matched standards was applied for quantitation.

2.5.2. Correlation study

To compare the analytical performance of the implemented immunoassay and the HPLC/MS/MS method, purified baby food extracts spiked at 1, 5, 15, 50, 250 and 500 $ng\,ml^{-1}$ with a mixture of carbaryl, carbofuran and methiocarb (see Section 2.3) were analysed by both methods. These testing samples were treated as if they were unknown samples, i.e. the con-

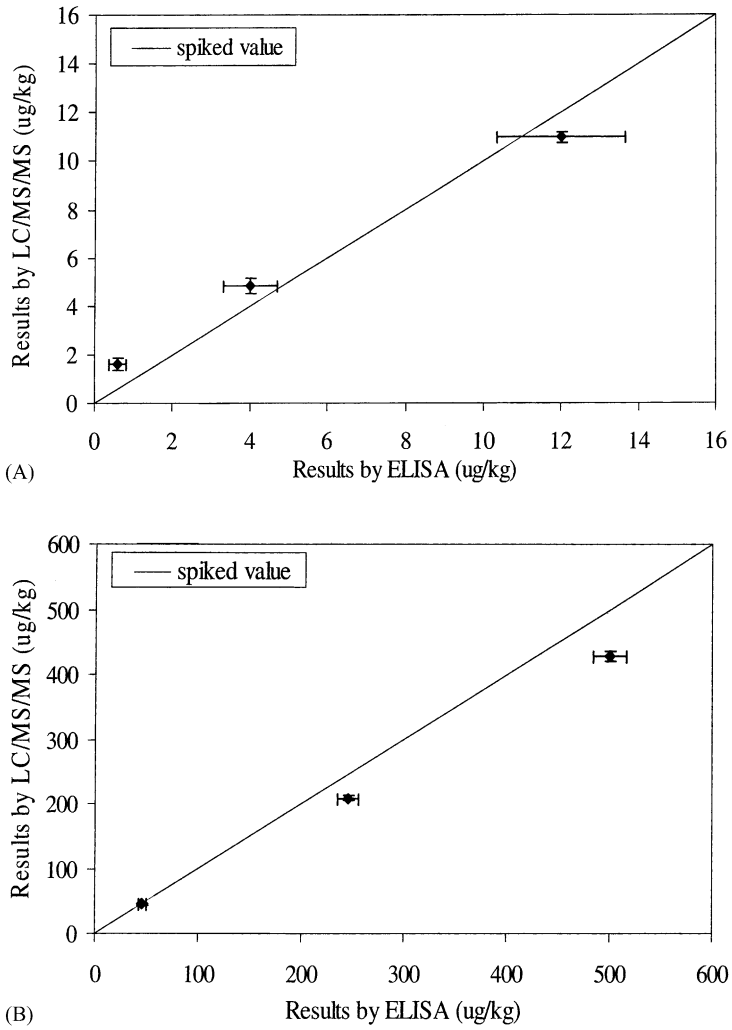

Fig. 1. Correlation between HPLC/MS/MS and ELISA values obtained for carbofuran ($n = 7$, $y = 0.85x + 1.83$, $r^2 = 0.991$) at (A) 1, 5 and 15 $ng\,ml^{-1}$, and (B) 50, 250, 500 $ng\,ml^{-1}$.

tent of *N*-methylcarbamates was quantified by using appropriate calibration procedure by both ELISA and LC/MS method. Whereas for ELISA standards in pure solvent could be used, for LC/MS matrix-matched standards were employed to compensate the so-called matrix effects occurring during LC/MS analysis [24,25]. Several different performance characteristics of both methods were evaluated and compared based on these results (see Section 3.2.).

When using ELISA, previous adjustment of the sample concentration to fit into the linear part of the calibration curve had to be carried out. It should be noted that further dilution of samples for immunoassay was necessary for another reason, i.e. the methanol content in the non-diluted target samples (methanol–water, 1:4, v/v) was too high for antibody–pesticide interactions. Therefore, in our experiments each primary test sample was subjected to seven independent dilutions and subsequently analysed by ELISA. The dilution factors applied for *N*-methylcarbamate analysis are shown in Table 1. Under this experimental setup, the precision parameter for ELISA comprised the uncertainty of both the sample dilution and the quantitative step. For LC/MS/MS, thanks to its much wider dynamic range, no sample dilution was necessary and therefore only the uncertainty of the quantitative step contributes to the results. This experimental approach aimed to reflect real-life situation in the analysis of baby food samples.

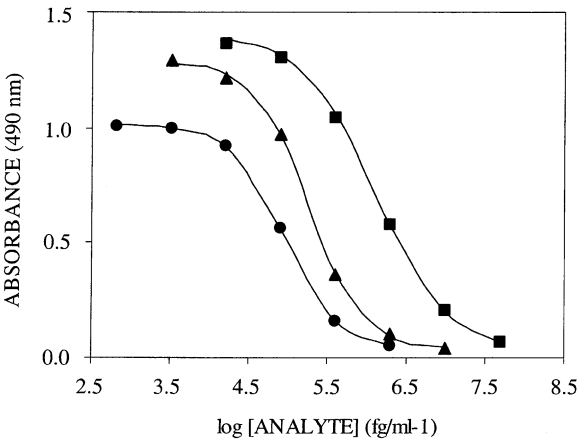

Fig. 2. Representative ELISA standard curves for carbofuran (■), carbaryl (▲) and methiocarb (●). Immunoassay conditions are described in Section 2.4.

The limit of detection (LOD) for ELISA was calculated as the analyte concentration that reduced absorbance to 90% of the maximum. For LC/MS/MS method the limits of detection were determined as a concentration corresponding to a signal to noise ratio of 3:1.

The confidence intervals in Figs. 1 and 2 were determined from the following equation:

$$\mu = \text{S.D.} \times \frac{t_{\text{crit}}(95)}{\sqrt{\text{number of analyses}}}.$$

3. Results and discussion

3.1. Overall recovery of the method

The suitability of the proposed extraction and clean-up procedures was evaluated on the basis of the overall recovery of the target analytes in samples spiked at 50 ng kg^{-1} (i.e. 50 μg ml^{-1}). Employing LC/MS/MS technique as the determination step, the recoveries of carbofuran, carbaryl and methiocarb were 76, 88 and 72%, with relative standard deviations (RSDs) of 13, 10 and 8%, respectively.

3.2. Correlation study

The experimental comparison of the analytical performance of ELISA and LC/MS/MS methods was carried out by simultaneous analysis of apple–strawberry baby food extracts spiked with *N*-methylcarbamates at six different concentrations: 1, 5, 15, 50, 250 and 500 ng ml^{-1} (i.e. 1, 5, 15, 50, 250 and 500 μg kg^{-1}). The lower part of this concentration range was chosen with respect to the maximum residue limit (MRL) for pesticides in baby food (10 μg kg^{-1}). The higher concentrations approximately correspond to the MRLs established for fruit and vegetables. In our experiments, the same analytical conditions as for routine unknown samples were applied.

For the purpose of methods comparison the following characteristics were assessed:

(1) Trueness—as the agreement of the measured concentrations in “unknown” samples with the spiked concentrations.

Table 2
Overview of the results obtained by analysis with ELISA and LC/MS/MS of strawberry baby food extracts spiked with carbaryl, carbofuran and methiocarb at 1, 5, 15, 50, 250 and 500 ng ml^{-1} ($n = 7$ replicates)

Analyte	Spiked level (ng ml^{-1})	ELISA			LC/MS/MS		
		Mean ± S.D. (ng ml^{-1})	Recovery (%)	RSD (%)	Mean ± S.D. (ng ml^{-1})	Recovery (%)	RSD (%)
Carbofuran	1	0.6 ± 0.2	60.0	40.0	1.6 ± 0.3[a]	160.0[a]	17.6[a]
	5	4.3 ± 0.8	86.0	17.6	4.8 ± 0.3	96.0	7.1
	15	12.3 ± 1.8	82.0	14.6	11.0 ± 0.2	73.3	5.8
	50	46.3 ± 3.9	92.6	8.4	45.0 ± 1.5	90.0	3.4
	250	246.0 ± 11.1	98.4	4.5	208.5 ± 3.8	83.4	1.8
	500	500.3 ± 18.1	99.9	3.6	428.7 ± 8.6	85.7	2.0
Carbaryl	1	1.0 ± 0.2	100.0	20.8	1.4 ± 0.3[a]	140.0[a]	18.7[a]
	5	5.0 ± 1.0	100.0	20.0	5.2 ± 0.4	104.0	8.2
	15	14.3 ± 1.0	95.3	6.7	13.6 ± 0.8	90.7	5.8
	50	46.9 ± 5.1	93.8	11.0	46.6 ± 1.6	93.2	3.4
	250	249.1 ± 12.3	99.6	4.9	243.8 ± 4.4	97.5	1.8
	500	484.4 ± 17.6	96.9	3.6	473.2 ± 8.2	94.6	1.7
Methiocarb	1	0.8 ± 0.2	80.0	27.6	1.2 ± 0.1[a]	120.0[a]	8.4[a]
	5	3.9 ± 0.9	78.0	23.3	4.9 ± 0.2	98.0	4.4
	15	13.9 ± 1.2	92.7	8.8	14.1 ± 0.7	94.0	4.7
	50	46.3 ± 5.0	92.6	10.8	50.8 ± 1.4	101.6	2.8
	250	234.9 ± 14.2	94.0	6.1	242.0 ± 5.3	96.8	2.2
	500	489.3 ± 32.0	97.9	6.5	452.3 ± 7.6	90.5	1.7

[a] Result at the detection limit of the method.

(2) Precision—as the relative standard deviation obtained from the analyses of seven replicate samples at each concentration.
(3) Limits of detection (LOD) and quantitation (LOQ). In addition, ELISA sensitivity was evaluated by means of I_{50} value (see below).

Table 2 summarises the repeatability and recovery values (the percentage ratio of ELISA or LC/MS/MS result to the spiked value) obtained. The term "recovery" discussed within this section should not be confused with the overall recovery of the extraction and clean-up method discussed in Section 3.1.

Irrespective of the spiked level, mean recovery values by immunoassay were 91.8, 97.6 and 89.2% for carbofuran, carbaryl and methiocarb, respectively. The RSDs for ELISA ranged from 3.6 to 27.6%. Nearly identical mean recoveries were obtained by LC/MS/MS (85.7, 96.0 and 96.2% for carbofuran, carbaryl and methiocarb, respectively) with lower RSDs ranging from 1.7 to 8.2%. The obtained recoveries of spiked samples indicate that no sample matrix problems or interferences occurred in the tested samples. For immunoassays, the 1 ppb concentration is within the linear working range of the carbofuran standard curve. The sample was therefore not diluted prior to ELISA, which resulted in a content of organic solvent higher than the optimal one. As a result, reduced carbofuran recovery value and higher RSD value (40%) was obtained. For LC/MS/MS, the lowest measured concentration corresponded to the LOD of the method (see below). Although relatively good results were obtained, in practice quantification should not be performed at levels lower than 4 ng ml^{-1}, which corresponds to the LOQ.

Fig. 1 shows the correlation analysis between the results obtained by ELISA and LC/MS/MS methods for carbofuran in spiked samples. For clarity reasons, the concentration range was divided into two charts for each pesticide. The values are presented with their corresponding confidence intervals. Similar results were found also for carbaryl and methiocarb. The linear regression equations for the whole concentration range were: $y = 0.85x + 1.83$, $y = 0.98x + 0.34$ and $y = 0.92x + 0.57$ for carbofuran, carbaryl and methiocarb, respectively. The excellent correlation

Table 3
Overview of the results obtained by analysis with ELISA of crude and purified extracts spiked with carbaryl, carbofuran and methiocarb at 10 and 250 ng ml^{-1} ($n = 7$ replicates)

Analyte	Spiked level (ng ml^{-1})	Crude extract			Purified extract		
		Mean ± S.D. (ng ml^{-1})	Recovery (%)	RSD (%)	Mean ± S.D.	Recovery (%)	RSD (%)
Carbofuran	10	8.5 ± 1.1	85.0	21.9	8.7 ± 0.8	87.0	14.6
	250	225.4 ± 35.6	90.1	14.4	199.1 ± 33.7	79.6	14.8
Carbaryl	10	9.5 ± 2.2	95.5	23.7	8.6 ± 1.4	86.4	16.6
	250	245.8 ± 20.7	98.3	8.8	216.6 ± 11.0	86.7	5.3
Methiocarb	10	9.6 ± 1.6	96.0	16.8	10.4 ± 1.3	104.4	12.7
	250	247.1 ± 24.6	98.8	10.3	253.1 ± 18.7	101.2	7.6

coefficients found (0.991, 0.999 and 0.998, respectively) indicate a high degree of correlation between both techniques.

The influence of sample pre-treatment on the analytical performance of immunoassay method was also assessed. For this experiment apple–strawberry baby food extracts spiked by *N*-methylcarbamates at two different concentrations (10 and 250 ng ml^{-1}, i.e. 10 and 250 μg kg^{-1}) were prepared in two duplicate sets—crude extracts and extracts purified by GPC. Table 3 summarises the obtained repeatability and recovery values. Good agreement between results obtained by analysis of crude extracts and purified extracts was found for carbofuran, carbaryl and methiocarb. The mean recoveries of carbaryl in crude and purified extracts were 96.9 and 86.6%, respectively, with RSDs ranging from 5.3 to 23.7%. The mean recoveries of methiocarb in crude extracts and purified extracts were 97.4 and 102.8%, respectively, with RSDs ranging from 7.6 to 16.8%. In the case of carbofuran the mean recoveries in crude and purified extracts were 87.0 and 84.0%, respectively, with RSDs ranging from 14.4 to 21.9%. As obvious from obtained results no significant influence of sample pre-treatment was observed for the mentioned carbamates.

The sensitivity of immunochemical methods is commonly expressed as a detection limit and by calculating the I_{50} value, i.e. the analyte concentration that decreases the assay signal to 50% of the maximum value. With respect to these criteria, the most sensitive of the three assays was ELISA for methiocarb, with an I_{50} value of 0.07 ng ml^{-1} (i.e. 0.07 μg kg^{-1}) and a detection limit of 0.02 ng ml^{-1} (i.e. 0.02 μg kg^{-1}). The carbaryl and carbofuran I_{50} values were 0.14 and 1.4 ng ml^{-1}, respectively. The detection limits for carbaryl and carbofuran were 0.04 and 0.3 ng ml^{-1}, respectively. For HPLC/MS/MS method the limits of detection for a calculated signal-to-noise ratio of 3 were 1 ng ml^{-1} (i.e. 1 μg kg^{-1}) for all three carbamates. In Table 4 linear ranges, LODs and I_{50} values of the compared methods are summarised. Fig. 2 shows the ELISA calibration curves for the three carbamates.

Table 5 gives an overview of time-demands for ELISA and LC/MS/MS methods. For LC/MS/MS ca. 40 min are required for the final analysis step. The ELISA technique usually includes overnight

Table 4
Comparison of linearity and limits of detection obtainable by ELISA and LC/MS/MS methods

	Linear range (ng ml^{-1})		Limit of detection (ng ml^{-1})		I_{50} (ng ml^{-1})
	ELISA	HPLC	ELISA	HPLC	ELISA
Carbofuran	10–0.4	500–4	0.3	1	1.4
Carbaryl	1.2–0.08	500–4	0.04	1	0.14
Methiocarb	0.4–0.02	500–4	0.02	1	0.07

Table 5
Comparison of time-demands for ELISA and LC/MS/MS methods

Analytical step	ELISA (min)	LC/MS/MS (min)
Extraction	15	15
Clean-up	35[a]	35[a]
Solvent exchange	10	10
Preparation of standards	10	20[b]
Dilution of samples	15	–
Quantitation step	240[c] (96 samples)	20 (1 sample)

[a] Can be omitted by ELISA.
[b] Matrix-matched standards are required for LC/MS/MS determination.
[c] Overnight incubation of the coating conjugate is not included.

incubation of coated microplate and thus requires a larger time of analysis. However, the problem of overnight incubation can be eliminated by storage of pre-coated plates in a refrigerator. In this way, the prepared plates are directly useable for analysis for 3 months. Using immunoassay, many samples can be analysed in each plate with minimum expense of reagents. Under the above described conditions, with a standard curve included in triplicate, 24 samples for one of mentioned pesticides can be determined in triplicate on each plate in less than 4 h. Considering the fact that four plates can be easily handled simultaneously, the number of samples handled per unit time is significant. Moreover, as discussed above, sample clean-up step can be omitted when using ELISA.

4. Conclusions

In the present work the potential of ELISA for the monitoring of selected pesticide residues in baby food samples was demonstrated. Immunoassay method provided excellent sensitivity and selectivity for the determination of carbaryl, carbofuran and methiocarb in baby food samples. Also a good correlation of results with the LC/MS/MS method has been proven in our experiments.

Whereas the initial price of an LC/MS/MS instrument is very high, ELISA technique can be performed at very low initial and operating costs. As regards time demands approximately 96 samples can be analysed in less than 4 h by ELISA, which considerably exceeds the time effectiveness of the LC/MS/MS method. In our study it has been demonstrated that practically no difference in the performance of ELISA exists between the analysis of purified and non-purified baby food samples. This is an additional advantage of the method, since the whole analytical procedure can be considerably simplified, which results in further savings in time and costs.

On the other hand, compared to LC/MS/MS, the number of compounds simultaneously analysed in one run is limited. However, the further development of multiresidue of immunoassay kits may overcome this particular disadvantage.

References

[1] J.C. Chuang, K. Hart, J.S. Chang, E. Boman, J.M. Van Emon, A.W. Reed, Anal. Chim. Acta 444 (2001) 87.
[2] G.S. Nunes, M.P. Marco, M.L. Ribeiro, D. Barcelo, J. Chromatogr. A 823 (1998) 109.
[3] Y. Picó, G. Font, J.C. Moltó, J. Manes, J. Chromatogr. A 882 (2000) 153.
[4] J.M. Fernandez, P.P. Vasquez, J.L.M. Vidal, Anal. Chim. Acta 412 (2000) 131.
[5] M. Honing, J. Riu, D. Barcelo, B.L.M. Baar, U.A.T. Brinkman, J. Chromatogr. A 733 (1996) 283.
[6] M.P. Marco, S. Chiron, J. Grascon, B.D. Hammock, D. Barcelo, Anal. Chim. Acta 311 (1995) 319.
[7] G.S. Nunes, M.P. Marco, M. Farre, D. Barcelo, Anal. Chim. Acta 387 (1999) 245.
[8] S.W. Jourdan, A.M. Scutellaro, J.R. Fleeker, D.P. Herzog, F.M. Rubio, J. Agric. Food Chem. 43 (1995) 2784.
[9] M.P. Marco, S.J. Gee, H.M. Cheng, Z.Y. Liang, B.D. Hammock, J. Agric. Food Chem. 41 (1993) 423.
[10] M.P. Marco, S. Chiron, J. Gascon, B.D. Hammock, D. Barcelo, Anal. Chim. Acta 311 (1995) 319.
[11] G.S. Nunes, M.P. Marco, M. Farre, D. Barcelo, Anal. Chim. Acta 387 (1999) 245.
[12] G.S. Nunes, M.P. Marco, M.L. Riberio, D. Barcelo, J. Chromatogr. A 823 (1998) 109.
[13] S. Wang, R.D. Allan, A.S. Hill, I.R. Kennedy, J. Environ. Sci. Health B 37 (2002) 521.
[14] S.J. Lehotay, R.J. Argauer, J. Agric. Food Chem. 41 (1993) 2006.
[15] J.C. Chuang, M.A. Pollard, M. Misita, J.M. Van Emon, Anal. Chim. Acta 399 (1999) 135.
[16] V. Lopez-Avila, C. Charan, J.M. Van Emon, Immunoassays for Residue Analysis (ACS Symposium Series No. 621), American Chemical Society, Washington, DC, 1996, pp. 439–446.
[17] A. Abad, M.J. Moreno, R. Pelegri, M.I. Martinez, A. Saez, M. Gamon, A. Montoya, J. Chromatogr. A 833 (1999) 3.
[18] A. Abad, J. Primo, A. Montoya, J. Agric. Food Chem. 42 (1994) 1818.

[19] A. Abad, M.J. Moreno, A. Montoya, J. Agric. Food Chem. 47 (1999) 2475.
[20] A. Abad, M.J. Moreno, A. Montoya, J. Agric. Food Chem. 46 (1998) 2417.
[21] A. Abad, M.J. Moreno, R. Pelegri, M.I. Martinez, A. Saez, M. Gamon, A. Montoya, J. Agric. Food Chem. 49 (2001) 1707.
[22] A. Abad, M.J. Moreno, A. Montoya, Anal. Chim. Acta 347 (1997) 103.
[23] A. Abad, A. Montoya, J. Agric. Food Chem. 45 (1997) 1495.
[24] J. Zrostlíková, J. Hajšlová, J. Poustka, P. Begany, J. Chromatogr. A 973 (2002) 13–26.
[25] J. Zrostlíková, J. Hajšlová, T. Kovalczuk, J. Poustka, J. AOAC Int. 86 (2003) 612–622.