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Determination of pesticides in vegetables using large-volume injection column liquid chromatography–electrospray tandem mass spectrometry

A.C. Hogenboom[a,*], M.P. Hofman[a], S.J. Kok[a], W.M.A. Niessen[b], U.A.Th. Brinkman[a]

[a]*Vrije Universiteit, Department of Analytical Chemistry and Applied Spectroscopy, De Boelelaan 1083, 1081 HV Amsterdam, Netherlands*

[b]*Hyphen MassSpec Consultancy, De Wetstraat 8, 2332 XT Leiden, Netherlands*

Abstract

Direct injection of a large volume (900 μl) of a sample extract onto a liquid chromatographic (LC) column, LC separation and electrospray tandem mass spectrometric detection were used for the quantitative analysis of a wide polarity range of pesticides in carrots and potatoes. Rapid sample preparation involved extraction of a small amount of sample (2 g) with a small volume of organic solvent (3 ml), clean-up over a filter and dilution of the organic extract with the aqueous LC eluent. The extraction efficiency for the selected pesticides was studied using methanol, acetone and acetonitrile as solvents. Evaluation of the performance of the overall method, using extraction with acetonitrile and detection in the selected-reaction-monitoring mode, showed excellent linearity in the range of 2–100 μg/kg with limits of detection of 0.5–2 μg/kg for both types of vegetable. With relative standard deviations of the MS peak area measurements of less than 6.5% ($n=8$) the repeatability of the method was fully satisfactory.

Keywords: Vegetables; Food analysis; Large-volume injection; Pesticides

1. Introduction

Insecticides, fungicides and herbicides are agrochemicals designed to combat the attacks of various pests on agricultural and horticultural crops. For obvious reasons, multi-residue monitoring is restricted to the pesticides used on a particular crop. Due to the ever-lower detection levels required by regulatory bodies and the complex nature of the matrices in which the target compounds are entrained, efficient sample preparation and trace-level detection and identification are important aspects in analytical method development. In the case of pesticides in vegetables and fruit, the maximum residue levels that are set by government agencies and the European Union are in the range of 100–1000 μg/kg, method development is focused on achieving the detection of 10–50 μg/kg [1,2].

Although gas chromatography (GC) is the technique preferably used for analyzing pesticide residues in agricultural food products [3,4], the use of LC for such applications is increasing [5]. LC is very effective in separating non-volatile and thermally labile compounds, but the conventional detection technique, ultraviolet absorption (UV), cannot be used for multi-residue screening, because it provides insufficient selectivity. Fluorescence detection may seem to be an interesting alternative but, unfortunately, most pesticides do not display native fluorescence. On the other hand, mass spectrometry

*Corresponding author. Fax: +31-20-444-544.

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(MS) and, especially, tandem mass spectrometry (MS–MS) detection are very sensitive and selective techniques for both multi-residue screening of a wide range of pesticides and for trace-level identification of target pesticides [6–8]. Currently, the routine application of LC–MS for pesticide analysis in vegetables and fruit is still limited, probably due to the high cost of the instrumentation [9–13].

Sample preparation aimed at isolating and enriching the target compounds is a prerequisite with all complex sample matrices. Many published procedures involve solvent partitioning, sometimes followed by clean-up on Florisil [14], or by means of gel permeation chromatography [15,16] or supercritical fluid extraction [17]. However, they are rather laborious and time-consuming and, in the former case, large volumes of toxic extraction solvents are used. Nowadays, solid-phase extraction (SPE) is increasingly being used in food analysis. The major reason is the possibility of automation and the high sample throughput that can be obtained [18]. In addition, more attention is focused on minimizing the sample preparation with, preferably, smaller sample sizes and extraction volumes and, occasionally, the analysis of crude samples. This is becoming a viable approach with the advent of better separation and, much more so, more selective detection techniques. Another promising approach is to apply sample (extract) volumes of some 0.5–10 ml directly on-column [8].

This paper describes the development of a sensitive and selective multi-residue method for the quantitative determination of pesticides selected from several compound classes in carrots and potatoes. The method involves a rapid and small-scale extraction procedure followed by on-line analyte enrichment by means of large-volume injection (LVI) on a 5 cm-long LC column coupled to electrospray (ESI) MS–MS, an ionization mode of MS detection which, in our hands, has shown excellent results in pesticides analysis (cf. [5,6,11,12]).

2. Experimental

2.1. Chemicals and samples

HPLC-grade methanol, acetone, phosphoric acid (85%, analytical reagent grade), sodium hydrogen phosphate and acetic acid were purchased from J.T. Baker (Deventer, Netherlands). Ammonium acetate, ammonium formate and formic acid were purchased from Riedel–de Haën (Seelze, Germany). Acetonitrile (HPLC-grade) was obtained from Fluka (Neu-Ulm, Germany). HPLC-grade water was demineralized in the laboratory and distilled in an all-quartz system. All pesticides were over 95% pure and were supplied by Riedel-de Haën. [$^2H_5$]Diazepam (over 98% pure) was used as internal standard in the MS experiments (Cambridge Isotope Labs., Andover, MA, USA).

Stock standard solutions of pesticides (1 mg/ml) were prepared by weighing and dissolving them in HPLC-grade methanol. The stock standard solutions were stored in the dark at −20°C. A standard mixture of 50 μg/ml was prepared by dilution of the stock solutions with HPLC-grade methanol and was used for preparing solutions for standard injections and spiking the extracts. They were stored at −20°C.

Nitrogen was used as drying and nebulizing gas (99.999% purity, Praxair, Oevel, Belgium) at flow-rates of 350 and 20 l/h, respectively.

2.2. Instrumentation and columns

The LC system consisted of a Hewlett-Packard (Waldbronn, Germany) 1100 Series liquid chromatograph equipped with a vacuum degasser, a quaternary solvent delivery system, an autosampler equipped with a 900-μl syringe for large-volume injections, a thermostated column compartment and a diode-array UV (DAD UV) detector. A computer using Hewlett–Packard ChemStation software controlled the whole LC system.

Large-volume injections and reversed-phase LC separations were carried out on a 50 mm×4.6 mm I.D. Zorbax SB-C_{18} column packed with 3.5 μm particles (Rockland Technologies, Nuenen, Netherlands).

Tandem mass spectrometry was performed on a Quattro II triple-stage quadrupole equipped with an ESI source (Micromass, Altrincham, UK). Masslynx software running under Windows NT was used for control of the system and data acquisition.

2.3. Analytical conditions and procedures

2.3.1. Sample preparation

Carrots and potatoes were cut into small pieces without any pretreatment such as washing or removing their skin. The vegetables were ground in a blender for 15 min and 2 g were extracted with 3 ml of an organic solvent (methanol, acetonitrile or acetone) in an ultrasonic bath for 45 min. The extract was filtered over a disposable-syringe Titan filter with 0.45 μm pores (Scientific Resources, Eatontown, NJ, USA). The extract was diluted with the buffer in an autosampler vial for 900-μl injection (for buffer/organic extract solvent ratios, see Section 3.1).

2.3.2. LC analyses

In order to select an LC eluent composition which would provide an overall optimum response for MS detection three aqueous solutions (solvent A; for details see Section 3.2) were selected, and two modifiers (solvent B; for details, see Section 3.2). Separation was performed using a single-step linear gradient. To save solvents, the flow-rate was set to 0.1 ml/min when no separation was carried out, and programmed to 1 ml/min during the first minute of the analysis. At that time the composition was solvent A–solvent B (85:15, v/v). Next, a linear gradient to 85% B was run in 13 min, with a final hold of 2 min. After the run, a sharp gradient was used to reach the initial composition again. During all separations the column was thermostated at 40°C.

A post-column splitter was placed between the LC and the ESI interface to maintain a flow of 120 μl/min entering the MS. The ESI source conditions were: source temperature, 100°C; capillary voltage, 3.5 kV in the positive-ion (PI), and −3.0 kV in the negative-ion (NI) mode. The sampling cone voltage was 30 V for the PI, and −25 V for the NI mode, with a skimmer lens offset of 5 V. Mass spectra were collected in the full-scan PI and NI modes (m/z 100–350; scan time, 100 amu/s; interscan time, 0.1 s). Selected-ion monitoring (SIM) was performed on $[M+H]^+$ in the PI mode and $[M-H]^-$ in the NI mode.

MS–MS experiments were carried out with an argon pressure of approx. 2.5×10^{-3} mbar in the collision cell. Cone voltage and collision energy values optimized for each of the compounds selected were used. Full-scan product-ion spectra of the $[M+H]^+$ or $[M-H]^-$ for PI and NI, respectively, were collected with the third quadrupole (Q3) scanning from m/z 40 up to 10 amu above the molecular mass of the compound (scan time, 100 amu/s; interscan time, 0.1 s). For selected reaction monitoring (SRM) experiments, both Q1 and Q3 were set at fixed m/z values, viz. $[M+H]^+$ or $[M-H]^-$ for Q1 for the PI and NI modes, respectively, and two of the most intense product ions for Q3 (dwell time, 0.1 s; interchannel delay, 0.01 s).

Automated and unattended data acquisition was performed using contact closure signals [8].

3. Results and discussion

3.1. Extraction solvents

Most multi-residue extraction procedures involve liquid–liquid extraction with large volumes of an organic solvent (acetone, dichloromethane, light petroleum or hexane) followed by evaporation and clean-up. The choice of the solvent is based on its extraction efficiency and its compatibility with introduction into a GC system. As a consequence, organic solvents such as methanol and acetonitrile, are often not considered and/or tested in extraction procedures for food samples. However, these solvents are widely used in LC eluents and are, therefore, compatible with LC-based procedures. Three solvents were selected, methanol, acetonitrile and acetone, to study their extraction efficiency for a wide polarity range of pesticides from carrots and potatoes.

With 900-μl injections of pure organic solvent, the more polar compounds eluted with the solvent peak and a dramatic decrease of peak area and deterioration of peak shape was observed for the more apolar compounds. To determine the maximum allowable amount of organic solvent in the injection volume, the pesticides were dissolved in various buffer–organic solvent mixtures ranging from (95:5, v/v) to (50:50, v/v) and 900 μl were injected and analysed by LC–DAD UV and full-scan MS. The main criteria were no loss of the first eluting compound (dimethoate), and no significant deterioration of peak shapes or decrease of peak areas. The maximum

allowable percentage of methanol was 25–50%. With acetonitrile and acetone, the ranges were lower, viz. 15–30 and 15–25%, respectively. To be on the safe side, the percentage of organic solvent in the 900-μl injection was set at 25% for methanol, and 15% for acetonitrile and acetone.

3.2. LC eluent composition

In order to select an LC eluent composition which would provide an overall satisfactory response for MS detection, two modifiers were selected, acetonitrile or methanol, and three aqueous solutions, a 10 m*M* phosphate buffer (pH 3), a 10 m*M* ammonium acetate (pH 4) solution and a 10 m*M* ammonium formate (pH 3) solution. In addition, the performance of two counter electrodes (built in the ESI source), the so-called ''pepperpot'' and ''crossflow'', were compared in terms of analyte response [19]. The manufacturer recommends the use of the crossflow if a large number of complicated samples containing non-volatiles have to be analysed or if a non-volatile buffer is present in the LC eluent**.**

Table 1 shows the relative peak areas of each of the analytes for different LC eluent compositions and the two counter electrodes. For each analyte, the highest responses obtained with the pepperpot and the crossflow were set at 100. One main conclusion is that the analyte responses vary widely with the LC eluent composition. The most extreme results were obtained for linuron, with relative responses ranging from 0 to 100% with both counter electrodes. Overall, for the analytes detected in the PI mode, gradient LC with aqueous ammonium formate–methanol gave the best results, with the pepperpot as well as the crossflow. The same was true for the two analytes detected in the NI mode, DNOC and dichlorprop, if the crossflow was used. However, in this case, ammonium acetate was a much better choice than ammonium formate for the pepperpot. Direct comparison of the results obtained with the pepperpot and the crossflow in the PI mode and using ammonium formate–methanol gradient LC showed response ratios which were about 50% better with the pepperpot for the first three compounds of Table 1, and some 50% better with the crossflow for the last three compounds. For DNOC and dichlorprop, on the other hand, no noticeable differences were observed. irrespective of the counter electrodes and with both ammonium acetate–methanol and ammonium formate–methanol LC eluents.

As regards the robustness of the several alternatives, when a large number of food samples was analyzed, the analyte responses remained constant over a long time period with the crossflow. No blocking of either the crossflow or the sample cone was observed. The crossflow was, therefore, selected for all further work, and also for a brief study of the system performance with an aqueous phosphate buffer, which probably is the buffer most frequently used in LC. Analyte responses were found to be constant over a period of time of about 12 h, but the

Table 1
Relative peak areas (%) of test analytes with different LC eluent compositions and detection by full-scan MS ($n=4$)[a]

LC eluent A[b]	LC eluent B[b]	PI						NI	
		Atraton	Atrazine	Diuron	Linuron	Metolachlor	Diazinon	DNOC	Dichlorprop
Pepperpot									
AA	ACN	45	50	15	0	70	65	95	90
AA	MeOH	65	80	50	60	**100**	**100**	**100**	**100**
AF	ACN	65	55	30	5	45	90	45	25
AF	MeOH	100	100	100	100	90	90	45	25
Crossflow									
AA	ACN	30	40	10	0	45	20	55	45
AA	MeOH	45	50	45	40	60	25	100	85
AF	ACN	60	55	55	65	95	55	80	75
AF	MeOH	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

[a] For each analyte, the highest response using the pepperpot and crossflow was set at 100 (in bold).
[b] AA, ammonium acetate; AF, ammonium formate; ACN, acetonitrile; MeOH, methanol.

responses were, typically, at least 10-fold lower than with ammonium formate, both with methanol and acetonitrile as organic modifier (data not shown).

Since the majority of the test compounds was detected in the PI mode, it was decided to continue the experiments in this mode. A new analyte mixture was selected which comprised the six compounds of interest from Table 1 and, in order to maintain a wide polarity range and variety in compound classes, dimethoate, metoxuron and carbofuran.

3.3. Sample analysis by on-line LC–MS and MS–MS

In order to test the practicality of the analytical procedures, 2-g samples were weighed into scintillation vials and 3 ml of extraction solvent (methanol) were added. The samples were spiked at the 100-μg/kg level directly after weighing. After filtering of the extract and dilution with buffer, 900-μl injections were performed into the LC–DAD UV and MS. In the UV chromatograms of the vegetable extracts spiked at this relatively high concentration level a large number of matrix interferences showed up which made identification and/or quantification of the compounds quite difficult. With full-scan MS, identification was possible by extracting ions (m/z values) characteristic of the compounds of interest. Although full-scan MS gave satisfactory results at spiking levels of 25–200 μg/kg, for ultra trace-level target analysis which is the principal goal of the present study, selected ion monitoring (SIM) in MS, and selected reaction monitoring (SRM) in MS–MS, are much better options, as will be demonstrated below.

The performance of SIM and SRM was compared in terms of sensitivity and selectivity for both aqueous standard solutions of the pesticides and spiked (5–10 μg/kg level) extracts of carrots. Comparison of Figs. 1A and 2A shows that the results obtained with SIM and SRM detection were closely similar with regard to both sensitivity and selectivity, if standard solutions were analysed. However, when vegetable extracts such as the carrot extract of Figs. 1B and 2B were analysed, there was a considerable difference. With detection in the SIM mode quite a number of interferences showed up at the m/z values of the pesticides, and the spiking level of 5–10 μg/kg was, occasionally (peaks 4–7), at or below the detection limits. No such problems were encountered with the alternative procedure, detection in the SRM mode. Actually, the ion traces of Figs. 2A and 2B are remarkably similar. The same results were obtained for the potato extract. Further evaluation of the procedure was done with LC–MS–MS using detection in the SRM mode.

3.4. Evaluation of LVI-LC–MS–MS procedure

3.4.1. Analytical data

Linearity was tested by performing LVI of standard pesticide solutions (with 15%, v/v acetonitrile and acetone, and 25%, v/v methanol) in the concentration range of 0.15–8.5 ng/ml (5–7 concentration levels in duplicate), which corresponds with spiking/concentration levels of 1–50 μg/kg on carrot and potato, and detection in the SRM mode. For all selected compounds and all sample compositions, calibration curves were linear with regression coefficients better than 0.999. The RSD values of the peak areas of the nine pesticides were satisfactory and were below 5% in methanol and acetone, and below 9% in acetonitrile (for all solvents, $n=4$; concentration level, 1.5 ng/ml). On average, the analyte responses in samples containing acetonitrile were about 10% higher than when methanol was used. With acetone, the analyte responses were about 20% lower than with methanol. The detection limits of the nine pesticides were in the range of 0.02–0.2 ng/ml.

The precision of the procedure was studied at two spiking levels of the carrot and potato extracts, 5 and 20 μg/kg. With acetonitrile and methanol, the RSDs of the peak areas of the pesticides almost invariably were less than 3.5% ($n=4$) for both vegetables. With acetone, somewhat less satisfactory results were obtained ($<11\%$ for carrots; $<6.5\%$ for potatoes). Not surprisingly, the present precision was fully sufficient to obtain reliable results in all instances. Considering all of the above, acetonitrile was selected as the extraction solvent for the evaluation and application of the total analytical procedure. One further remark should be made. Since no appropriate (deuterated) pesticide standard was available, $^{2}H_{5}$-diazepam was used to study changes in the MS response. The RSD values of the peak areas of this

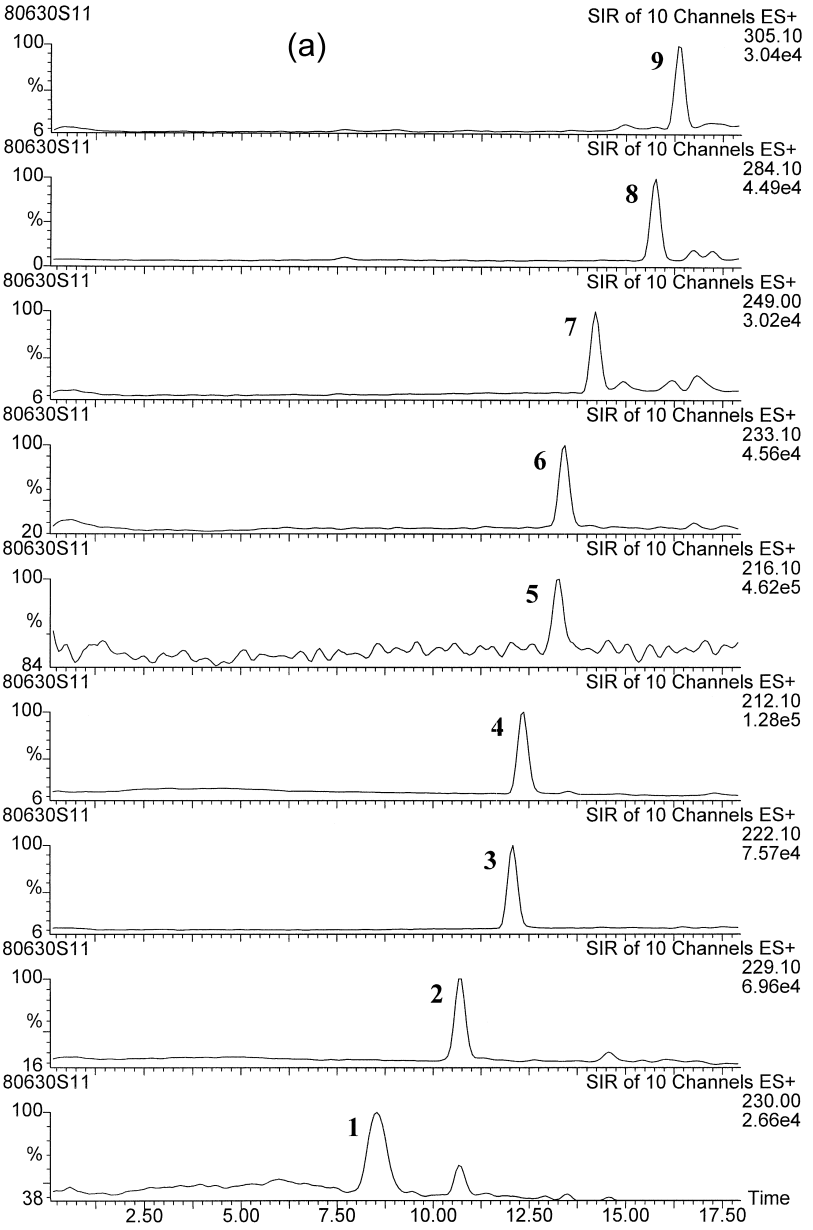

Fig. 1. LVI-LC–MS (PI mode; SIM, for m/z values, see below) of (A) standard solution of the pesticide mixture and (B) carrot extract spiked at the 5-μg/kg level. For sample preparation, see Section 2. LC conditions: gradient elution with 10 m*M* ammonium formate–methanol (85:15 to 15:85, v/v) in 13 min.. Peak assignment: (1) dimethoate (m/z 230), (2) metoxuron (m/z 229), (3) carbofuran (m/z 222), (4) atraton (m/z 212), (5) atrazine (m/z 216), (6) diuron (m/z 233), (7) linuron (m/z 249), (8) metolachlor (m/z 284) and (9) diazinon (m/z 305). Time scales in min.

standard were less than 5% (acetonitrile) and 13% (methanol and acetone; $n=8$ in all cases). In addition, for all extraction solvents studied, the response of the internal standard was found to be $50\pm5\%$ (one spiking level, $n=18$ in carrot and potato) of the response observed with the standard pesticide solution. This indicates that considerable signal suppression in the ESI process occurred for the internal

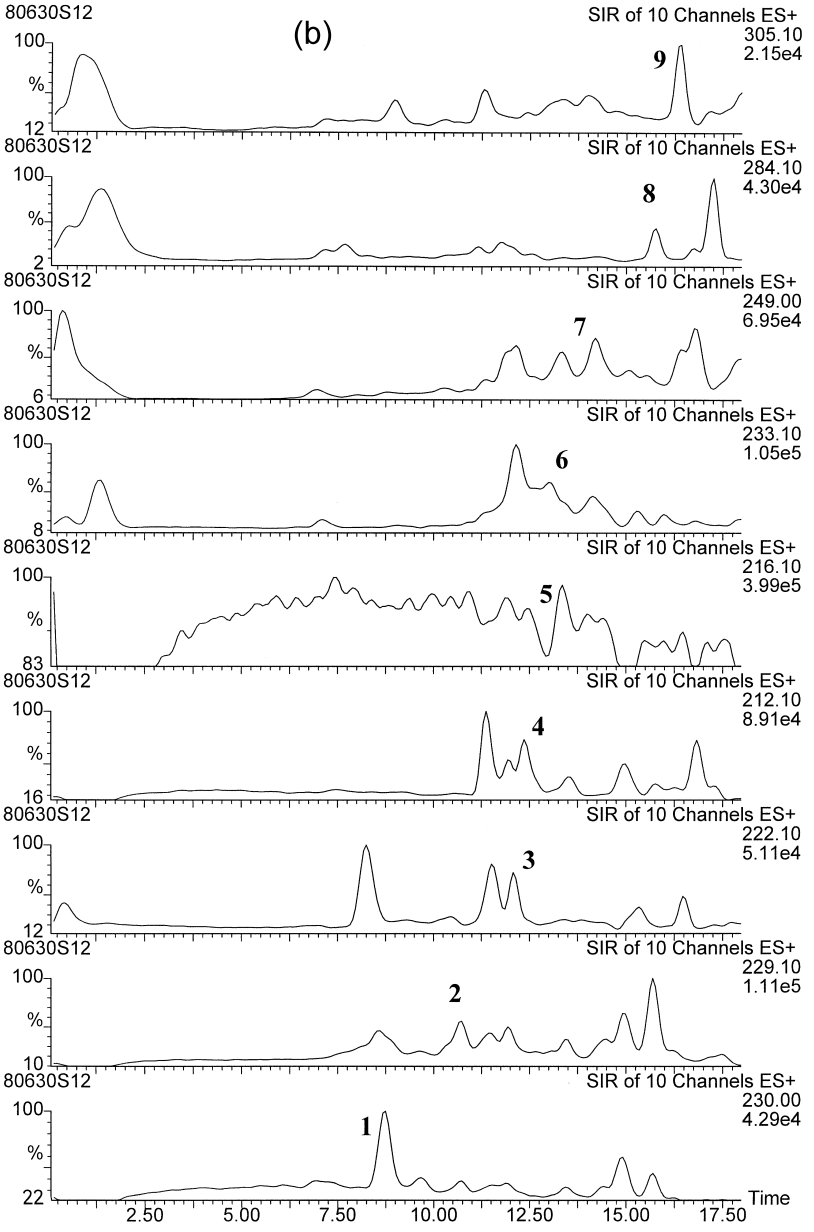

Fig. 1. (*continued*).

standard, probably, also for the pesticides. However, because of the good quality of the analytical data for both standard solutions (cf. above) and real-life samples (see below), this aspect was not studied in detail. Actually, if the pesticide recoveries were calculated by comparing 900-μl injection of real-life extracts and standard (and assuming a, not fully justified, identical quenching as for the internal standard) values of 70–90% were obtained at the 5-μg/kg spiking level for all but metoxuron and diuron (30–50%).

3.5. Evaluation of total analytical procedure

3.5.1. Analytical data

The final procedure involved an extraction of

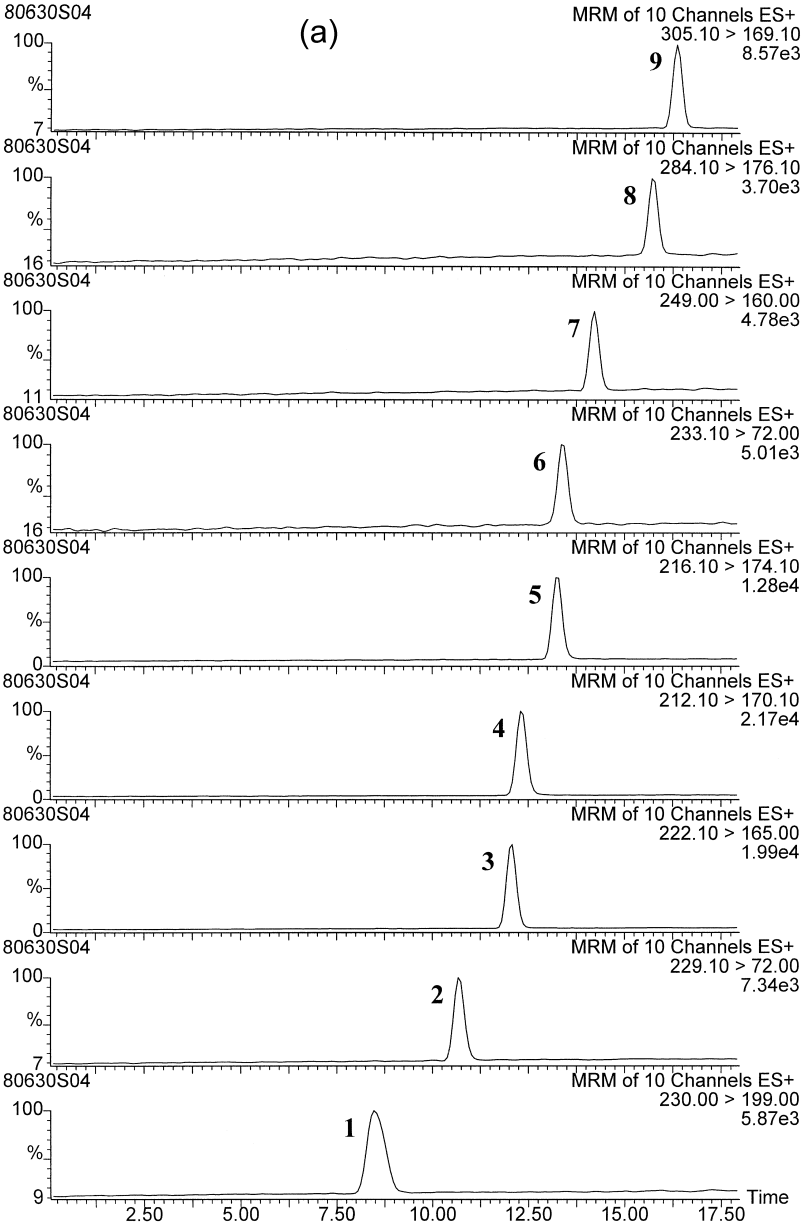

Fig. 2. LVI-LC–MS–MS (PI mode; SRM, for m/z transitions, see Table 2) of (A) standard solution of the pesticide mixture and (B) carrot extract spiked at the 10-μg/kg level. For experimental conditions and peak assignment, see legend to Fig. 1. Time scales in min.

carrot or potato with acetonitrile followed by filtration, dilution with ammonium formate to ammonium formate–acetonitrile (85:15, v/v) and LVI onto the 5-cm LC column, gradient elution and detection in the SRM PI mode. Results of the performance of the optimized procedure are presented in Table 2. Linearity was studied for all nine pesticides over a range of spiking levels of 2–100 μg/kg on carrot and potato. For all analytes, the calibration curves were linear with regression coefficients of at least 0.999. In addition, a repeatability study was performed at the 20-μg/kg spiking level by analysing

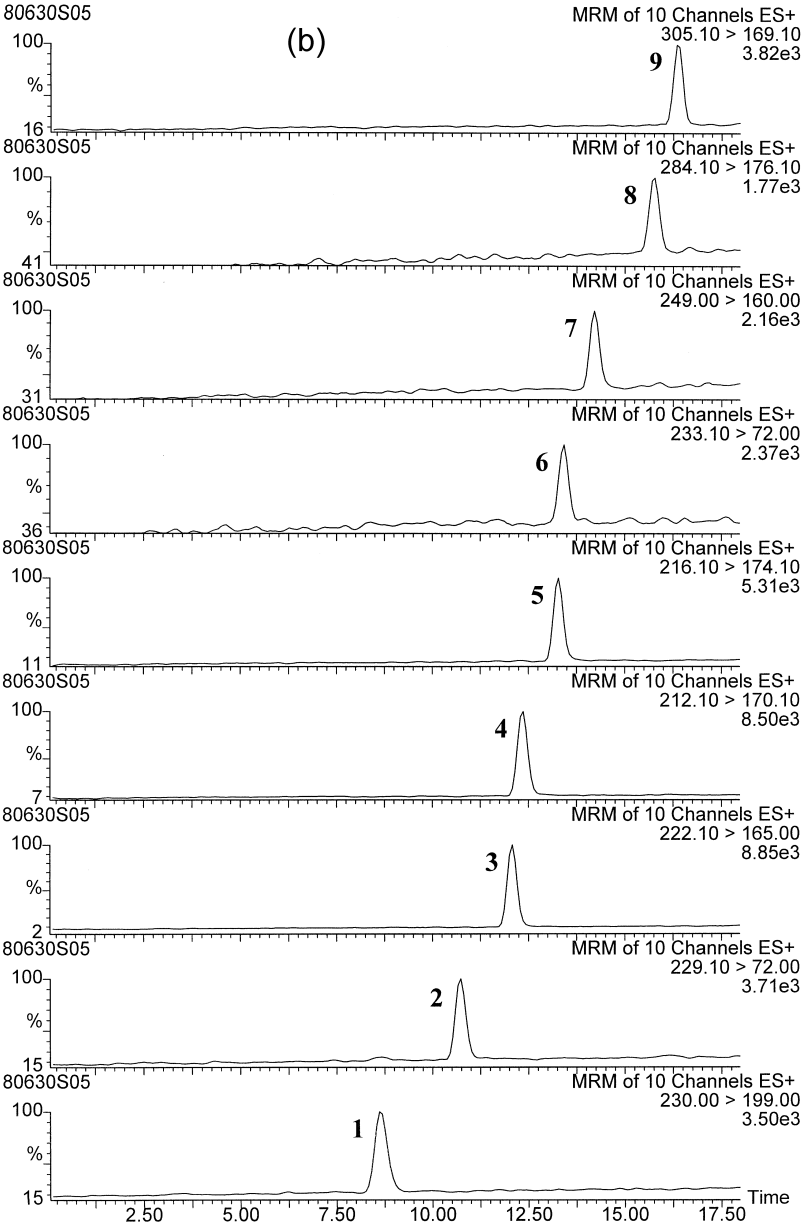

Fig. 2. (*continued*).

eight samples of vegetable extract. The RSD values of the peak areas of pesticides on carrot and potato were less than 4 and 6.5%, respectively. The RSD values of the peak areas of $^{2}H_{5}$-diazepam were 2 and 6% ($n=8$) for carrot and potato extracts, respectively.

The limits of detection for all analytes were in the range of 0.5–2 μg/kg for both carrots and potatoes.

3.5.2. Application

Fig. 3 shows a typical result obtained after LVI-LC–MS–MS of a potato extract spiked with 5-μg/

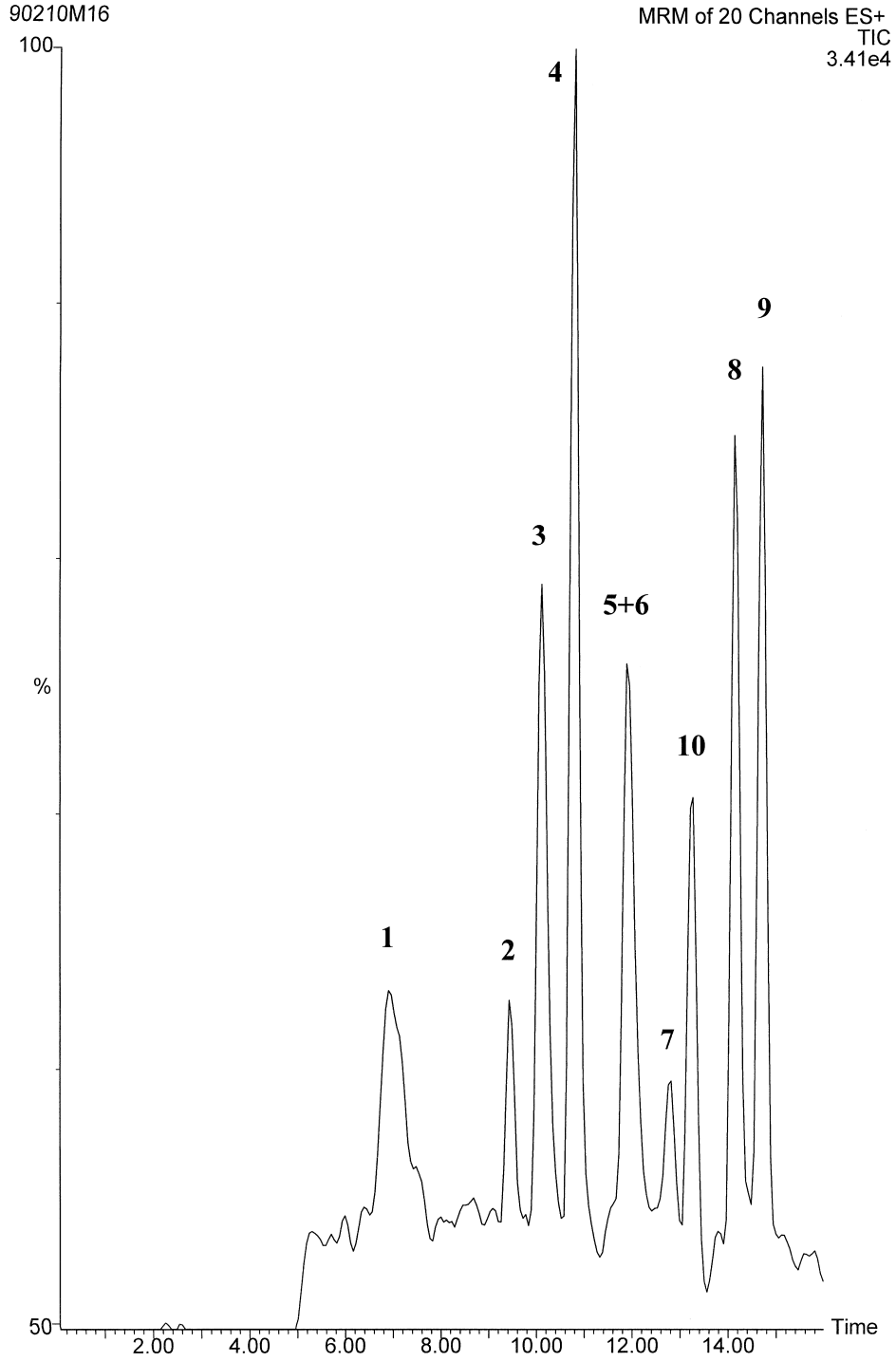

Fig. 3. LVI-LC–MS–MS (PI mode; SRM, for m/z transitions, see Table 2) of a potato extract spiked at the 5-μg/kg level. For experimental conditions and peak assignment, see legend to Fig. 1; peak No. 10, internal standard $^{2}H_{5}$-diazepam. Time scale in min.

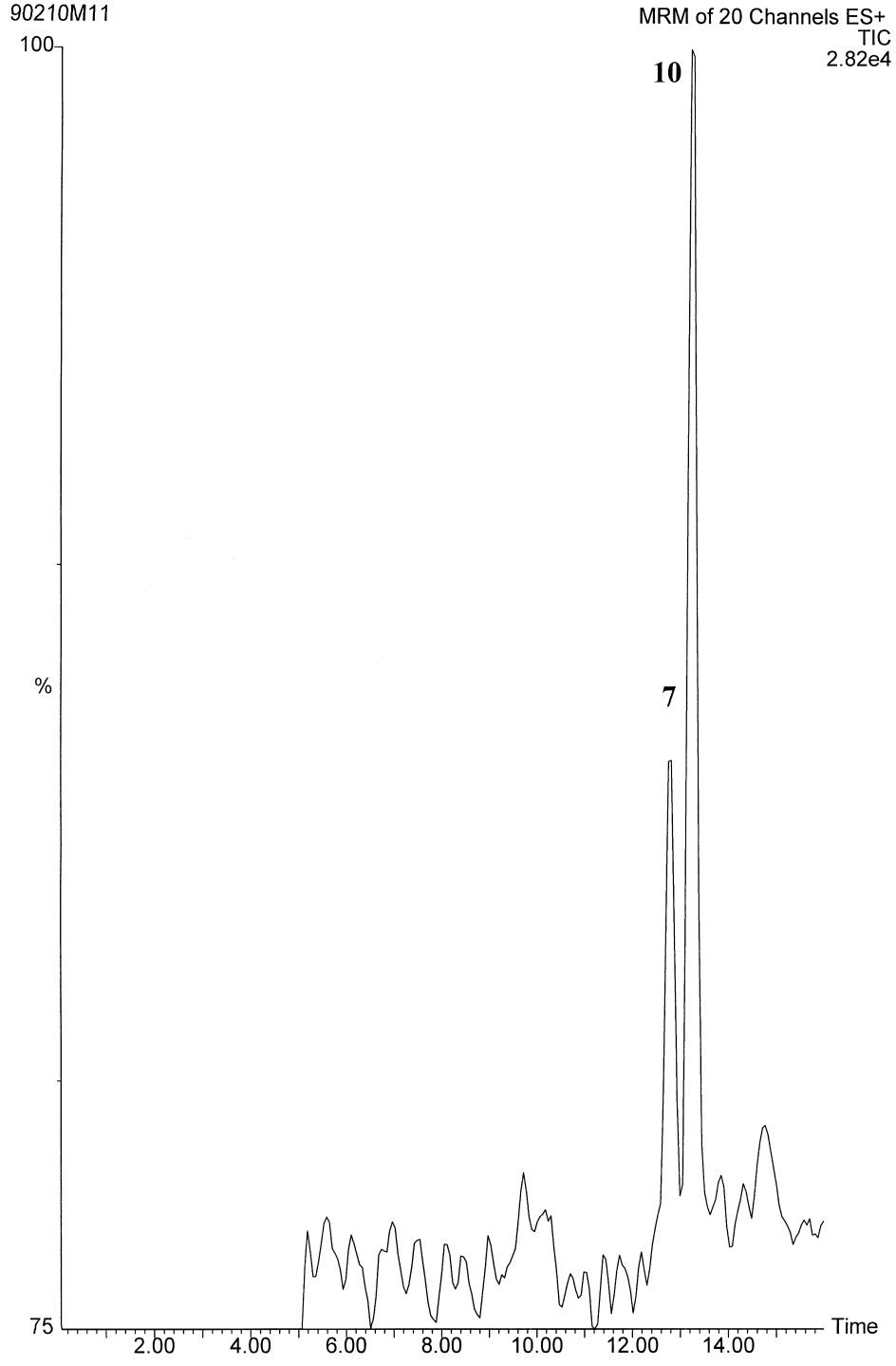

Fig. 4. LVI-LC–MS–MS (PI mode; SRM, for m/z transitions, see Table 2) of a carrot extract. For experimental conditions and peak assignment, see legends to Figs. 1 and 3. Time scale in min.

Table 2
Calibration and detection limits results for the analysis of pesticides in carrot (range, 2–100 μg/kg; $n=7$; data points in duplicate) with LC–MS–MS in SRM PI mode

Compound	Calibration equation[a]	R^2	Detection limits (μg/kg)	SRM transitions
Dimethoate	$y=917(18)x-114(77)$	0.998	2	230→199, 171
Metoxuron	$y=797(11)x-95(45)$	0.999	2	229→72, 46
Carbofuran	$y=5408(145)x-487(605)$	0.998	0.5	222→165, 123
Atraton	$y=2804(34)x-12(14)$	0.999	1	212→170, 100
Atrazine	$y=2122(37)x-45(55)$	0.999	1	216→174, 96
Diuron	$y=492(6)x-24(26)$	0.999	2	233→72, 46
Linuron	$y=624(10)x-7(14)$	0.999	2	249→185, 160
Metolachlor	$y=4045(17)x+120(70)$	0.999	0.5	284→252, 176
Diazinon	$y=2819(22)x+302(93)$	0.999	0.5	305→169, 153
[$^2H_5$]Diazepam (I.S.)				290→198, 154

[a] y=area; x=spiking level (μg/kg); SD values between brackets.

kg of each of the pesticides. None of the target compounds was detected/identified in the non-spiked extract. In addition, a number of carrot extracts was analyzed. Target analysis allowed the identification of linuron (peak 7 in Fig. 4). The linuron concentration calculated from the calibration data was found to be 3 μg/kg of carrot.

4. Conclusions

The trace-level determination of pesticides in vegetables can be performed by combining the extraction of a small amount of sample (2 g) with an organic solvent (3 ml) with a 900-μl LVI on a 5-cm LC column, and ESI-MS–MS detection in the SRM mode. Optimum detectability is obtained with an aqueous 10 m*M* ammonium formate–methanol gradient. The maximum percentage of extraction solvent in the LVI varies from 15 (acetonitrile) to 25% (methanol).

With the proposed procedure, which was tested for nine pesticides, linearity was observed in the range of 2–100 μg/kg, and precision (RSD values <6.5%; $n=8$) was fully satisfactory. The detection limits of 0.5–2 μg/kg are well below the maximum residue levels laid down by European regulatory bodies. Even if we would like to argue that simple (in-)homogeneity can become a problem for very small sample sizes, this leaves the central message intact: 900-μl LVI-LC–MS–MS enables the low ng/g level quantification of microcontaminants in small samples, and with little organic solvent consumption.

References

[1] EC Directive, No. 95-61, European Community, Brussels, 1995.
[2] Pesticide Residues in Food, Evaluations (131/1, 131/2), FAO, Rome/WHO, Geneva, 1994.
[3] H.-J. Stan, M. Linkerhägner, J. Chromatogr. A 750 (1996) 369.
[4] F. Hernández Hernández, J.M. Grases, J. Beltrán, J.V. Sancho, Chromatographia 29 (1990) 459.
[5] M. Careri, A. Mangia, M. Musci, J. Chromatogr. A 727 (1996) 153.
[6] J. Slobodník, A.C. Hogenboom, J.J. Vreuls, J.A. Rontree, B.L.M. van Baar, W.M.A. Niessen, U.A.Th. Brinkman, J. Chromatogr. A 741 (1996) 59.
[7] T. Cairns, E.G. Siegmund, J.J. Stamp, Mass Spectrom. Rev. 8 (1989) 127.
[8] A.C. Hogenboom, P. Speksnijder, R.J. Vreeken, W.M.A. Niessen, U.A.Th. Brinkman, J. Chromatogr. A 777 (1997) 81.
[9] C.-H. Liu, G.C. Mattern, X. Yu, R.T. Rosen, J.D. Rosen, J. Agric. Food Chem. 39 (1991) 718.
[10] W.M. Newsome, B.P.-Y. Lau, D. Ducharme, D. Lewis, J. AOAC Int. 78 (1995) 1312.
[11] D. Volmer, J. Chromatogr. A 794 (1998) 129.
[12] E. Lacassie, M.-F. Dreyfuss, J.L. Daguet, M. Vignaud, P. Marguet, G. Lachâtre, J. Chromatogr. A 830 (1999) 135.
[13] H.G.J. Mol, R.J.C. van Dam, R.J. Vreeken, O.M. Steijger, J. Chromatogr. A 833 (1999) 53.
[14] M.A. Luke, J.E. Forbeng, H.T. Masumoto, J. AOAC Int. 58 (1975) 1020.
[15] K. Fodor-Csorba, J. Chromatogr. 624 (1992) 353.
[16] A. Balinova, J. Chromatogr. A 823 (1998) 11.
[17] S.J. Lehotay, J. Chromatogr. A 758 (1997) 289.
[18] M. Hiemstra, J.A. Joosten, A. De Kok, J. AOAC Int. 78 (1995) 1267.
[19] W.M.A. Niessen, J. Chromatogr. A 794 (1999) 407.