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Determination of aldicarb, aldicarb sulfoxide and aldicarb sulfone in some fruits and vegetables using high-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry☆

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Abstract

An analytical method for the determination of aldicarb, and its two major metabolites, aldicarb sulfoxide and aldicarb sulfone in fruits and vegetables is described. Briefly the method consisted of the use of a methanolic extraction, liquid–liquid extraction followed by solid-phase extraction clean-up. Afterwards, the final extract is analyzed by liquid chromatography–atmospheric pressure chemical ionization mass spectrometry (LC–APCI-MS). The specific fragment ion corresponding to $[M-74]^+$ and the protonated molecular $[M+H]^+$ ion were used for the unequivocal determination of aldicarb and its two major metabolites. The analytical performance of the proposed method and the results achieved were compared with those obtained using the common analytical method involving LC with post-column fluorescence detection (FL). The limits of detection varied between 0.2 and 1.3 ng but under LC–FL were slightly lower than when using LC–APCI-MS. However both methods permitted one to achieve the desired sensitivity for analyzing aldicarb and its metabolites in vegetables. The method developed in this work was applied to the trace determination of aldicarb and its metabolites in crop and orange extracts.

Keywords: Fruits; Vegetables; Food analysis; Pesticides; Aldicarb

1. Introduction

Aldicarb [2-methyl-2-(methylthio)propionaldehyde-*O*-(methylcarbamoyl)oxime; Temik] is a systemic insecticide widely used for the protection of numerous fruit and vegetable cultures. After its application, aldicarb (A) gradually is transformed to the metabolites, aldicarb sulfoxide (AX) and aldicarb sulfone

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(AN), and can be further degraded to oxime and nitrile forms. Although in some cases these two degradation products can undergo subsequent hydrolysis to their respective non toxic oximes [1], such products are known to be most toxic overall. Also, we can detach aldicarb sulfoxide as the most important compound, accounting for the high systemic activity and the long-term persistence of the insecticide activity in soil [2,3]. The wide use of A in Brazilian agriculture – mainly in potato culture – has led to increasing demand for monitoring of its residues in both crops and the environment [4–8], but the lack of a integrated control program all over the country is still observed.

The market basket survey of foods conducted by the Brazilian Agriculture and Supply Ministry requires determination of residues at concentrations orders of magnitude below the maximum legal residue limit [9] to determine actual intake values. Due to their frequent use and inherent toxicity, combined with the often inadequate form of application in some parts of Brazil, data are sought on residues of these compounds in foods. Both gas chromatography (GC) and high-performance liquid chromatography (HPLC) methods have been used to measure A and its metabolites. HPLC is often a preferred method because A and its metabolites are thermally labile and of relatively polar nature. In some cases, UV absorbance has been used as a detection technique in HPLC determination of *N*-methylcarbamate pesticides [10], but this technique is subject to interferences of other co-extractives taken from the sample and also to the lack of sensitivity for some compounds at below 205 nm [8,10]. In this respect, the development of diode-array detectors has been the most important advance in HPLC quantification of such compounds, since they provide an opportunity to explore all wavelengths in the UV range and choose the monitoring wavelength which maximizes instrumental sensitivity [11,12]. Krause [13] developed a multiresidue method for the determination of carbamate and their main metabolites, involving the use of a post-column with fluorogenic reactions. This method was further validated [14], and satisfactory recoveries were demonstrated to below 50 ppb. Post-column derivatisation affords low detection limits, and optimization has yielded determination limits of 5–50 ppb [6]. Although the sample preparation step of this fully automated LC–fluorescence (FL) method has involved solid-phase extraction (SPE) clean-up that is fast and provides a based-routine method for carbamate analysis in crude extracts, the main disadvantage of this analytical methodology is still the lack of confirmation of positive responses. Mass spectrometry (MS) has been used for the analysis of carbamates and their degradation products separated by LC. Various LC–MS systems with different interface have been applied in the determination of such compounds [7]. Several techniques for sample introduction and MS interfaces were compared by Pleasance et al. [15]. The authors concluded that a heated nebuliser interface coupled to an atmospheric pressure chemical ionization (APCI) source provides a clear advantage in terms of sensitivity, linearity, and range of compounds to which it is applicable. Preliminary studies with food samples indicate that the APCI interface can improve the sensitivity of conventional LC methods, as for example for analysis of the carbaryl and its metabolite, α-naphthol, in crop samples [16]. This paper contains the results of a study involving the analysis of aldicarb and its degradation products in samples of fruits and vegetables. One aim of the present study is to extend the analytical methodology for sample preparation that we have reported previously [8,16], by adding the use of LC–APCI-MS. Therefore, separation and operational chromatographic conditions were optimized again, and they were slightly different from the above-mentioned studies. Finally, the LC–MS results were compared to the standard LC–FL methodology for carbamate determination.

2. Experimental

2.1. Materials and reagents

The pesticide standards used in this work (aldicarb, aldicarb sulfoxide and aldicarb sulfone) were purchased from Dr. Ehrenstorfer (Augsburg, Germany) and ranged in purity from 97 to 99.5%. Stock and work solutions were made up in methanol and were stored in the dark at −20°C. All solvents and water were of HPLC-grade (Merck, Augsburg, Germany), and they were filtered through a 0.45-μm

membrane in a Millipore device (Bedford, MA, USA) before use. Ammonium formate was purchased from Aldrich (Steinheim, Germany). All reagents used for sample preparation methodology were of analytical grade. A CN cartridge was obtained from Waters–Millipore (Bedford, MA, USA).

2.2. Sample preparation

The pesticides were extracted from the matrices of potato, tomato and orange fortified at 100 μg kg^{-1} by following the general procedure described by Nunes et al. [8]. This was based on the analytical methodology adopted by the US Food and Drug Administration for *N*-methylcarbamate determination [17], but numerous modifications, including reduction of sample and solvents and miniaturisation of clean-up step with a SPE procedure were proposed in order to simplify the general methodology. Briefly, extraction was performed with 10 ml methanol (10 g crop sample), followed by washing with acetonitrile (twice, 5 ml) during filtration in a Bückner funnel. The liquid–liquid extraction (LLE) with liquid petroleum (b.p. 45–47°C) was carried out three times, and after separation of most of the pigments and other co-extractives. An off-line SPE using a CN-SPE cartridge (500 mg, Waters) was used for the clean-up, and the elution was performed with dichloromethane–methanol (98:2, v/v). The final extract was firstly concentrated in a rotator evaporator at 35°C until dryness, and then the residue was redissolved with methanol to perform a pre-concentration of about 10–20-times, and then filtered on a Millex device (0.22-μm, Millipore) before LC analysis. LC–APCI-MS and LC–FL analyses were performed in duplicate in spiked (100 ppb) and organic samples, in order to verify the method efficiency and to detect the contamination in Brazilian agricultural products from different origins (supermarket, farms, etc.), if present.

2.3. LC–APCI-MS

A volume of 20 μl of the extract was injected by a U6K Rheodyne type universal LC injector (CA, USA). For chromatographic separation, a C_{18}-Zorbax column (15 cm×4.6 mm I.D.) packed with 5 μm particles was used, and an acetonitrile–water (water containing 0.05 *M* ammonium formate) mixture at 1.0 ml min^{-1} was used as mobile phase. The elution process started at 5% acetonitrile, increasing linearly during 10 min until 30% acetonitrile, and then returning to 5% in the last 20 min (total run time: 30 min) in order to separate the more polar metabolites. The eluent was delivered by a gradient system from Waters 616 pumps coupled to a Waters 600S controller (Waters, Milford, MA, USA). Details of the experimental set-up for MS are described elsewhere [16]. A VG Platform mass spectrometer (Micromass, Manchester, UK) equipped with an APCI interface was used. Briefly, this interface consists of a heated nebuliser probe and the standard atmospheric pressure source configured with a corona discharge needle. The LC eluent enters the probe, where it is pneumatically converted into an aerosol (using a nebulising gas flow-rate of 10 l h^{-1}) and rapidly heated into the vapor/gas phase at the probe tip. The resulting vapor is carried toward the counter electrode by a nitrogen gas flow at ~300 l h^{-1} (drying gas). After expansion of the gas containing the sample and reagent ions into the intermediate pressure region, an excess of energy (in this case obtained by applying a potential of 30 V) between collisions can be gained by the ions. Mobile phase molecules rapidly react with ions from the corona discharge to produce stable reagent ions. In this study, the corona voltage was set at ~3.2 kV. Analyte molecules introduced into the mobile phase react with the reagent ions at atmospheric pressure and typically become protonated (positive ion mode). The ion source and the APCI probe temperatures were set at 150°C and 350°C, respectively. Finally, the sample and reagent ions pass through the chicane counter electrode prior to being expanded through an sample cone and skimmer assembly into the mass spectrometer. The LC–MS system was controlled by the MassLynx data system. Chromatograms were recorded under time-scheduled SIR (selected ion recording) conditions, within the mass range of 80–250, in order to monitoring protonated molecular and fragmentation ions of each compound.

2.4. LC–fluorescence detection

The LC–FL system was composed of a Waters

Carbamate Analysis System (CAS). The CAS consists of a Model 600E multisolvent delivery system including a gradient controller, coupled to a post-column reagent delivery system (PCRDS). The PCRDS contains two separate, single-piston, cam-driven, pulse-dampened pumping systems, one each for the NaOH and *o*-phthalaldehyde (OPA)–mercaptoethanol (ME) post-column reagent solutions (0.5 ml min^{-1} flow-rate to each reagent). The temperatures of column and post-column ovens were controlled by a temperature control module and a column heater and they were fixed at 30 and 80°C, respectively. The detection system was a Model 470 fluorescence detector. The fixed parameters were: excitation wavelength 339 nm, emission wavelength 445 nm, with bandwidth=18 nm. A Model U6K universal LC injector was used for the introduction of 100 μl of the extract or standard solution. The chromatographic column was a Supelcosil-C_{18} (25 cm×4.6 mm I.D.) packed with 5-μm spherical silica particles (supplied by Supelco, UK). The CAS also used a Waters Chromatography Workstation to automate system control, and a Waters Model 745B recording integrator to peak monitoring and data processing. A water–methanol–acetonitrile mixture at 1.5 ml min^{-1} flow-rate was used as mobile phase. The mobile phase gradient program consisted of decreasing polarity from water–methanol–acetonitrile (88:12:0) to (68:16:16) during the first 4.1 min, and then to (30:35:35) until 16.1 min, and returning to (88:12:0) during the last 2.9 min (total run time: 19 min). This is the standard gradient program used to separate most commonly used *N*-methylcarbamates and their metabolites in a chromatographic run. The spurge gas (helium) was kept at 200 ml min^{-1} throughout. All LC injections were performed in duplicate.

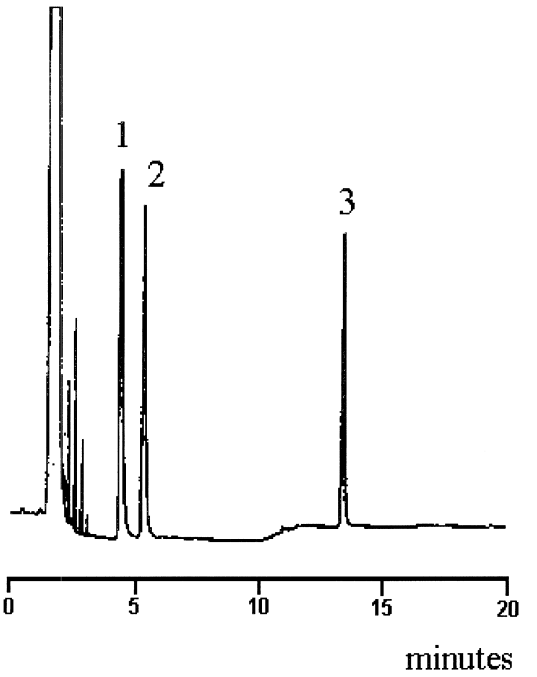

Fig. 1. Liquid chromatography with fluorescence detection of potato extract spiked with aldicarb sulfoxide (1), aldicarb sulfone (2) and aldicarb (3) in such mode that final concentration is around 100 ppb. Chromatographic conditions as described in the Experimental section.

3. Results and discussion

3.1. Instrumental sensitivity

Typical chromatograms are shown in Figs. 1 and 2 for fluorescence and mass spectrometric detection, respectively. Table 1 lists the mass ion values (m/z) used for peak confirmation of the analyzed compounds by LC–APCI-MS in the selected ion monitoring (SIM) mode. For aldicarb and aldicarb sulfoxide, $[M-74]^+$ ions were most abundant, and for aldicarb sulfone, 100% of abundance was obtained for protonated molecular ion. In order to avoid interferences from matrix, detached ions (Table 1) were taken for future confirmations in SIM mode, when real samples were analyzed (Fig. 3). With the data of Table 2 we can compare the sensitivities of the fluorescence and mass spectrometric detectors for such compounds, determined with standard solutions within typical on-column injection. As we can see, APCI-MS was less sensitive than fluorescence detection and gave slightly lower values than those reported by Pleasance et al. [15], but comparable with those of other laboratories [18,19]. Also, the former study showed that some instrumental parameters such as reproducibility, linearity and instrumental detection limits were excellent for residue monitoring of such compounds in commodities without

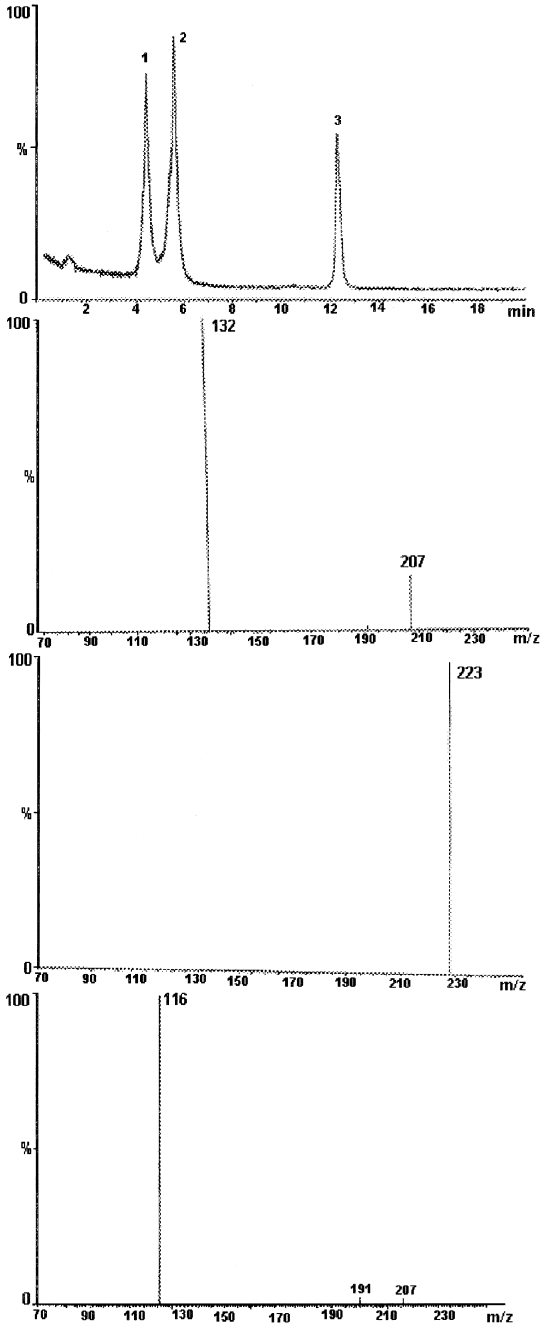

Fig. 2. Liquid chromatography with APCI-MS detection for aldicarb sulfoxide (1), aldicarb sulfone (2) and aldicarb (3) used for peak confirmation throughout SIM mode by using standard solutions containing 100 ppb of each compound. Six ions were shown to provide best selectivity in real samples were used for the identification of the compounds.

necessitating the use of isotopically labeled internal standards.

3.2. Degradation pathways

Fig. 4 shows the metabolic pathway of aldicarb under environmental conditions. Initial metabolic attack is rapid and complete oxidative conversion to aldicarb sulfoxide followed by a much slower oxidation to the sulfone. We can establish two important facts regarding the pesticide residue chemistry. Firstly, the complete conversion of aldicarb to its sulfoxide and sulfone resulted in a increase in the cholinesterase inhibition. Secondly, the persistence of sulfoxide in the plant, combined with slow oxidation to the corresponding sulfone can account for the already observed prolonged systemic activity [20,21]. These facts have to be considered in the determination of such compounds, because in some cases the parent compound is not present in the studied matrix. Thus, the toxicity could be effectively more acute if the metabolites are present at higher concentrations.

3.3. Analytical methodology

Assuming a 20-fold concentration step via a solid-phase cartridge and an injection volume of 20 μl of the final LC–MS extract into the chromatographic system, a minimum of 100–200 ng has to be detected by LC–APCI-MS to verify the presence of residues of such pesticides in foods. These quantification limits are much lower than the tolerance levels for most cases where carbamate insecticides are present in the analyzed matrix. Considering the volume of injection and loop size in the chromatographic analysis, we can extrapolate previous limit of detection (LOD) results for some carbamates, including aldicarb and its metabolites, and an around 500 ng minimal content has been detected by applying other analytical methods [15]. For these compounds, the criterion of quantification limit could not be achieved with full-scan analysis in the present study, due to the lower sensitivity of this fragmentation technique. In addition, the experiments were extended to time-scheduled selected ion monitoring measurements in order to lower the detection limits of the method to the low ppb. Moreover, the factor of the extract pre-concentration could be increased to

Table 1
Spectral data for confirmation of aldicarb and its metabolites

Compound	*m/z*	Abundance (%)	Ion/fragmentation
Aldicarb	116	100	$[M-101]^+$ (101→CH=N-O-CO-NH-CH_3)
	89	83	[M−74] (74→-O-CO-NH-CH_3)
	191	9	$[M+1]^+$
	132	100	[M−74] (74→-O-CO-NH-CH_3)
Aldicarb sulfoxide	207	36	$[M+1]^+$
	223	100	$[M+1]^+$
Aldicarb sulfone	148	56	[M−74] (74→-O-CO-NH-CH_3)
	166	37	[M−56] (56→CO-$NHCH_3$)

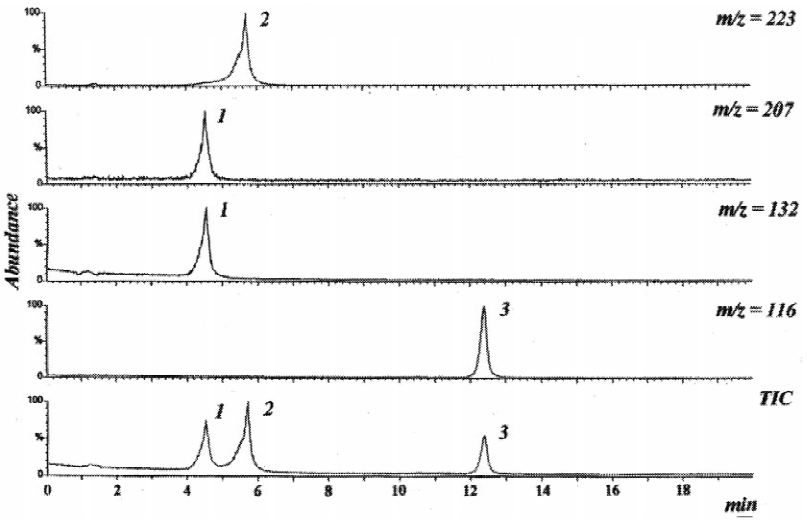

Fig. 3. Liquid chromatogram for aldicarb sulfoxide (1), aldicarb sulfone (2) and aldicarb (3) present in a spiked orange extract. MS peak confirmation by SIM mode of five selected channels. Final extract 20-fold concentrated (see sample preparation in Section 2.2).

attain content values at trace levels. For LC–FL these limits are still better, and the LODs obtained for the analyzed compounds were suitable for the determination of minimal pesticide contents in real samples. It is convenient to consider here the enormous possibility to increase the presence of interferences in the LC extract with the use of higher pre-concentration factors. This is particularly important when we are determining pesticide residues in complex matrices, such as foodstuffs. Regarding the fragmentation mode, in general, although sensitivity varies widely from compound to compound, positive ion APCI analysis was shown to be more sensitive than negative ion in case of these pesticides. This was demonstrated in other studies involving detection of carbamate pesticides by LC–MS [16,18]. We have also performed negative ion studies under APCI for carbamates with the results

Table 2
Sensitivities for LC analysis of aldicarb and its metabolites with fluorescence and APCI-MS detection

Compound	Linearity (ng)	LOD (ng)		RSD (%)
		LC–MS	LC–FL	
Aldicarb	1–250	0.5	0.2	3.5
Aldicarb sulfoxide	0.6–200	0.5	0.2	4.2
Aldicarb sulfone	2–500	1.3	0.3	7.0

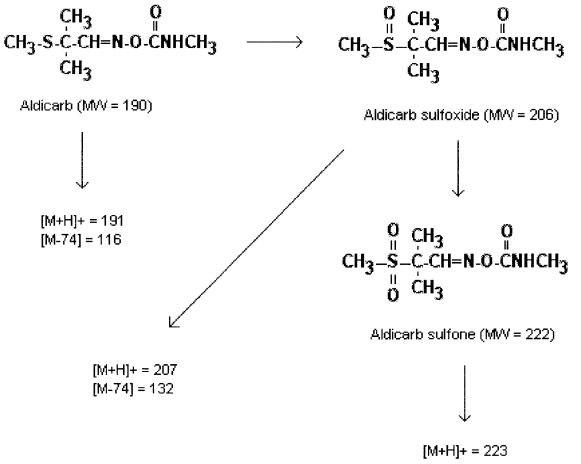

Fig. 4. Degradation pathway of aldicarb to its degradation products under environmental conditions.

obtained also being in the similar to those of previous studies and for this reason are not shown in this report.

3.4. Method accuracy

Method accuracy can be evaluated through the recovery values for some spiked samples listed in Table 3. It can be observed that detection technique has enormously influenced the final recovery results, since that in this case the obtained chromatographic extracts, when analyzed by both techniques, were the same. In general, LC–FL showed better accuracy and reproducibility. In contrast, it is convenient to mention the additional advantage of LC–MS for the unequivocal confirmation of the analyzed compound. Not rarely, in some agricultural farms, the equivocal practice to prepare *cocktails* containing different agrochemicals taking out the same protection function is still observed, mainly in developing countries. It is clear that such practice is profoundly noxious, does not improve the response in terms of combat to plagues, and just contributes to the increase in the number of pesticides present in foodstuffs at residue levels. The best alternative to effectively confirm the compounds identity is still by use of MS.

Table 3
Efficiency of the LC detection techniques applied for the analysis of aldicarb and its metabolites in crop extracts[a]

Sample	Compound	Recovery (%)	
		APCI-MS	LC–FL
Potato	Aldicarb	68 (12.3) A	100 (5.2) B
	Aldicarb sulfoxide	83 (8.4) A	90 (6.3) B
	Aldicarb sulfone	75 (13.0) A	85 (5.0) B
Orange	Aldicarb	70 (18.4) A	95 (4.0) B
	Aldicarb sulfoxide	89 (10.0) A	91 (7.5) B
	Aldicarb sulfone	–	–
Tomato	Aldicarb	81 (16.5) A	81 (9.6) A
	Aldicarb sulfoxide	78 (8.5) A	85 (4.7) B
	Aldicarb sulfone	89 (6.8) A	92 (6.0) B

[a] Data in parentheses: RSDs ($n=3$). Samples spiked with 100 ppb of each compound. Averages followed by the same letter, in horizontal line, are not statistically different at 5% probability by the Tukey test.

4. Conclusions

LC–APCI-MS can be used as a routine method for the trace determination of aldicarb and its two major metabolites, aldicarb sulfone and aldicarb sulfoxide in fruits and vegetables. The method showed high selectivity and allowed unequivocal confirmation of the target analytes. Method performance of the newly proposed analytical methodology agrees well with the commonly used LC–post-column reaction–fluorescence detection method.

References

[1] N.R. Andrawes, W.P. Bagley, R.A. Herrett, J. Agric. Food Chem. 19 (1971) 731.
[2] F.A. Rickey, W.J. Bartley, K.P. Sheets, J. Agric. Food Chem. 25 (1977) 47.
[3] A.K. Hassal, The Biochemistry and Uses of Pesticides – Structure, Metabolism, Mode of Action and Uses in Crop Protection, 2nd ed., VCH, Weinheim, New York, 1990.
[4] D. Chaput, J. Assoc. Off. Anal. Chem. 71 (1988) 542.
[5] S. Lesage, LC–GC 7 (1989) 268.
[6] A. de Kok, M. Hiemstra, J. AOAC Int. 75 (1992) 1063.
[7] W.H. Newsome, B.P.-Y. Lau, D. Ducharme, D. Lewis, J. AOAC Int. 78 (1995) 1312.
[8] G.S. Nunes, M.L. Ribeiro, L. Polese, D. Barceló, J. Chromatogr. A 795 (1998) 43.
[9] AGROFIT/97, Secretary of Agricultural Defense, Department of Defense and Crop Inspection, (5 diskettes), Ministry of the Agriculture, Brazil, 1997.

[10] B. McGarvey, J. Chromatogr. 642 (1993) 89.
[11] P.R. Loconto, J. Liq. Chromatogr. 14 (1991) 1297.
[12] P. Parrilla, J.L. Martínez-Vidal, A.R. Fernandez-Alba, J. Liq. Chromatogr. 16 (1993) 4019.
[13] R.T. Krause, J. Assoc. Off. Anal. Chem. 63 (1980) 1114.
[14] R.T. Krause, J. Assoc. Off. Anal. Chem. 68 (1985) 726.
[15] S. Pleasance, J.F. Anacleto, M.R. Bailey, D.H. North, J. Am. Soc. Mass Spectrom. 3 (1992) 378.
[16] G.S. Nunes, M.-P. Marco, D. Barceló, M.L. Ribeiro, J. Chromatogr. 823 (1998) 109.
[17] Pesticide Analytical Manual, US Food and Drug Administration (FDA), 1992, pp. 400.1–401.b1, Chapter 4: Selective NMCs.
[18] D.R. Doerge, S. Bajic, Rapid Commun. Mass Spectrom. 6 (1992) 663.
[19] A. Slobodnik, M.E. Jager, S.J.F. Hoeskstra-Oussoren, M. Honing, B.L.M. van Baar, U.A.T. Brinkman, J. Mass Spectrom. 32 (1997) 43.
[20] T. Cairns, E.G. Siegmund, T.S. Savage, Bull. Environ. Contam. Toxicol. 32 (1984) 274.
[21] W.J. Bartley, N.R. Andrawes, E.L. Chancey, W.P. Bagley, H.W. Spurr, J. Agric. Food Chem. 18 (1970) 446.