Journal of Chromatography A, 1217 (2010) 119–126

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Determination of organochlorine pesticides in vegetable matrices by stir bar sorptive extraction with liquid desorption and large volume injection-gas chromatography–mass spectrometry towards compliance with European Union directives

M. Barriada-Pereira[a,b], P. Serôdio[c], M.J. González-Castro[a,b], J.M.F. Nogueira[c,d,*]

[a] Universidade da Coruña, Facultade de Ciencias, Departamento de Química Analítica, Campus da Zapateira, 15071 A Coruña, Spain
[b] Universidade da Coruña, Instituto Universitario de Medio Ambiente, Pazo de Lóngora, Santa Eulalia de Liáns, 15179 Oleiros, A Coruña, Spain
[c] Universidade de Lisboa, Faculdade de Ciências, Departamento de Química e Bioquímica, Campo, Grande Ed. C8, 1749-016 Lisboa, Portugal
[d] Universidade de Lisboa, Faculdade de Ciências, Centro de Química e Bioquímica, Campo Grande Ed. C8, 1749-016 Lisboa, Portugal

ARTICLE INFO

Article history:
Received 9 April 2009
Received in revised form
30 September 2009
Accepted 23 October 2009
Available online 30 October 2009

Keywords:
Vegetable matrices
Organochlorine pesticides
Stir bar sorptive extraction
Liquid desorption
Large volume injection
GC–MS(SIM)
EU directives

ABSTRACT

A novel analytical approach to determine trace levels of 20 organochlorine pesticides (OCPs) in nine vegetable matrices (lettuce, spinach, green bean, green pepper, tomato, broccoli, potato, carrot and onion) is proposed, based on stir bar sorptive extraction followed by liquid desorption and large volume injection-gas chromatography coupled to mass spectrometry using the selected-ion monitoring mode acquisition (SBSE-LD/LVI-GC–MS(SIM)). The experimental procedure consists of a previous ultrasonic extraction of the freeze-dried vegetable samples (100.0 mg) with methanol (2 mL) followed by centrifugation and dissolution in aqueous media prior to SBSE-LD/LVI-GC–MS(SIM) under optimised conditions. Assays were performed on 30 mL aqueous samples using stir bars coated with 47 μL of polydimethylsiloxane, an equilibrium time of 180 min (1000 rpm; 20 °C) and acetonitrile as back-extraction solvent, providing convenient analytical performance to monitor OCPs in vegetable matrices at the trace level. Besides the selectivity reached, the data obtained clearly demonstrate that the matrices involved have a strong effect on the recovery yields (10–110%) of the OCPs under study, in particular the green vegetables especially the leafy ones. By using the standard addition methodology, good linearity ($r^2 > 0.99$) and convenient precisions (RSD < 20%) were found for almost all cases, depending on the particular OCP and vegetable matrix involved. Furthermore enough sensitivity was also achieved (limit of detection <10 μg kg^{-1}) for all OCPs under study towards compliance with the European Union regulations for the maximum residue limits of pesticides in agricultural vegetables. The methodology showed to be easy of work-up, fast, almost solventless with low sample amount requirement, when compared with conventional methods of sample preparation to screen pesticides in vegetable matrices.

1. Introduction

Organochlorine pesticides (OCPs), effective in the control of pest and diseases, have been extensively used in agriculture around the world. These compounds are considered among the most dangerous contaminants because of their toxicity, low biodegradability and great stability. Their long biological half-life and high liposolubility result in high bioaccumulation and biomagnification along the food chain, involving a wide range of trophic levels [1]. The capability to produce adverse effects at very low concentrations as endocrine disrupting chemicals or as carcinogens [2] supports the statement that OCPs are a significant risk to natural ecosystems and human health. Consequently, the production and the use of OCPs have been restricted or even banned for some decades in most countries. However, they are still found widespread in the environment because of their extreme stability and probable indiscriminate use in the past [3,4]. Some of them have been included as persistent organic pollutants in the Stockholm Convention, which is a global treaty to protect human health and the environment from these compounds [5]. Moreover, the accumulation of pesticides in agriculture products is of great concern because plants act as intermediates in the transport of contaminants from soil, water and air to humans and fauna. This situation has led to regulations setting maximum residue limits (MRLs) of pesticides in different agricultural commodities. The values established by the European Union (EU) and the governments of its member countries can be

* Corresponding author at: Universidade de Lisboa, Faculdade de Ciências, Departamento de Química e Bioquímica, Campo Grande Ed. C8, 1749-016 Lisboa, Portugal. Tel.: +351 217500899; fax: +351 217500088.
E-mail address: nogueira@fc.ul.pt (J.M.F. Nogueira).

0021-9673/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2009.10.076

as low as 10 μg kg^{-1} depending on the particular pesticide and matrix type [6]. Due to the low detection usually required by regulatory bodies and the complex nature of these matrices, efficient sample preparation and convenient instrumentation are important issues for trace level analysis. The more frequently used methodologies for the analysis of OCPs in plant matrices employ solvent extraction procedures such as Soxhlet [7–9], shake-flask [10–12], sonication [13–15], supercritical fluid extraction [16,17], pressurized liquid extraction [18–20] and microwave assisted extraction [21], prior to gas chromatography with selective and sensitive detection such as mass spectrometry (GC–MS). Nevertheless, these approaches need, in general, a clean-up step to decrease the presence of interferences in the final extracts in order to reduce the detection limits of the methods and to avoid overlapping during chromatographic separation [22], which is time consuming, many times expensive and simultaneously decreases the precision of the methodologies involved. In the last few years the trends for simplifying the analytical procedures have driven to the development of new analytical approaches which enable the determination of pollutants in complex matrices such as plant materials with improved capabilities, reduced clean-up and concentration steps, the avoidance of toxic solvents and improved detection limits. In this context, sorptive extraction techniques like solid-phase extraction (SPE), matrix solid-phase dispersion (MSPD), solid-phase microextraction (SPME) and stir bar sorptive extraction (SBSE), appear to be appropriate and they have been applied for trace analysis of OCPs in vegetable matrices [23–25]. Whereas SPE and MSPD need a concentration step, SPME and SBSE allow carrying out the extraction and concentration in a single step. The SBSE technique, first described in the nineties by Baltussen et al. [26], is a relatively novel and efficient technique for the extraction and concentration of organic compounds from aqueous samples using a thick film of polydimethylsiloxane (PDMS). Since SBSE is an equilibrium process, the analyte is extracted by partitioning between the aqueous phase and the PDMS phase according to its distribution constant, which is correlated with the octanol–water distribution coefficient ($K_{O/W}$). This technique is based on the same mechanisms of SPME, but SBSE enables a much higher capacity because of the large amount of polymeric phase (24–126 μL) compared to SPME (0.5 μL). Although SBSE methods have been developed for a variety of applications including the determination of OCPs in different type of matrices such as water [27–30], soil [31] and food [32,33], references in vegetables are still scarce and furthermore very few pesticides are included in these studies. In this contribution, a novel analytical approach is proposed for determining trace levels of 20 OCPs in nine vegetable matrices (lettuce, spinach, green bean, green pepper, tomato, broccoli, potato, carrot and onion) using SBSE followed by liquid desorption and large volume injection-gas chromatography coupled to mass spectrometry using selected-ion monitoring mode acquisition (SBSE-LD/LVI-GC–MS(SIM)). For this purpose, important parameters affecting the extraction process such as extraction time, ionic strength, and organic modifier are fully discussed. The performance of the proposed method was evaluated in terms of accuracy, precision, linearity and limits of detection towards compliance with the EU directives for the MRLs of pesticides in vegetable matrices, as well as the comparison with other conventional dedicated analytical methodologies.

2. Experimental

2.1. Chemicals and materials

A mix of organochlorine pesticides named "CLP Organochlorine Pesticide Mix" containing: α-chlordane, methoxychlor, γ-chlordane, endrin ketone, aldrin, α-HCH, β-HCH, γ-HCH, δ-HCH, *p,p′*-DDD, *p,p′*-DDE, *p,p′*-DDT, dieldrin, α-endosulfan, β-endosulfan, endosulfan sulfate, endrin, endrin aldehyde, heptachlor, and heptachlor epoxide (isomer B) (2 mg mL^{-1} each in toluene:hexane (1:1)), was obtained from Supelco (Bellefonte, PA, USA). HPLC-grade acetone, acetonitrile (ACN), methanol (MeOH) and hexane were supplied from Fluka Chemie AG (Buchs, Switzerland). Hydrochloric acid (37%) and sodium chloride were purchased from Merck (Darmstadt, Germany). Ultra-pure water was obtained from Milli-Q (Millipore, Bedford, MA, USA) water purification system. The stir bars (Twister) with a length of 20 mm and coated with a 0.5 mm PDMS layer (47 μL) were obtained from Gerstel (Mülheim and der Ruhr, Germany). Prior to use, the stir bars were conditioned into a vial containing ACN and treated by sonication and then thermically desorbed in a deactivated liner overnight at 300 °C with a helium flow of 100 mL/min.

2.2. Samples

Fresh vegetables (lettuce, spinach, green bean, green pepper, tomato, broccoli, potato, carrot and onion) were purchased at several local markets in A Coruña city, NW-Spain. A 1–2 kg sample of each vegetable was chopped and homogenized. An aliquot of about 100 g was weighted on an Erlenmeyer flask and freeze-dried. Subsequently, the samples were grounded in a mill and stored in glass bottles out of light exposure until their analysis.

2.3. SBSE-LD procedure

In typical assays, 100.0 mg aliquots of freeze-dried samples were weighted into a glass vials (Mettler Toledo AG135) and spiked with OCPs standards in acetone to the desirable concentration. Pre-extractions were performed twice with 2 mL of MeOH each time by sonication (Branson 3510, Branson Ultrasonic Corporation, Danbury, USA) for 30 min (2 × 15 min). Then the mixtures were centrifuged for 5 min at 4000 rpm (Hermle Z300). The obtained supernatants were introduced into glass vials (Macherey-Nagel, Düren, Germany), diluted with ultra-pure water to make a total volume of 30 mL and the stir bars were placed and then closed with a seal using a manual crimper. SBSE assays were carried out in a fifteenth agitation point plate (Variomag Multipoint) for 180 min with a stirring rate of 1000 rpm and at room temperature (20 °C). After extraction the stir bars were removed with a clean tweezers and dried with a lint-free tissue and placed into a 2 mL glass vial filled with 1.5 mL of ACN ensuring the total immersion. Analytes were desorbed by sonication during 15 min, concentrated to dryness under a gentle stream of nitrogen and redissolved with 120 μL of hexane. Subsequently, the vials were closed with seals using a hand crimper and placed into the automatic liquid sampler tray for LVI-GC–MS analysis. All assays were performed at least in triplicate and blank assays were also performed using the same procedure as above and vegetable samples without spiking. For quantification, the standard addition methodology was used to suppress possible matrix effects in concentrations ranging from 0.1 μg kg^{-1} to 5000.0 μg kg^{-1} in freeze dry basis.

2.4. LVI-GC–MS operating conditions

Large volume injection GC–MS analysis were performed on a Agilent 6890 Series gas chromatograph equipped with an Agilent 7683 automatic liquid sampler coupled to a Agilent 5973 *N* mass selective detector (Agilent Technologies, Little Falls, DE, USA). A programmed temperature vaporization injector (PTV) with a septumless sampling head having a baffled liner (SLH; Gerstel, Mülheim a/d Ruhr, Germany) was used, operating in the solvent vent mode with liquid nitrogen as inlet cooling. Through the electronic pneumatic control (EPC), the solvent vent injection mode was per-

formed (vent time: 0.30 min; flow: 150 mL/min; pressure: 0 psi; purge: 60 mL/min@2 min), for which the inlet temperature was programmed from 40 °C (0.35 min) to 320 °C at a rate of 600 °C/min (held 3 min) and subsequently decreased to 200 °C (held until end) at a rate of 50 °C/min. The injection volume and speed were 20 μL and 100 μL/min, respectively. GC analysis was performed on a TRB-5MS (30 m × 0.25 mm I.D., 0.25 μm df) capillary column (5% diphenyl, 95% dimethylpolysiloxane; Teknokroma, Spain) and helium as carrier gas maintained in the constant pressure mode (17.30 psi) was used. The oven temperature was programmed from 70 °C (held 2 min) at 25 °C/min to 200 °C, and then at 8 °C/min to 280 °C (held 10 min). The transfer line, ion source and quadrupole analyzer temperatures were maintained at 280 °C, 230 °C and 150 °C, respectively and a solvent delay of 5 min was selected. In the full-scan mode, electronic ionization mass spectra in the range 35–550 Da were recorded at 70 eV with an ionization current of 34.6 μA. In the selected-ion monitoring (SIM) mode acquisition, several groups having target ions were monitored at different time windows defined by the corresponding retention times, maintaining a dwell time of 100 ms. Two ions of each OCP were chosen, according to the mass spectra characteristic features obtained in the full-scan mode and by comparison with the Wiley's library spectral data bank (G1035B; Rev D.02.00; Agilent Technologies). Data recording and instrument control were performed by the MSD ChemStation software (G1701CA; version C.00.00; Agilent Technologies).

3. Results and discussion

3.1. SBSE-LD/LVI-GC–MS(SIM) method optimisation

Twenty OCPs (α-chlordane, methoxychlor, γ-chlordane, endrin ketone, aldrin, α-HCH, β-HCH, γ-HCH, δ-HCH, *p,p′*-DDD, *p,p′*-DDE, *p,p′*-DDT, dieldrin, α-endosulfan, β-endosulfan, endosulfan sulfate, endrin, endrin aldehyde, heptachlor and heptachlor epoxide (isomer B)) were selected as model compounds for the present study. Since the very beginning we started to establish the best GC–MS(SIM) instrumental conditions for these compounds. In a first approach, the mass spectral fragmentation pattern of each pesticide was evaluated by analysing the standard mixture by GC–MS in the full-scan mode, where target (base peak) and qualifier ions (*m*/*z*) were properly chosen to attain high sensitivity in the SIM mode acquisition, as previously reported [28]. By monitoring those selected ions (Table 1), high response, remarkable selectivity and excellent peak shape could be achieved under the established chromatographic conditions, in a suitable analytical time (<30 min). Additionally, for sensitivity enhancement on real matrices, large volume injection (LVI) was implemented during GC–MS(SIM) analysis, by using *n*-hexane in the solvent vent mode. Thus, an injection volume of 20 μL was set, once larger sample volumes could lead to an increment of solvent background and therefore a lower signal-to-noise ratio was obtained at the trace level. Furthermore, from instrumental calibration, excellent linear dynamic ranges, precision and convenient limits of detection (LOD) at the low ppb levels were achieved for the 20 OCPs under study, according to previous data [28]. Table 1 summarizes the OCPs under study, the corresponding octanol–water partition coefficients ($\log K_{O/W}$), retention times (RT) as well as the ions (*m*/*z*) selected for quantification in SIM mode acquisition by LVI-GC–MS, under the established instrumental conditions.

While the application of SBSE to water samples can be easily achieved to monitor OCPs and other chemical pollutants [27,28,34], in solid matrices is a much more difficult task due the very high level of interfering compounds. As a matter of fact, SBSE in very complex samples such as vegetable matrices must be carried out in a first approach with a suitable organic solvent via a conventional liquid–solid extraction. For the present work, the best pre-extraction of pesticides from vegetable matrices was performed by ultrasonic treatment with MeOH, according to several authors [32–35]. Other solvents were also tested, *e.g.* acetone and equimolar volumes with MeOH, but without reaching better performance than with MeOH alone. Regarding this preconcentration step, previous experiments were performed under ultrasonic extraction during 30 min in a unique step; however, later assays showed that the efficiency was improved with two ultrasonic extraction cycles of 15 min each. Subsequently, the methanolic extracts (2 mL) were centrifuged and the supernatants introduced into glass vials and diluted with ultra-pure water to make a total volume of 30 mL for SBSE-LD implementation. This analytical approach is significantly influenced by the extraction time, agitation speed, pH, addition of an organic modifier and ionic strength, as well as by the LD conditions during back-extraction, as previously reported [27–32]. It must be emphasised that the non-polar PDMS polymer is ideal to recover the OCPs under study since these compounds presenting $\log K_{O/W}$ equal or higher than 3.5 (Table 1). During method development we have decided to test experimental conditions that could be a compromise between analytical time and performance. Thus, we started to assess different extraction times (60 min, 120 min and 180 min) to obtain the sorption profiles on the nine vegetable matrices (lettuce, green bean, onion, broccoli, carrot, spinach, potato, tomato and green pepper).

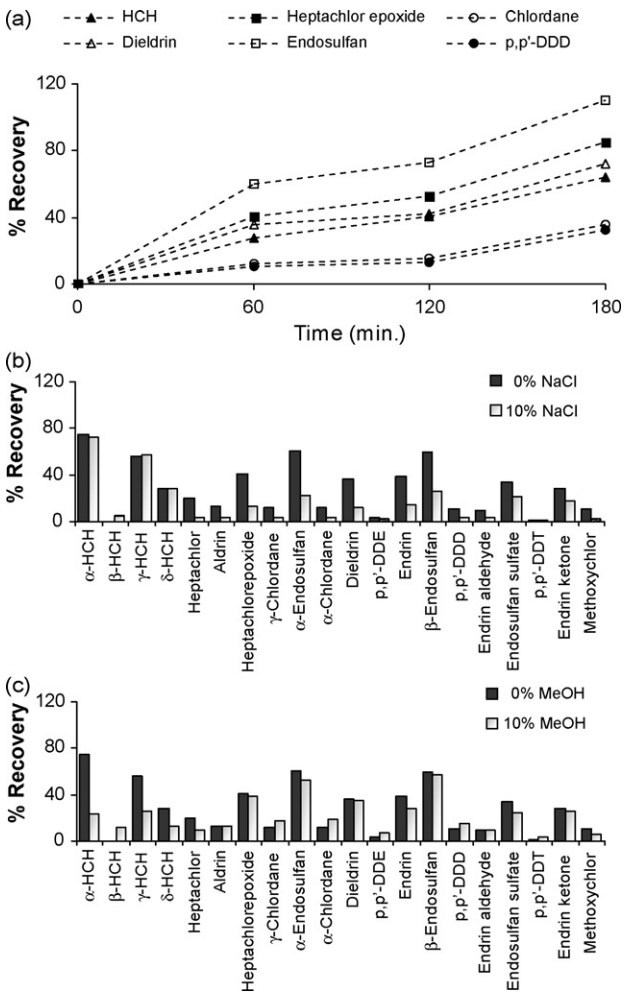

Fig. 1. Assays exemplifying the effect of extraction time (a), salt addition (b) and methanol modifier (c) on the SBSE average efficiency of OCPs in spiked tomato matrix.

Table 1
OCPs studied, corresponding octanol–water partition coefficients ($\log K_{O/W}$), retention time (RT) under locked conditions and ions (m/z) selected for quantification in SIM mode acquisition, MRLs in horticultural products, analytical recoveries (% ± RSD), correlation coefficients (r^2) from linear dynamic range and LOD achieved ($\mu g\,kg^{-1}$ of fresh sample) in several vegetable matrices by SBSE-LD/LVI-GC–MS(SIM), under optimised experimental conditions.

OCPs	$\log K_{O/W}$[a]	RT (min)[b]	Ions (m/z)[c]	MRLs ($\mu g\,kg^{-1}$)[d]	Lettuce			Green bean			Onion		
					Recovery (% ± RSD)[e]	r^2[f]	LOD ($\mu g\,kg^{-1}$)	Recovery (% ± RSD)[e]	r^2[f]	LOD ($\mu g\,kg^{-1}$)	Recovery (% ± RSD)[e]	r^2[f]	LOD ($\mu g\,kg^{-1}$)
α-HCH	4.26	12.22	*219*/181	10	52.1 ± 12.4	0.9609	0.53	32.7 ± 19.1	0.9779	0.50	96.6 ± 12.0	0.9924	0.80
β-HCH	4.26	13.32	*219*/181	10	3.2 ± 0.0	–	0.87	3.1 ± 10.5	0.9039	0.34	110.2 ± 6.8	0.9975	0.05
γ-HCH	4.26	13.57	*219*/181	10	35.6 ± 10.8	0.9650	0.59	27.0 ± 20.2	0.9471	0.65	111.0 ± 8.1	0.9899	7.61
δ-HCH	4.26	15.02	*219*/181	10	18.2 ± 0.0	0.9738	0.51	14.0 ± 14.9	0.9373	0.35	97.3 ± 6.3	0.9993	0.53
Heptachlor	5.86	16.93	*272*/274	10	26.7 ± 3.7	0.9231	4.31	27.5 ± 25.5	0.9734	2.66	64.0 ± 15.5	0.9964	2.21
Aldrin	6.75	18.67	*263*/265	10	39.4 ± 8.7	0.9890	1.03	16.8 ± 13.8	0.9943	0.82	41.8 ± 19.6	0.9972	0.60
Heptachlor epoxide	4.56	20.82	*353*/355	-	49.4 ± 5.9	0.9897	0.69	44.5 ± 19.9	0.9860	0.62	77.7 ± 0.6	0.9962	0.69
γ-Chlordane	6.26	22.14	*373*/375	10	18.4 ± 3.1	0.9950	2.36	19.9 ± 9.9	0.9917	1.75	59.4 ± 19.5	0.9970	1.18
α-Endosulfan	3.50	22.73	*239*/237	50[g]	55.5 ± 7.0	0.9929	0.58	50.5 ± 18.3	0.9880	0.54	80.5 ± 1.3	0.9949	0.64
α-Chlordane	6.26	22.94	*373*/375	10	20.8 ± 1.5	0.9953	2.33	21.8 ± 9.0	0.9911	1.75	60.4 ± 17.4	0.9970	1.28
Dieldrin	5.45	23.94	*263*/265	10	49.8 ± 14.5	0.9943	1.64	42.8 ± 7.4	0.9892	1.43	88.1 ± 1.6	0.9848	1.45
p,p′-DDE	6.00	24.17	*246*/318	50	10.1 ± 4.0	0.9945	1.61	11.5 ± 15.0	0.9967	1.12	80.1 ± 8.6	0.9888	0.50
Endrin	5.45	24.81	*263*/265	10	68.3 ± 6.2	0.9958	0.43	56.1 ± 11.2	0.9872	0.32	77.0 ± 12.2	0.9766	0.70
β-Endosulfan	3.50	25.20	*237*/235	50[g]	42.1 ± 17.7	0.9564	1.26	39.0 ± 13.9	0.9876	0.77	74.7 ± 1.5	0.9963	0.79
p,p′-DDD	5.87	25.81	*235*/237	50	16.7 ± 5.0	0.9727	2.71	35.9 ± 13.8	0.9943	1.53	65.1 ± 17.4	0.9944	1.18
Endrin aldehyde	4.80	25.99	*345*/235	10	6.6 ± 2.6	0.9996	1.36	12.3 ± 1.5	0.9901	1.11	16.6 ± 6.4	0.9764	1.27
Endosulfan sulfate	3.64	26.87	*272*/274	50[g]	22.9 ± 8.9	0.9479	3.42	17.2 ± 18.4	0.9481	2.30	55.5 ± 11.5	0.9985	1.68
p,p′-DDT	6.79	27.13	*235*/237	50	12.5 ± 2.4	0.9762	44.50	17.9 ± 20.6	0.9223	3.11	52.3 ± 7.7	0.9759	0.69
Endrin ketone	4.99	28.28	*317*/345	10	25.5 ± 4.4	0.9902	1.04	24.8 ± 12.1	0.9927	0.81	47.6 ± 1.3	0.9951	0.79
Methoxychlor	5.67	28.99	*227*/274	10	11.6 ± 4.0	0.9350	6.03	24.9 ± 13.4	0.9573	0.80	76.3 ± 1.9	0.9967	0.40

OCPs	Broccoli			Carrot			Spinach		
	Recovery (% ± RSD)[e]	r^2[f]	LOD ($\mu g\,kg^{-1}$)	Recovery (% ± RSD)[e]	r^2[f]	LOD ($\mu g\,kg^{-1}$)	Recovery (% ± RSD)[e]	r^2[f]	LOD ($\mu g\,kg^{-1}$)
α-HCH	88.5 ± 20.1	0.9884	0.93	69.3 ± 19.2	0.9453	0.69	74.3 ± 4.3	0.9951	0.73
β-HCH	108.8 ± 6.5	0.9528	1.12	59.3 ± 4.7	0.9695	0.04	58.3 ± 15.4	0.9886	0.05
γ-HCH	55.6 ± 8.8	0.9892	1.55	108.1 ± 1.5	0.9853	0.27	113.9 ± 4.8	0.9618	7.26
δ-HCH	94.3 ± 17.3	0.9570	0.58	51.2 ± 3.7	0.9373	0.56	30.5 ± 8.6	0.9944	0.60
Heptachlor	65.6 ± 18.7	0.9547	6.96	96.1 ± 18.9	0.9426	1.83	74.4 ± 3.7	0.9934	4.29
Aldrin	102.3 ± 22.5	0.9307	0.98	60.2 ± 19.3	0.9618	0.56	48.0 ± 19.0	0.9946	1.12
Heptachlor epoxide	89.0 ± 21.0	0.9891	0.88	98.6 ± 2.5	0.9854	0.70	79.1 ± 18.0	0.9965	0.97
γ-Chlordane	100.1 ± 20.5	0.9795	2.67	83.3 ± 4.6	0.9803	1.33	42.2 ± 14.8	0.9947	3.19
α-Endosulfan	68.0 ± 12.1	0.9910	1.02	93.8 ± 2.7	0.9897	0.68	62.6 ± 4.9	0.9974	0.86
α-Chlordane	105.7 ± 23.4	0.9734	2.57	83.4 ± 5.1	0.9813	1.45	49.4 ± 13.8	0.9953	2.86
Dieldrin	110.4 ± 7.9	0.9824	1.84	92.4 ± 5.9	0.9913	1.78	87.0 ± 16.1	0.9944	2.16
p,p′-DDE	99.7 ± 11.8	0.9852	2.03	53.3 ± 2.3	0.9820	0.89	19.8 ± 10.0	0.9911	1.54
Endrin	97.4 ± 9.4	0.9906	0.56	73.5 ± 6.5	0.9721	0.59	62.7 ± 14.4	0.9967	0.85
β-Endosulfan	101.6 ± 10.9	0.9850	1.92	81.5 ± 2.3	0.9967	1.00	68.1 ± 17.1	0.9962	1.40
p,p′-DDD	85.9 ± 3.6	0.9981	2.51	65.3 ± 10.0	0.9785	1.55	28.7 ± 8.7	0.9907	2.65
Endrin aldehyde	86.0 ± 20.0	0.9933	2.88	26.9 ± 2.6	0.9821	1.18	12.1 ± 4.5	0.9982	2.16
Endosulfan sulfate	78.6 ± 9.3	0.9786	3.39	50.2 ± 12.5	0.9694	3.13	44.7 ± 4.2	0.9952	2.56
p,p′-DDT	70.8 ± 10.1	0.9147	15.59	32.6 ± 13.5	0.9666	1.47	10.8 ± 6.2	0.9611	3.28
Endrin ketone	84.5 ± 8.1	0.9676	1.35	79.7 ± 6.4	0.9892	1.20	55.7 ± 9.5	0.9955	1.25
Methoxychlor	63.1 ± 6.6	0.9141	3.09	91.6 ± 5.5	0.9793	0.71	82.9 ± 18.1	0.9863	1.00

OCPs	Potato			Tomato			Green pepper		
	Recovery (% ± RSD)[e]	r^2[f]	LOD ($\mu g\,kg^{-1}$)	Recovery (% ± RSD)[e]	r^2[f]	LOD ($\mu g\,kg^{-1}$)	Recovery (% ± RSD)[e]	r^2[f]	LOD ($\mu g\,kg^{-1}$)
α-HCH	146.4 ± 23.2	0.9794	1.40	88.9 ± 4.3	0.9928	0.28	46.7 ± 7.3	0.9904	0.89
β-HCH	–	–	–	–	–	–	–	–	–
γ-HCH	96.9 ± 4.0	0.9867	0.45	72.2 ± 9.1	0.9896	0.07	34.3 ± 5.3	0.9753	0.38
δ-HCH	117.5 ± 21.0	0.9924	8.32	49.2 ± 33.5	0.9974	1.63	17.5 ± 25.2	0.9943	4.90
Heptachlor	238.7 ± 14.2	0.9794	0.02	95.2 ± 11.2	0.9896	0.05	35.9 ± 14.5	0.9912	0.02
Aldrin	132.2 ± 2.8	0.9881	0.02	52.8 ± 13.5	0.9972	0.01	34.9 ± 17.2	0.9951	0.02
Heptachlor epoxide	126.8 ± 3.3	0.9938	0.02	120.8 ± 8.0	0.9966	0.01	75.3 ± 0.3	0.9975	0.03
γ-Chlordane	106.7 ± 1.9	0.9928	0.01	65.8 ± 19.4	0.9977	0.11	40.9 ± 17.3	0.9969	0.02
α-Endosulfan	131.0 ± 4.3	0.9971	4.50	–	-	-	110.7 ± 0.9	0.9969	0.90
α-Chlordane	103.9 ± 2.6	0.9927	0.01	70.7 ± 19.5	0.9975	0.11	43.7 ± 16.1	0.9970	0.02
Dieldrin	113.6 ± 1.6	0.9973	0.93	111.2 ± 11.6	0.9982	1.10	73.5 ± 3.4	0.9981	0.78
p,p′-DDE	60.2 ± 4.2	0.9898	0.20	28.6 ± 21.5	0.9952	0.13	1.2 ± 4.5	0.9980	7.23
Endrin	105.2 ± 6.1	0.9954	1.90	141.9 ± 11.2	0.9921	2.60	83.5 ± 2.0	0.9941	2.02
β-Endosulfan	132.2 ± 4.8	0.9946	6.80	–	-	-	124.3 ± 3.0	0.9969	2.38
p,p′-DDD	106.4 ± 1.5	0.9915	0.12	65.7 ± 19.6	0.9961	0.05	49.3 ± 26.5	0.9955	0.12
Endrin aldehyde	30.8 ± 31.8	0.9863	6.20	19.6 ± 20.3	0.9976	1.10	12.8 ± 13.5	0.9976	2.34
Endosulfan sulfate	248.5 ± 12.3	0.9887	5.20	112.4 ± 16.4	0.9972	0.64	23.2 ± 17.6	0.9968	0.65
p,p′-DDT	72.6 ± 3.9	0.9891	1.01	23.2 ± 31.9	0.9972	1.06	7.7 ± 37.1	0.9900	8.68
Endrin ketone	169.0 ± 7.1	0.9938	1.40	93.4 ± 12.4	0.9979	0.28	51.8 ± 3.5	0.9988	0.63
Methoxychlor	97.8 ± 0.3	0.9794	2.50	92.7 ± 17.2	0.9816	2.50	39.3 ± 15.6	0.9802	0.27

[a] From Ref. [42].
[b] Retention times.
[c] Target ion in *italic*.
[d] According to EU.
[e] Assays performed at the 1500.0 $\mu g\,kg^{-1}$ level.
[f] SAM ranging from 0.1 $\mu g\,kg^{-1}$ to 5000.0 $\mu g\,kg^{-1}$ ($l = 5$).
[g] Except in tomato (500 $\mu g\,kg^{-1}$) and pepper (1000 $\mu g\,kg^{-1}$).

Fig. 1a exemplifies the effect of the extraction time on the SBSE efficiency for six OCPs in spiked tomato matrix. Although equilibrium was not definitely attained for the compounds involved, a 180 min extraction time was selected to avoid unreasonable analytical time. This option is in good agreement with several authors that have point out this extraction time for the determination of OCPs in soils [31], although other reports have suggested longer periods (4–6 h for hexachlorocyclohexane isomers and 24 h for methoxychlor) to getting the equilibrium [27]. Thereby, a period of time of 180 min with an agitation rate of 1000 rpm was selected for the extraction of OCPs in the nine vegetable matrices at 20 °C since temperature is not a critical parameter for the SBSE procedure. During experimental development, the pH of the samples was not adjusted since OCPs are non-ionizable compounds in aqueous medium and this parameter is not significantly affected by changing the value [31]. Ionic strength is also an important parameter that could play a decisive role to enhance efficiency, by decreasing the affinity of the less non-polar OCPs to the aqueous matrix rather than its affinity for the PDMS coating of the stir bar [32,34]. Thus, the "salting-out effect" was performed through the addition of 10% (w/v) of NaCl, where it was observed that the recovery yields were negatively affected, as can be observed in Fig. 1b, exemplifying this effect on the efficiency of SBSE in spiked tomato matrices. This effect was also observed by other authors when assaying organophosphorous insecticides [36], pesticides including OCPs [31] and polycyclic aromatic hydrocarbons [37] and might be explained because the addition of salt besides increasing the matrix polarity, reducing the affinity of OCPs towards to the PDMS of the stir bar due the occurrence of lipids, proteins and organic acids that may compete with polymeric phase. Therefore, further experiments were performed without salt addition.

Several authors have point out that hydrophobic compounds tend to be adsorbed in the walls of the containers used in the handling of aqueous samples and also by sample compounds such as lipids, proteins and organic acids [38,39]. Therefore, in order to prevent this effect the addition of an organic solvent, such as MeOH [27–32] or ACN [37], is recommended to increase analytical recoveries. However, for the more polar pesticides, the addition of an organic solvent may reduce the recovery yields because it can reduce partitioning coefficients between PDMS phase and aqueous media [27,28,32]. In addition, the polarity of the matrix mixture can also condition the amount of OCPs that can be extracted by SBSE [32]. To evaluate the effect of dilution factor on the SBSE, 10% (v/v) of MeOH was added to the sampling extract in all vegetable matrices under study. Fig. 1c exemplifies the effect of MeOH addition on the extraction efficiency in spiked tomato matrix. As it can be observed, the influence of MeOH addition was also negative for almost all OCPs under study, in particular for the most polar ones. Other authors have reported that the addition of organic solvent affects negatively the sorption of analytes on the stir bar, especially the most polar compounds [31]. This fact could be due to the increase of OCPs solubility in the MeOH/water mixture and subsequent reduction of OCPs partitioning in favour of the PDMS phase. Therefore, MeOH addition was also discarded. During method development, we also assess the LD conditions, which ensure the best back-extraction for the 20 OCPs from the stir bar polymeric phase. In a first approach, 1.5 mL is essential to ensure the total immersion and a enough phase ratio in between the PDMS and the stripping solvent volumes for a better back-extraction process, according to previous reports [28]. Covering a wide range of polarities, we try to assess the higher capacity of the solvents to remove the OCPs from the stir bars polymeric phase. LD solvents such as ACN, acetone and mixtures of both were tested in order to survey the best stripping performance, using standard conditions. Sonification treatment was also implemented at this stage, to accelerate the stripping efficiency of the OCPs from the stir bars and a period of 15 min

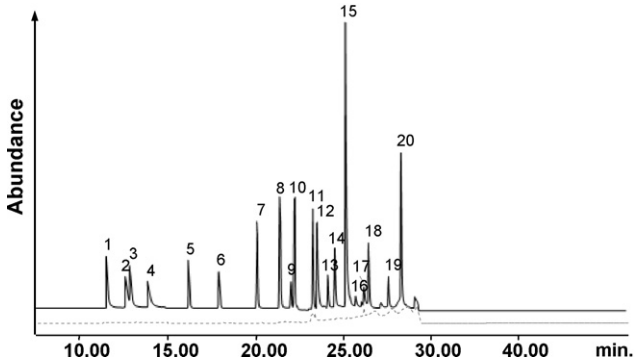

Fig. 2. Total ion fragmentogram exemplifying a carrot blank matrix (dashed line) and after spiking with OCPs obtained by SBSE-LD/LVI-GC–MS(SIM), under established experimental conditions. (1: α-HCH, 2: β-HCH, 3: γ-HCH, 4: δ-HCH, 5: heptachlor, 6: aldrin, 7: heptachlor epoxide, 8: γ-chlordane, 9: α-endosulfan, 10: α-chlordane, 11: dieldrin, 12: *p,p′*-DDE, 13: endrin, 14: β-endosulfan, 15: *p,p′*-DDD, 16: endrin aldehyde, 17: endosulfan sulfate, 18: *p,p′*-DDT, 19: endrin ketone, 20: methoxychlor).

was established for back-extraction. From the data obtained, it was observed that ACN presents a much higher stripping capacity than the other solvents for all OCPs and therefore, this solvent was chosen for the LD procedure in agreement with previous reports [28]. Furthermore, no evidence of contamination was observed during blank assays and the PDMS phase of the stir bars showed very high stability after several tens of sampling experiments. Solvent switch, *i.e.* evaporation to dryness followed by reconstitution in an organic solvent more convenient for LVI, was required in order to obtain acceptable peak shape and enough reproducibility. Thereby, the residues were redissolved in *n*-hexane (120 μL), injected into the LVI-GC–MS(SIM) system and the corresponding data compared with those obtained with standard controls. During this stage, we had observed non-significative losses of OCPs as previously reported [28] since we are dealing with semi-volatile compounds. In short, the experimental conditions established to monitor OCPs in vegetable matrices involves a pre-extraction step of each freeze-dried sample (100.0 mg) with MeOH (2 mL) under sonication (2 × 15 min), followed by centrifugation (4000 rpm; 5 min) and dissolution of the methanolic extract to 30 mL with ultra-pure water; the SBSE-LD conditions are as following: extraction time: 180 min (1000 rpm; 20 °C); back-extraction solvent: ACN (1.5 mL), 15 min under sonication and *n*-hexane (120 μL) as solvent switch. Fig. 2 exemplifies a total ion fragmentogram from a carrot blank matrix and after spiking (1500 $\mu g\,kg^{-1}$) with OCPs obtained by SBSE-LD/LVI-GC–MS(SIM) showing the remarkable selectivity achieved by the proposed methodology.

3.2. SBSE-LD/LVI-GC–MS(SIM) method performance

After establishing the most convenient experimental conditions, the performance of the proposed methodology was evaluated for the 20 OCPs through accuracy assays, precision, linear dynamic range and LODs for each vegetable matrix. In order to evaluate the accuracy, recovery assays ($n = 3$) of freeze-dried uncontaminated samples (100.0 mg each) were spiked with the target OCPs at a concentration level of 1500.0 $\mu g\,kg^{-1}$ and assayed according to the established conditions described in the previous section. Table 1 summarizes the average recoveries with the relative standard deviation (% ± RSD), correlation coefficients and LODs achieved for the 20 OCPs extracted from the nine spiked vegetable matrices by the present methodology. In a first approach, the data obtained clearly demonstrate that the matrices involved seem to have a strong effect on the recovery behaviour of these pesticides by the proposed methodology, where ranges with some

variability are notice in particular for the lower yields; 3.2 ± 0.0 to 68.3 ± 6.2% (lettuce), 3.1 ± 10.5 to 56.1 ± 11.2% (green bean), 16.6 ± 6.4 to 111.0 ± 8.1% (onion), 55.6 ± 8.8 to 108.8 ± 6.5% (broccoli), 26.9 ± 2.6 to 108.1 ± 1.5% (carrot), 10.8 ± 6.2 to 113.9 ± 4.8% (spinach), 30.8 ± 31.8 to 132.2 ± 4.8% (potato), 19.6 ± 20.3 to 120.8 ± 8.0% (tomato) and 1.2 ± 4.5 to 110.7 ± 0.9% (green pepper). This observation is in good agreement with several authors [40,41,45], who have pointed out that differences in the plant constitution, such as water content, fat, pigments, metabolites and texture influences very much the extraction efficiency of the analytical methodologies. In general, lower yields were obtained from green vegetables, especially the leafy ones, which can be explained through the epicuticular wax that cover the plants can have remarkable influence on the SBSE process. For instance, the recovery yields obtained from potato matrix present for some OCPs (α-HCH, heptachlor, endosulfan sulfate and endrin ketone) abnormal yields than those expected, and for other ones, *i.e.* potato, tomato and green pepper, β-HCH present very low or even the absence of recovery. This observation can be attributed to the occurrence of strong interfering metabolites that could have a negative influence on those particular OCPs. According to SBSE theory [26], since the analyte partition coefficient between PDMS and water ($K_{PDMS/W}$) is strongly correlated with the corresponding $K_{O/W}$, theoretical recoveries of pesticides from aqueous samples by SBSE can be calculated from the corresponding $\log K_{O/W}$ and the sample–PDMS volume phase ratio (β) involved. Consequently, it is expected that the non-polar OCPs ($\log K_{O/W} \geq 3.5$) should present considerable affinity to the PDMS coating of the stir bars, the only commercially available polymeric phase. Thus, the $K_{O/W}$ of each pesticide under study can be calculated with the SRC-KOWWIN software package (Syracuse Research, Syracuse, NY, USA), according to a fragment constant estimation methodology [42]. Fig. 3 exemplifies the theoretical and experimental average recovery data (Table 1) against $\log K_{O/W}$ obtained for five OCPs (α-endosulfan, endrin ketone, methoxychlor, *p,p′*-DDT and γ-chlordane) in the nine vegetable matrices by SBSE-LD/LVI-GC–MS(SIM), under the established conditions. The equilibrium theoretical line was calculated taking into consideration that 30 mL of aqueous sample (V_W) and a stir bar coated with 47 μL (V_{SBSE}) of PDMS were used, for which a phase ratio ($\beta = V_W/V_{SBSE}$) value of 638.3 was established. Thus, for a specific OCP having a $\log K_{O/W}$ of 5.67 (*e.g.* methoxychlor), a theoretical recovery of 99.8% should be expected. Nevertheless, the experimental data obtained for this particular OCP shows that the recovery depends of the vegetable matrix involved, demonstrating values ranging from 11.6 ± 4.0% (lettuce) to 97.8 ± 0.3% (potato). As stated before, the deviations observed for this particular case could be attributed to the matrix effects due the occurrence of many interfering compounds as well as synergisms that could play a negative role on recovery yields. As a general trend, the experimental average efficiencies obtained for almost all OCPs present deviations from the theoretical line in particular the green vegetables, as can be seen for α-endosulfan, endrin ketone, methoxychlor, *p,p′*-DDT and γ-chlordane (Fig. 3), presenting a similar behaviour to those obtained from drinking water matrices [28]. It must be emphasized that the theoretical recoveries represent only indicative values because the extraction time of 180 min is not long enough to reach the equilibrium and matrix effects are not taken into consideration. Although the recoveries obtained are in many cases lower than 70%, they are comparable or even better to those provided by other authors for the determination of some of these priority pollutants by SBSE in fruits and vegetables [32,33].

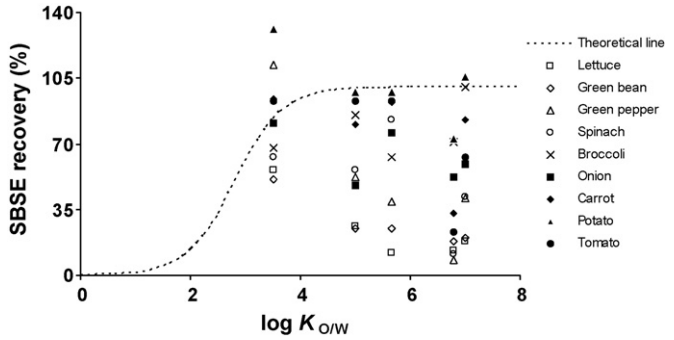

Fig. 3. Theoretical and experimental average recovery data (Table 1) against $\log K_{O/W}$ obtained for five OCPs (α-endosulfan, endrin ketone, methoxychlor, *p,p′*-DDT and γ-chlordane) in the nine vegetable matrices by SBSE-LD/LVI-GC–MS(SIM), under optimised conditions.

Due the strong matrix effects observed, the linearity of the proposed method was evaluated through the standard addition methodology, by spiking all freeze-dried matrices at five concentration levels ranging from 0.1 to 5000.0 μg kg^{-1} and performed in triplicate. From the data obtained (Table 1), the resulted dynamic ranges showed suitable linearity of the response for the 20 OCPs, with correlation coefficients (r^2) higher than 0.99 in almost all cases. Subsequently, the LODs were also calculated as the lowest concentration of the pesticides in spiked matrices giving at least response with a signal-to-noise ratio of three in the total ion chromatograms. Table 1 shows the LODs (μg kg^{-1} of fresh sample) obtained in all matrices under study, where it can be observed that almost all of these values are towards compliance with the requirements by EU directives [6,43]. Even so, for the particular case of *p,p′*-DDT in lettuce and broccoli matrices, slightly higher LODs are observed, although lower than the MRL (50 μg kg^{-1}), which can be attributed to possible degradation. Nevertheless, the LODs obtained show that the proposed methodology seems to be very useful in the control of OCP residues in several vegetable matrices as the MRLs established by the EU and the governments of its member countries can be as low as 10 μg kg^{-1} depending on the particular pesticide and vegetable type [6]. By comparing the LODs obtained by the proposed methodology (SBSE-LD/LVI-GC–MS(SIM)) with conventional techniques that use Soxhlet [8] or shake-flask [10] extraction, much better sensitivity is attained, presenting the same order of magnitude to those methods that use novel solvent extraction approaches such as pressurized liquid extraction [18] or microwave assisted extraction [19], for the determination of OCPs in horticultural matrices. On the other hand it must be taken into account that other advantages are definitely found. For instance, the amount of matrix processed by the proposed methodology is 20–50 times lower than that used for the conventional techniques and 3–8 times lower than that used for novel solvent extraction approaches. Additionally, the LODs obtained for endosulfans (α and β) by these conventional techniques are higher than those provided by SBSE existing methods for the determination of OCPs in fruits with the exception for potato matrix [44]. In short, the proposed methodology showed to be easy of work-up, fast, almost solventless with low sample amount requirements, when compared with conventional methods of sample preparation. The LD approach demonstrated a noteworthy back-extraction performance and is a cost-effective option, due eliminate the need for the expensive thermal desorption devices. The remarkable selectivity and sensitivity provided by SBSE-LD/LVI-GC–MS(SIM) for determining OCPs in different vegetable matrices could be established as a suitable protocol to screen trace levels towards compliance with the EU directives.

4. Conclusions

Stir bar sorptive extraction followed by liquid desorption and large volume injection-gas chromatography coupled to mass spectrometry using selected-ion monitoring mode acquisition (SBSE-LD/LVI-GC–MS(SIM)) have been successfully applied to

monitor 20 OCPs in nine vegetables (lettuce, spinach, green bean, green pepper, tomato, broccoli, potato, carrot and onion). The main experimental parameters influencing the extraction efficiency were optimised provided the best analytical performance to monitor OCPs in vegetable matrices at the trace level. The data obtained clearly demonstrate that the matrices involved have a strong effect on the recovery behaviour of the OCPs under study, in particular the green vegetables especially the leafy ones. By using the standard addition methodology good linearity, enough recovery yields and precision were attained for almost all cases, depending on the particular OCP and vegetable matrix involved. The sensitivity achieved is towards compliance with the European Union regulations for the maximum residue limits of OCPs in agricultural vegetables. The main advantages of this methodology when compared with the conventional sample preparation approaches to screen pesticides in vegetable matrices are the avoidance of clean-up and concentration procedures, as well as the significant reduction of organic solvents, thus decreasing costs and analytical time.

Acknowledgements

M. Barriada-Pereira wishes to thank the Xunta de Galicia for a grant and Laboratory of Chromatography & Capillary Electrophoresis of the Chemistry and Biochemistry Department (FCUL) for technical assistance. P. Serôdio wishes to thank Fundação para a Ciência e a Tecnologia for a grant (SFRH/BD/9108/2002).

References

[1] B.C. Kelly, F. Gobas, Environ. Sci. Technol. 35 (2001) 325.
[2] M.A. Mendez, L. Arab, Pure Appl. Chem. 75 (2003) 1973.
[3] E. Concha-Graña, M.I. Turnes-Carou, S. Muniategui-Lorenzo, P. López-Mahía, D. Prada-Rodríguez, E. Fernández-Fernández, Chemosphere 64 (2006) 588.
[4] P. Manirakiza, A. Covaci, L. Nizigiymana, G. Ntakimazi, P. Schepens, Environ. Pollut. 117 (2002) 447.
[5] Off. J. Eur. Union 209 L (2006) 3.
[6] Off. J. Eur. Union 70 L (2005) 1.
[7] M. Gonzalez, K.S.B. Miglioranza, J.E.A. de Moreno, V.J. Moreno, Food Chem. Toxicol. 43 (2005) 261.
[8] R. Sanghi, V. Tewari, Bull. Environ. Contam. Toxicol. 67 (2001) 587.
[9] A. Zohair, A.B. Salim, A.A. Soyibo, A.J. Beek, Chemosphere 63 (2006) 541.
[10] A. Agüera, M. Contreras, J. Crespo, A.R. Fernández-Alba, Analyst 127 (2002) 347.
[11] B. Kumari, V.K. Madan, J. Singh, S. Singh, T.S. Kathpal, Environ. Monit. Assess. 90 (2004) 65.
[12] J.L. Martínez-Vidal, F.J. Arrebola, M. Mateu-Sánchez, J. Chromatogr. A 959 (2002).
[13] C.M. Lino, L.M.C. Guarda, M.I.N. Silveira, J. AOAC Int. 82 (1999) 1206.
[14] C.M. Lino, M.I.N. d. Silveira, J. Chromatogr. A 769 (1997) 275.
[15] P. Manirakiza, A. Covaci, P. Schepens, Chromatographia 52 (2000) 787.
[16] S.J. Lehotay, K.I. Eller, J. AOAC Int. 78 (1995) 821.
[17] C. Quan, S.F. Li, S.J. Tian, H. Xu, A.Q. Lin, L. Gu, J. Supercrit. Fluid 31 (2004) 149.
[18] K. Adou, W.R. Bontoyan, P.J. Sweeney, J. Agric. Food Chem. 49 (2001) 4153.
[19] M. Barriada-Pereira, M.J. Gonzalez-Castro, S. Muniategui-Lorenzo, P. Lopez-Mahia, D. Prada-Rodriguez, E. Fernandez-Fernandez, Talanta 71 (2007) 1345.
[20] S. Tao, F.L. Xu, X.J. Wang, W.X. Liu, Z.M. Gong, J.Y. Fang, L.Z. Zhu, Y.M. Luo, Environ. Sci. Technol. 39 (2005) 2494.
[21] M. Barriada-Pereira, M.J. Gonzalez-Castro, S. Muniategui-Lorenzo, P. Lopez-Mahia, D. Prada-Rodriguez, E. Fernandez-Fernandez, Int. J. Environ. Anal. Chem. 85 (2005) 325.
[22] J. Tekel, T. Hudecová, K. Pecníková, Eur. Food Res. Technol. 213 (2001) 250.
[23] C. Ferrer, M.J. Gomez, J.F. Garcia-Reyes, I. Ferrer, E.M. Thurman, A.R. Fernandez-Alba, J. Chromatogr. A 1069 (2005) 183.
[24] D. Stajnbaher, L. Zupancic-Kralj, J. Chromatogr. A 1015 (2003) 185.
[25] L. Wennrich, P. Popp, G. Köller, J. Breuste, J. AOAC Int. 84 (2001) 1194.
[26] E. Baltussen, P. Sandra, F. David, C. Cramers, J. Microcolumn Sep. 11 (1999) 737.
[27] V.M. León, B. Álvarez, M.A. Cobollo, S. Muñoz, I. Valor, J. Chromatogr. A 999 (2003) 91.
[28] P. Serôdio, J.M.F. Nogueira, Anal. Chim. Acta 517 (2004) 21.
[29] P. Serôdio, J.M.F. Nogueira, Anal. Bioanal. Chem. 382 (2005) 1141.
[30] P. Serôdio, J.M.F. Nogueira, Water Res. 40 (2006) 2572.
[31] R. Rodil, P. Popp, J. Chromatogr. A 1124 (2006) 82.
[32] N. Ochiai, K. Sasamoto, H. Kanda, T. Yamagami, F. David, B. Tienpont, P. Sandra, J. Sep. Sci. 28 (2005) 1083.
[33] P. Sandra, B. Tienpont, F. David, J. Chromatogr. A 1000 (2003) 299.
[34] N. Ochiai, K. Sasamoto, H. Kanda, S. Nakamura, J. Chromatogr. A 1130 (2006) 83.
[35] W.M. Liu, Y. Hu, J.H. Zhao, Y. Xu, Y.F. Guan, J. Chromatogr. A 1095 (2005) 1.
[36] C. Blasco, M. Fernández, Y. Picó, G. Font, J. Chromatogr. A 1030 (2004) 77.
[37] M.S. García-Falcón, B. Cancho-Grande, J. Simal-Gándara, Water Res. 38 (2004) 1679.
[38] D. Barceló, M.-C. Hennion, Trace Determination of Pesticides and Their Degradation Products in Water, Elsevier, The Netherlands, 1997.
[39] E. Concha-Graña, M.I. Turnes-Carou, S. Muniategui-Lorenzo, P. López-Mahia, E. Fernández-Fernández, D. Prada-Rodríguez, Chromatographia 54 (2001) 501.
[40] H. Obana, K. Akutso, M. Okihashi, S. Hori, Analyst 126 (2001) 1529.
[41] J. Tekel, S. Hatrík, J. Chromatogr. A 754 (1996) 397.
[42] W.M. Meylan, P.H. Howard, J. Pharm. Pharm. Sci. 84 (1995) 83.
[43] Off. J. Eur. Union L 350 (1990) 71.
[44] C. Bicchi, C. Cordero, C. Iori, P. Rubiolo, P. Sandra, J.H. Yariwake, V.G. Zuin, J. Agric. Food Chem. 51 (2003) 27.
[45] C. Przybylski, F. Hommet, J. Chromatogr. A 1201 (2008) 78.