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Review

Derivatization reactions applicable to pesticide determination by high-performance liquid chromatography☆

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Abstract

The literature dealing with HPLC analytical methods for pesticides employing derivatization reactions is reviewed. Included are methods for insecticides, acaricides, nematicides, antimicrobials, herbicides and a rodenticide. Derivatization reactions are employed mainly for the purpose of increasing sensitivity or selectivity for the UV or, more frequently, fluorescence detectors. Of the pre-column and post-column derivatization methods reviewed, post-column methods are the more commonly used.

Contents

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List of abbreviations

Abs	Absorption
AMPA	(Aminomethyl)phosphonic acid
2,4-D	2,4-Dichlorophenoxyacetic acid
2,4-DP	2-(2,4-Dichlorophenoxy)-propionic acid
DMF	Dimethylformamide
EBDC	Ethylenebisdithiocarbamate
ETU	Ethylenethiourea
FDNB	1-Fluoro-2,4-dinitrobenzene
FH	Following hydrolysis
Fl	Fluorescence
GC	Gas chromatography
HPLC	High-performance liquid chromatography
MBC	Methyl 1H-benzimidazol-2-ylcarbamate
MBC-PIC	1-(*n*-Propylcarbamoyl)-2-benzimidazole carbamate
MMC	4-Bromomethyl-7-methoxycoumarin
NMIM	N-Methylimidazole
NMC	N-Methylcarbamate
NPB	2-Naphthacyl bromide
OPA/MERC	*o*-Phthalaldehyde/2-mercaptoethanol
PCRS	Post-column reaction system
2,4,5-T	2,4,5-Trichlorophenoxyacetic acid
TFAA	Trifluoroacetic anhydride

1. Introduction

High-performance liquid chromatography (HPLC) and gas chromatography (GC) are the major techniques used for the determination of pesticide residues. The usefulness of GC is limited by the fact that many pesticides are not volatile enough for introduction onto the GC column. Derivatization of insufficiently volatile compounds is therefore common in GC methods of analysis. Since analytes do not have to be volatilized for introduction onto an HPLC column, derivatization for this purpose is not required in HPLC methods; this gives HPLC a tremendous advantage over GC for these compounds. Derivatization is therefore not nearly as common in HPLC methods as in GC methods.

One weakness of HPLC in comparison with GC, however, has been the lack of sensitivity of the available HPLC detectors. Derivatization has therefore been applied to many pesticides to increase sensitivity and perhaps selectivity by addition of a chromophore or fluorophore for detection by UV absorption or fluorescence detectors, respectively.

Derivatization may be performed either before or after separation on the HPLC column. Pre-column derivatization is normally performed off-line prior to injection onto the HPLC. It requires extra sample preparation time and may introduce interferences from reagents or coextractives that were not initially present. Post-column derivatization normally occurs on-line after separation and prior to detection and requires hardware such as pumps, heaters or solid-phase reactors to be installed between the column and detector.

In spite of potential problems, however, some HPLC analyses require the use of derivatization in order to furnish the required level of sensitivity. This review will survey derivatization methods which have been applied to HPLC determination of pesticides. Only those derivatization reactions in which a new compound is formed by the addition of one chemical species to another are included in this review. That is, such procedures as hydrolysis, photodegradation, ion-pairing or post-column pH adjustment are not included. The review is intended to be comprehensive, but omissions have no doubt inadvertently occurred. The units ppm and ppb are reported as used in the references.

2. Insecticides, acaricides and nematicides

HPLC methods for insecticides, acaricides and nematicides employing derivatization reactions are summarized in Table 1.

Table 1
Derivatization techniques for HPLC determination of insecticides, acaricides and nematicides

Derivatization reagent	Detector	Detection limit	Reference
N-methylcarbamates			
1-Fluoro-2,4-dinitrobenzene (pre-col, FH)	Abs, 222 nm	0.3 mg/kg	1
1-Fluoro-2,4-dinitrobenzene (pre-col)	Abs, 280 nm	2–4 ng	2
4-Aminoantipyrene (pre-col, FH)	Abs, 460 nm	3 ng	3
Diazotized sulphanilic acid (post-col, FH)	Abs, 280, 506 nm	3.4 ng	4,5
Dansyl chloride (pre-col)	Fl	1–10 ng	6–10
o-Phthalaldehyde, 2-mercaptoethanol (post-col, FH)	Fl	0.1–2.0 ng	11–50
o-Phthalaldehyde, thiofluor (post-col, FH)	Fl	0.2–0.5 ng	51
Organophosphates			
Dansyl chloride (pre-col, FH)	Fl	5–10 ng	52
2,3,4,5,6-Pentafluorobenzyl bromide (pre-col)	Abs, 260 nm	–	53
Avermectins			
Acetic anhydride (pre-col)	Fl	1 ng	54–59
Trifluoroacetic anhydride (pre-col)	Fl	0.02–1.0 ng/ml	60–63
Trichloroacetic acid, 2-naphthalene-sulfonic acid 1-hydrate (post-col)	Abs, 570 nm	1 μg	64

Abs = absorption; FH = following hydrolysis; Fl = fluorescence.

2.1. N-Methylcarbamates

The N-methylcarbamates comprise a group of pesticides having in common a hydrolyzable N-methyl group and including such commonly used compounds as aldicarb, carbaryl, carbofuran, methomyl and oxamyl among many others. Pre-column and post-column derivatization methods have been reported for HPLC analysis of N-methylcarbamate (NMC) pesticides using both UV absorption and fluorescence detection.

1-Fluoro-2,4-dinitrobenzene (FDNB) was used for pre-column derivatization of butocarboxim and its oxidation products [1]. The compounds were hydrolyzed to release methylamine, which was then reacted with FDNB to form N-methyl-2,4-dinitroaniline which was determined by HPLC-UV at 222 nm. The detection limit in soil and crops was 0.3 mg/kg. The phenolic metabolites of carbofuran were also reacted with FDNB to form 2,4-dinitrophenyl ether derivatives, with detection limits of 2–4 ng using UV detection at 280 nm [2].

Pietrogrande *et al.* [3] hydrolyzed carbaryl and derivatized the resulting 1-naphthol with 4-aminoantipyrine. The derivative was detected by absorption at 460 nm, a wavelength at which coextractive compounds are not likely to absorb. The detection limit was 3 ng.

A post-column reaction for UV detection of carbaryl involved the delivery of 3 reagents to accomplish hydrolysis of carbaryl to 1-naphthol with NaOH, diazotization of sulphanilic acid with $NaNO_2$, and coupling of 1-naphthol with diazotized sulphanilic acid [4,5]. Derivatization provided stronger absorption at 280 nm and also allowed monitoring of the chromatogram at 506 nm, thus minimizing the possibility of interference from coextractives. The flow cell of the UV detector was packed with C_{18} bonded silica (60–100 μm) which served to retain and concentrate the derivative in the flow cell; the detection limit was <3.5 ng.

A pre-column derivatization reaction for fluorescence detection of NMC pesticides was also reported [6,7]. Fourteen NMCs were rendered

fluorescent by derivatization with dansyl chloride prior to injection. Detection limits were between 1 and 10 ng. The same approach was used to determine carbaryl [8,9] and carbofuran [10] residues in vegetables.

Moye *et al.* [11] introduced a two-stage post-column reaction system (PCRS) for NMCs. Sodium hydroxide introduced by a post-column reagent-delivery pump was used to hydrolyze the NMC at 90°C and release methylamine. The released methylamine was subsequently reacted with a mixture of *o*-phthalaldehyde and 2-mercaptoethanol (OPA/MERC), introduced by a second post-column pump, to form a highly fluorescent derivative identified as (1-hydroxyethylthio)-2-methylisoindole [12]. The detection limit for methomyl was as low as 0.1 ng.

Krause refined the derivatization parameters of the PCRS (Fig. 1) [13,14], introduced a complex extraction and clean-up procedure for crop samples [15] and validated the method through collaborative studies [16,17]. The post-column derivatization method was rapidly adopted by a large number of researchers for determination of various NMCs in a variety of substrates including water [18–23], soil [18,24], plant tissue [24–35] and bovine, swine and duck liver [36,37]. The method formed the basis for EPA Method 531.1 [38] for determination of NMC pesticides in water.

Use of a catalytic solid-phase reactor consisting of a column packed with anion-exchange resin maintained at 100–120°C for hydrolysis of NMCs eliminated the need for the NaOH post-column reagent-delivery pump and allowed detection of as little as 0.1 ng aldicarb and 0.85 ng methiocarb [39–41]. This technique was used with a less expensive anion exchanger to determine carbaryl in polluted water samples; detection limits were 0.4–2.0 ng [42]. Band broadening was reduced to zero by the use of magnesium oxide in place of anion exchanger [43]. Detection limits in crop samples ranged from 1 to 10 ppb. Jansen *et al.* [44] miniaturized the solid-phase reactor to render it compatible with narrow-bore LC on 1 mm I.D. columns and observed detection limits of 0.4 ng for methomyl and 1.0 ng for propoxur. De Kok and Hiemstra [45] completely automated the clean-up and analysis steps using on-line coupling of automated solid-phase extraction and HPLC.

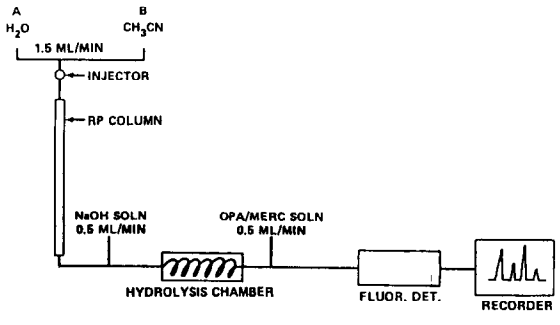

Fig. 1. HPLC post-column fluorometric system. OPA = *o*-phthalaldehyde; MERC = 2-mercaptoethanol. Hydrolysis chamber: 3-m coil. Fluorometric detector: excitation wavelength 340 nm, emission wavelength 455 nm. (From ref. 14, with permission.)

Another approach to hydrolysis of NMCs prior to derivatization with OPA/MERC was proposed by Miles and Moye [46,47] who employed a photolytic reactor consisting of a UV lamp inserted in the centre of a woven coil of Teflon tubing. This also eliminated the need for one post-column pump. Detection limits were *ca.* 1.0 ng.

A useful simplification of the post-column derivatization technique which eliminated the need for both the NaOH post-column pump and solid-phase or photolytic reactors was reported by McGarvey [48]. In this approach the hydrolysis and derivatization steps were combined by the use of a single reagent consisting of OPA/MERC in 0.01 *M* KOH, which was delivered by a single post-column pump (Fig. 2). The detection limit for 11 NMCs using the single-stage PCRS was *ca.* 0.1 ng. Reproducibility of retention times and peak heights was good, coefficients of variation averaging 0.2% and 2.3%, respectively. Chromatograms obtained using the single-stage and two-stage methods are compared in Fig. 3. This technique has been used to determine oxamyl in potatoes [49], and oxamyl and methomyl in crops and water [50]. Simon *et al.* [51] modified the method by substituting N,N-dimethyl-2-mercaptoethylamine hydrochlo-

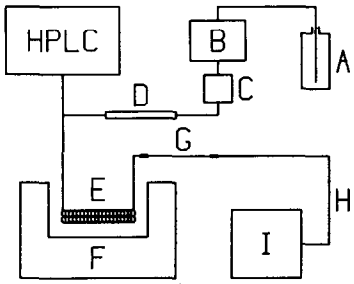

Fig. 2. Schematic diagram of single-stage post-column reactor. A = derivatization reagent reservoir; B = reagent-delivery pump; C = pulse damper; D = spent PRP-1 column (to give back pressure to pulse damper); E = delay tube; F = hydrolysis heater; G = single-bead string reactor; H = 1 m × 0.5 mm I.D. Teflon tubing; I = fluorescence detector. (From ref. 47, with permission.)

ride (thiofluor) for 2-mercaptoethanol in the derivatization reagent.

2.2. Organophosphates

A pre-column derivatization method for HPLC determination of organophosphate insecticides including fenthion, crufomate, fenchlorphos, methylparathion and fenitrothion was reported by Lawrence *et al.* [52]. The compounds were hydrolyzed to the corresponding phenols using sodium hydroxide. The phenols were reacted with dansyl chloride for 90 min to give fluorescent derivatives which were determined by HPLC with fluorescence detection. The detection limits ranged from 5 to 10 ng.

Bardarov and Mitewa [53] proposed a method for dialkylphosphate, dialkylthiophosphate and dialkyldithiophosphate metabolites of organophosphate pesticides in urine. Reaction with 2,3,4,5,6-pentafluorobenzyl bromide produced pentafluorobenzyl esters which were determined by HPLC-UV at 260 nm.

2.3. Avermectins

The avermectins, including ivermectin and abamectin, are macrocyclic lactones prepared by fermentation. Ivermectin is used as an antiparasitic agent in animals and humans, and abamectin as an insecticide and acaracide. HPLC-UV methods lack sensitivity for avermectins. A pre-column derivatization method for avermectins in plasma developed by Tolan *et al.* [54] involved reaction with acetic anhydride in pyridine, which acted both as catalyst and solvent, for 24 h. This formed a fluorescent compound which was determined by reversed-phase HPLC with fluorescence detection. The detection limit was 1 ng.

This method was improved by substituting N-methylimidazole (NMIM) as the catalyst and dimethylformamide (DMF) as the solvent [55], which allowed formation of the fluorophore in 1 h at 95°C, with more reproducible yields. The limit of detection in cattle and sheep tissues was 1–2 μg/kg. The method was subsequently applied to a variety of tissues and plasma [56–58]. Additional silica column clean-up was required after derivatization. A confirmatory technique was reported by Downing [59] in which ivermectin was hydrolyzed to the monosaccharide (using 1% sulfuric acid in isopropanol) or to the aglycone (using 1% sulfuric acid in methanol) prior to derivatization with acetic anhydride.

The derivatization method was further shortened by the use of trifluoroacetic anhydride (TFAA) as the derivatization reagent, NMIM as the catalyst, and acetonitrile as the solvent [60]. The reaction occurred virtually instantaneously at ambient temperature and no additional clean-up was required after derivatization. The detection limit in animal plasma was 20 pg/ml.

Prabhu *et al.* [62] applied this method to the determination of 4″-deoxy-4″-(epimethylamino)avermectin B_1 benzoate (MK-0224) and its delta 8,9-isomer, one of its major photodegradation products, in celery and lettuce [61] and to the determination of abamectin and its delta 8,9-isomer in tomatoes. Limits of detection were 2 μg/kg.

Abamectin was reacted with TFAA and NMIM in DMF for 1 h at 30°C for determination of residues on cotton gloves and foliage and in air [63]. Ammonium hydroxide in methanol was added to remove an unstable trifluoroacetyl group at one of the sugar moieties of abamectin.

A post-column derivatization method for

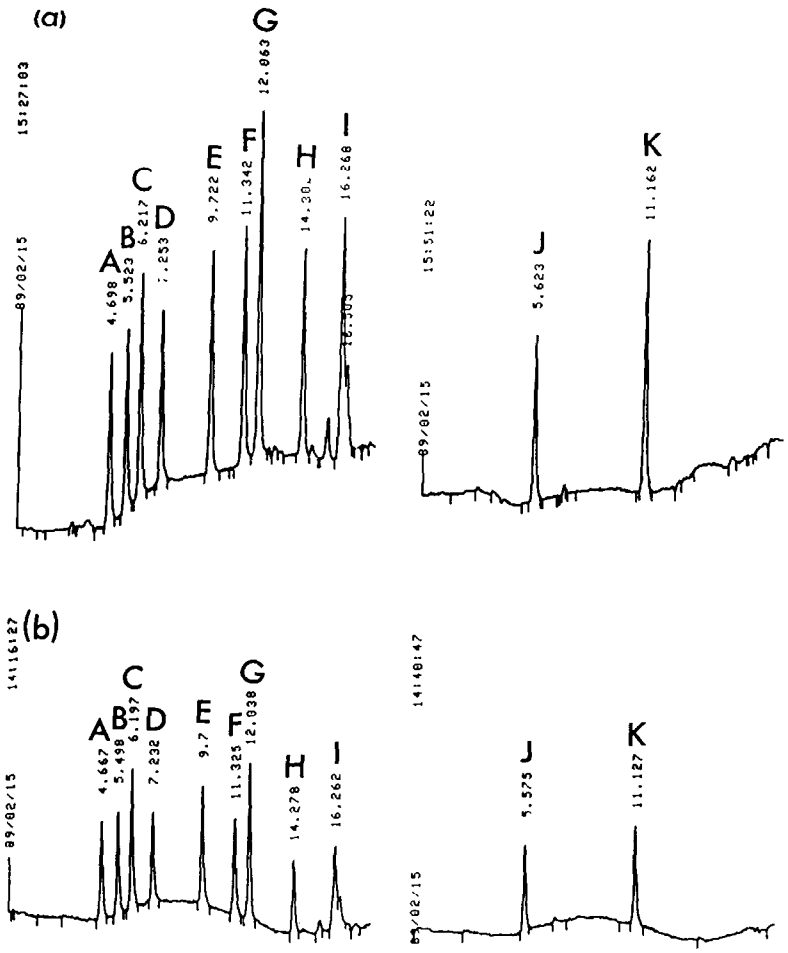

Fig. 3. Chromatograms of carbamate pesticides (0.2 μg/ml each): (a) single-stage method; (b) two-stage method. A = aldicarb sulfoxide; B = aldicarb sulfone; C = methomyl; D = 3-hydroxycarbofuran; E = aldicarb; F = carbofuran; G = carbaryl; H = methiocarb; I = bufencarb; J = oxamyl; K = propoxur. (From ref. 47, with permission; numbers at individual peaks represent retention times in min.)

abamectin-8,9-oxide was reported by Demchak and MacConnell [64] in which, following normal-phase HPLC separation, the analyte was reacted on-line at 45°C in a 5-ml reaction coil with trichloroacetic acid in 1,2-dichloroethane saturated with 2-naphthalenesulfonic acid 1-hydrate introduced by a post-column pump. The reaction product was detected by absorption at 570 nm and detection of 1 μg was reported. This is not sufficiently sensitive for residue determination, though it was not specifically expressed as the detection limit.

3. Antimicrobial agents

Derivatization reactions for HPLC determination of antimicrobial agents, including fungicides and antibiotics, are summarized in Table 2.

3.1. Ethylenebisdithiocarbamates and thiuram disulfides

An HPLC method for ethylenebisdithiocarbamate (EBDC) fungicides including nabam, zineb, maneb, mancozeb and propineb was re-

Table 2
Derivatization techniques for HPLC determination of antimicrobial compounds

Derivatization reagent	Detector	Detection limit	Reference
Ethylenebisdithiocarbamates and thiuram disulfides			
Methyl iodide (pre-col, in EDTA solution)	Abs, 727 nm	10–20 ng/g	65–67
o-Phthalaldehyde, 2-mercaptoethanol (post-col, FH, nabam only)	Fl	1 ng	68
Copper (post-col)	Abs, 435 nm	10 ng/g	69,70
Ethyleneurea			
Pentafluorobenzoyl chloride (pre-col)	Abs, 254 nm	0.1 μg/g	71
2-Imidazoline			
p-Nitrobenzoyl chloride (pre-col)	Abs, 254 nm	0.02 μg/g	72
Ethylenethiourea			
p-Nitrophenacyl bromide (pre-col)	Abs, 264	0.04 ng	73
Thiabendazole			
p-Nitrobenzyl bromide (pre-col)	Abs, 305	2–20 ng/g	74
Methyl 1H-benzimidazol-2-ylcarbamate (MBC)			
n-Propyl isocyanate (pre-col)	Abs, 254 nm	0.2 μg/g	75
Dithianon			
Sodium sulphide (post-col)	Abs, 375 nm	20 ng	76,77
Mildiomycin			
Fluorescamine (pre-col)	Fl	0.06–0.7 μg/g	78,79
Zhongshengjunsu			
o-Phthalaldehyde (pre-col)	Fl	–	80

Abs = absorption; FH = following hydrolysis; Fl = fluorescence.

ported by Gustafsson and Thompson [65]. This method involved extraction with an alkaline EDTA solution followed by addition of hydrochloric acid and tetrabutylammonium hydrogen sulfate. The solubilized EBDCs were extracted and methylated in chloroform–hexane (3:1, v/v) containing methyl iodide to form *S*-methyl dithiocarbamates which were analyzed by HPLC with UV detection at 272 nm. Nabam, however, either present in the sample or formed from zineb, maneb or mancozeb by treatment with EDTA, reacted with coextractives and with thiram. Gustafsson and Fahlgren [66] overcame this problem by adding L-cysteine to the EDTA solution. Limits of detection for zineb, ziram and thiram were below 0.02, 0.01 and 0.01 mg/kg, respectively. This method was applied to the determination of zineb and maneb in air and on cotton gloves, and to the determination of dislodgable zineb residues on carnation leaves [67].

Miles and Zhou [68] employed a PCRS and fluorescence detection for determination of nabam in several crops. Nabam was solubilized using a solution of EDTA and 2-mercaptoethanol and analyzed by HPLC. A two-stage PCRS consisting of two post-column pumps and woven Teflon reaction coils was used to derivatize nabam. The first pump delivered 0.05 *M* H_2SO_4 to hydrolyze nabam to ethylenediamine which was then fluorogenically labelled using

OPA/MERC in borate buffer delivered by a second pump. The detection limit was *ca.* 1 ng. Efforts to recover mancozeb or maneb as nabam from solution or from fortified crops proved unsuccessful.

Irth *et al.* [69,70] determined thiram and disulfiram as copper(II) dimethyldithiocarbamate using absorption at 435 nm after post-column complexation with copper(II) in a solid-state reactor packed with metallic copper prepared by reduction of copper(I) chloride. The high detection wavelength made it possible to determine thiram without interference from coextractives. The detection limit for thiram in soil was 10 ppb.

Ethyleneurea is a major metabolite of EBDCs. In a pre-column derivatization method for ethyleneurea, extraction and preliminary clean-up on alumina were followed by derivatization with pentafluorobenzoyl chloride to form a pentafluorobenzamide which was determined by HPLC-UV at 254 nm [71]. The volatility of the derivative permitted subsequent confirmation by GC and mass spectrometry.

2-Imidazoline is a degradation product of ethylenethiourea (ETU), an intermediate in the decomposition of EBDCs. Newsome and Panopio [72] reported a method which involved extraction of the sample with 0.1 *M* HCl, preliminary clean-up on a cation-exchange resin, and derivatization of 2-imidazoline with *p*-nitrobenzoyl chloride. After further clean-up on silica gel, the derivative was analyzed by HPLC-UV at 254 nm. The detection limit was 0.02 ppm.

A pre-column derivatization method for determination of ETU employing phenacyl bromides was developed to increase the sensitivity and selectivity of HPLC-UV for this compound [73]. ETU was refluxed in ethanol with *p*-nitrophenacyl bromide for 2 h. The mixture was cooled, made basic and extracted with chloroform. The extract was evaporated to dryness and redissolved in mobile phase for HPLC determination. The limit of detection was 0.04 ng using 264 nm.

3.2. Benzimidazoles

A confirmatory pre-column derivatization method for thiabendazole residues in fruits was reported by Tafuri *et al.* [74]. Thiabendazole was reacted with *p*-nitrobenzyl bromide at 110°C for 3 h and the resulting *p*-nitrobenzyl derivative was determined by normal-phase HPLC-UV at 305 nm. The detection limit in pears and bananas was 0.002 mg/kg and in oranges was 0.02 mg/kg. Determination of thiabendazole residues without derivatization by either normal- or reversed-phase was also reported.

Methyl 1H-benzimidazol-2-ylcarbamate (MBC) is a fungicidal degradation production of the fungicide benomyl. A pre-column derivatization method for the determination of MBC in the presence of benomyl was reported by Chiba and Veres [75] in which MBC was extracted with chloroform containing *n*-propyl isocyanate at 1°C. This quantitatively converted MBC to methyl 1-(*n*-propylcarbamoyl)-2-benzimidazole carbamate (MBC-PIC) and allowed its extraction, along with benomyl residues, into chloroform. Benomyl was stabilized by the addition of *n*-butyl isocyanate to the extract, and benomyl and MBC-PIC were determined simultaneously by normal-phase HPLC with UV detection at 254 nm. The detection limit for both compounds in apple leaves was 0.2 ppm.

3.3. Dithianon

Dithianon is a non-systemic fungicide effective against many foliar diseases of pome and stone fruit. A reversed-phase HPLC method involving post-column derivatization was reported by Kojima *et al.* [76] and Baker and Clarke [77]. The column eluent was mixed with a 3% sodium sulfide solution delivered using air pressure and the mixture was passed through a reaction tube packed with glass beads. The derivative was detected using UV at 375 nm. The detection limit was 20 ng. HPLC determination of dithianon was also possible without derivatization, but the PCRS, though more subject to baseline drift, was less subject to interference.

3.4. Mildiomycin

Mildiomycin is an antibiotic which is effective against powdery mildews on many types of plants. A pre-column derivatization method in-

volved extraction with saturated sodium pyrophosphate solution and clean-up by column chromatography, after which mildiomycin was reacted at pH 8.0 with fluorescamine, and determined by ion-pairing reversed-phase HPLC with fluorescence detection [78,79]. The limits of detection in cucumber, tobacco foliage and soil were 0.06, 0.2 and 0.7 ppm, respectively.

3.5. Zhongshengjunsu

Zhongshengjunsu is an antibiotic effective against some bacterial diseases of crops and vegetables. Lin *et al.* [80] reported a reversed-phase ion-pairing HPLC method in which zhongshengjunsu was reacted with *o*-phthalaldehyde before injection onto the HPLC system. The resulting fluorescent derivative was detected with a fluorescence detector using an excitation wavelength of 340 nm and an emission wavelength of 425 nm.

4. Herbicides

Derivatization techniques for HPLC determination of herbicides are summarized in Table 3.

4.1. Glyphosate

Glyphosate is a widely used nonselective post-emergence herbicide. (Aminomethyl)phosphonic acid (AMPA) is its major degradation product in plants, water and soil. Moye and Boning [81] reported a pre-column derivatization method for glyphosate and AMPA in which both compounds were reacted with 9-fluorenylmethyl chloroformate for 20 min prior to determination by

Table 3
Derivatization techniques for HPLC determination of herbicides

Derivatization reagent	Detector	Detection limit	Reference
Glyphosate			
9-Fluorenylmethyl chloroformate (pre-col)	Fl	0.1 ng	81–86
o-Phthalaldehyde, 2-mercaptoethanol (post-col, following cleavage)	Fl	0.5–2 ng	82,87–96
1-Fluoro-2,4-dinitrobenzene (pre-col)	Abs, 405 nm	0.05 μg/g	97
Ninhydrin and hydrindantin (post-col)	Abs, 570 nm	0.01–0.1 μg/g	98
p-Toluenesulfonyl chloride (pre-col)	Abs, 240	0.2 ng	99,100
Al^{3+}-morin (post-col)	Fl	14–40 ng	101
Phenoxyacids			
2-Naphthacyl bromide (pre-col)	Abs, 254 nm	0.2–0.3 ng	102,103
4-Bromomethyl-7-methoxycoumarin (pre-col)	Fl, Abs, 340 nm	0.5–0.7 ng	102,103
Thionyl chloride, diphenylamine (pre-col)	Abs, 240 nm	–	104
9-Anthryldiazomethane (pre-col)	Fl	1–2 ng	105,106
Phenylureas			
o-Phthalaldehyde, 2-mercaptoethanol (post-col, FH)	Fl	1–5 ng	107,108
Diquat and paraquat			
Sodium hydrosulfite (post-col)	Abs, 379 nm	1 ng/g	109
Amitrole			
Sodium nitrite, sulfamic acid, 8-amino-1-naphthol-3,6-disulfonic acid (pre-col)	Abs, 546 nm	5–10 ng/g	110

Abs = absorption; FH = following hydrolysis; Fl = fluorescence.

anion-exchange HPLC and fluorescence detection.

Moye and St. John [82] reported a post-column derivatization method for the determination of glyphosate and AMPA and critically compared it with the aforementioned pre-column method. The post-column method was similar to that reported for N-methylcarbamates [11]. An oxidative calcium hypochlorite reagent was delivered by the first post-column reagent-delivery pump. This allowed cleavage of glyphosate to produce a primary amine which rapidly reacted with OPA/MERC reagent, introduced by a second reagent-delivery pump, to produce a fluorophore. The authors concluded that though both pre-column and post-column methods produced highly fluorescent derivatives, the post-column method was preferable because it produced fewer interferences; the pre-column method results in derivatization of both primary and secondary amines as well as alcohols, whereas the post-column method is specific for primary amines.

The pre-column method was used for the determination of glyphosate and AMPA in straw [83], soil and water [84] and water [85,86]. The sensitivity of the post-column method was subsequently improved by Moye *et al.* [87] (detection limit 0.5 ng) and the method was validated in inter-laboratory studies [88,89] and further refined [90]. It was used for the determination of glyphosate in blackberries [91], cereals and vegetables [92], environmental water [93], lentils [94], and cereals, oilseeds and pulses [95], and formed the basis for EPA Method 547 [96] for determination of glyphosate in water.

In another pre-column derivatization method for glyphosate and AMPA the compounds were extracted with aqueous triethylamine solution and derivatized with FDNB in the dark at room temperature for 1 h [97]. Determination was by reversed-phase ion-pairing HPLC with absorption detection at 405 nm. The limit of detection in soil was 0.05 μg/g for glyphosate and 0.1 μg/g for AMPA.

A post-column derivatization method for glyphosate and AMPA employed a derivatization reagent consisting of ninhydrin and hydrindantin which was introduced by a post-column reagent-delivery pump [98]. The reagent had to be prepared and stored under nitrogen atmosphere. Derivatization of glyphosate and AMPA occurred in a post-column reactor at 100°C. Detection was by absorption at 570 nm. Limits of detection ranged from 0.01 μg/g dry mass for sediments to 0.1 μg/g dry mass for foliage.

Pre-column derivatization of glyphosate and AMPA with *p*-toluenesulphonyl chloride was employed by Kawai *et al.* [99]. The reaction required only 5 min at 50°C in phosphate buffer (pH 11.0). Determination was by reversed-phase HPLC with UV detection at 240 nm. The detection limit for standard solutions was 0.2 ng for glyphosate and 0.16 ng for AMPA. The method was applied to the determination of glyphosate and AMPA in human serum and detection limits were 0.3 μg/ml and 0.2 μg/ml, respectively [100].

An indirect detection method for glyphosate and AMPA involving post-column reaction was reported by Lovdahl and Pietrzyk [101]. Following their separation by ion-exchange HPLC, glyphosate and AMPA interacted with Al^{3+}-morin (3,5,7,2′,4′-pentahydroxyflavone) reagent introduced by a post-column pump. Glyphosate and AMPA competed favorably with morin to form a complex with Al^{3+}. The presence of glyphosate or AMPA was therefore detected as a decrease in the baseline fluorescence due to the Al^{3+}-morin reagent, which was proportional to the analyte concentration provided that Al^{3+}-morin was in excess. The detection limits, 14 ng for glyphosate and 40 ng for AMPA, were not adequate for residue analysis.

4.2. Phenoxyacids

This class of herbicides includes such compounds as 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2-(2,4-dichlorophenoxy)propionic acid (2,4-DP), 2-methoxy-3,6-dichlorobenzoic acid (dicamba) and 2-(2,4,5-trichlorophenoxy)propionic acid (phenoprop). Though the underivatized acids are readily separated by HPLC, sensitivity by UV absorption is poor.

Two pre-column derivatization procedures and

a procedure not involving derivatization were compared in an attempt to ascertain the optimum HPLC methodology for these compounds [102,103]. Acids were reacted with either 2-naphthacyl bromide (NPB) or 4-bromomethyl-7-methoxycoumarin (MMC) in acetone for 45 min at 35°C in the dark. Acids and derivatives were determined using UV detection (acids at 280 nm, NPB derivatives at 254 nm and MMC derivatives at 340 nm) and MMC derivatives were also determined by fluorescence detection. Though nine underivatized acids were easily separated by reversed-phase HPLC, neither the NPB or MCC derivatives of the same nine acids could be completely separated in a single chromatographic run. Sensitivity of UV detection of MMC and NPB derivatives was 5–7 and 10–25 times better than that for the underivatized acids, respectively. Fluorescence detection was slightly less sensitive than UV detection for the MMC derivatives. The limit of detection by UV of NPB derivatives was better by a factor of 5–20 than that for MMC derivatives. However, derivatization with NPB produced so many side-products that a clean-up was required before HPLC determination. Thus no procedure was clearly superior to the others.

Blessington and Crabb [104] prepared diphenylamide derivatives of two chiral and one non-chiral phenoxyacid herbicides for chiral separation by HPLC on a Pirkle column with UV detection at 240 nm. One drop of thionyl chloride was added to 2 mg of herbicide, which was then heated over a steam bath for 10 min. After evaporation to dryness, 1 ml of 2 mg/ml diphenylamine in chloroform was added. After 10 min the mixture was evaporated to dryness and redissolved in a solvent appropriate for HPLC.

In another pre-column derivatization method the acids were reacted with 9-anthryldiazomethane in acetone for 60 min in the dark at 40°C [105,106]. Determination was by reversed-phase HPLC using fluorescence detection with a detection limit of 1–2 ng, allowing detection of 0.2–0.4 μg/l in a 20-ml water sample. A chromatogram obtained on sampling 20 ml of ground water spiked with 0.5 μg/l of each herbicide is shown in Fig. 4.

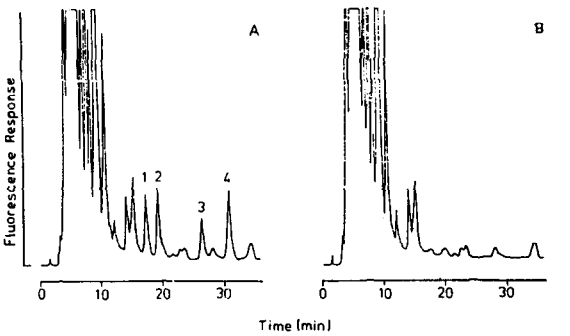

Fig. 4. Chromatograms of ground water extracts treated with 9-anthryldiazomethane. (A) Ground water sample spiked with 0.5 μg/l concentrations of phenoxy acid herbicides. Peaks: 1 = 2,4-D; 2 = (4-chloro-2-methylphenoxy)acetic acid; 3 = 2-(4-chloro-2-methylphenoxy)propionic acid; 4 = (4-chloro-2-methylphenoxy)butyric acid. (B) Blank ground water sample. (From ref. 105, wih permission.)

4.3. Phenylureas

A two-stage post-column derivatization method was reported by Luchtefeld [107] for substituted phenylurea herbicides including chlorbromuron, chloroxuron, diuron, fluometuron, linuron, and metobromuron. Following separation by reversed-phase HPLC, the phenylureas were hydrolyzed in a photolytic reactor consisting of a UV lamp surrounded by a woven coil of Teflon capillary tubing. The methylamine produced during hydrolysis was then reacted with OPA/MERC reagent introduced by a post-column reagent-delivery pump to produce a fluorescent isoindole which was detected by a fluorescence detector. The method was subsequently applied to the determination of phenylurea herbicide residues in a variety of fruits and vegetables [108] with limits of detection of 1–5 ng/g.

4.4. Diquat and paraquat

An HPLC assay for diquat and paraquat employing direct UV detection and confirmation by UV detection following post-column reaction was reported by Simon and Taylor [109]. Following HPLC separation on silica gel using an acidic aqueous mobile phase containing tetramethylammonium and ammonium ions, diquat was detected at 310 nm and paraquat at 255 using a diode-array UV detector. Downstream from the

diode-array detector, sodium hydrosulfite in sodium hydroxide solution was delivered by a post-column pump. Following post-column reaction in a 2-m coil of woven Teflon tubing at room temperature, derivatives of diquat and paraquat were detected using UV at 379 nm. The method allowed detection of diquat and paraquat at a concentration of 1 μg/kg in a 20-ml sample of well water.

4.5. Amitrole

The herbicide amitrole (3-amino-1,2,4-triazole) does not absorb significantly in the UV or visible range. A pre-column derivatization method for amitrole was reported in which amitrole in cleaned-up crop extracts containing 3.5 *M* sulfuric acid was diazotized with 0.1 *M* sodium nitrite at room temperature [110]. After 2 min, 0.1 *M* sulfamic acid was added, followed by 1 m*M* 8-amino-1-naphthol-3,6-disulfonic acid (H-acid) in 50% ethanol. Following additional clean-up, the coloured reaction products were determined by HPLC with absorption detection at 546 nm. HPLC of the reaction products showed two peaks, and amounts of amitrole above 25 μg resulted in the appearance of an additional two peaks. With increasing amounts of amitrole, the heights of the former two peaks decreased and the height of the fourth peak increased. In addition, the formation of derivatives of amitrole was found to be dependent on the quality of the preceding clean-up. The detection limit was 0.005–0.01 mg/kg in potatoes and fodder beets. However, the aforementioned problems would appear to limit the value of this method for the determination of amitrole residues.

5. Rodenticide

5.1. Warfarin

Warfarin, an anticoagulant rodenticide, is a racemic mixture of which the (*S*)-isomer shows 7-fold greater rodenticidal activity than the (*R*)-isomer. While non-stereospecific analysis of warfarin is possible using direct determination by HPLC with fluorescence detection, a method involving pre-column derivatization allowed stereospecific determination [111]. Warfarin, in extracts of plasma or urine, was reacted with carbobenzyloxy-L-proline for 2 h in the presence of imidazole and dicyclohexylcarbodiimine, forming diastereoisomeric esters. The esters were separated using normal-phase HPLC and detected by UV absorption at 313 nm. The detection limit for each isomer was 0.1 μg.

The diastereoisomeric esters were also detected using post-column derivatization and fluorescence detection [112,113]. Following normal-phase HPLC separation of the esters, *n*-butylamine–methanol (1:1, v/v) was introduced by a reagent-delivery pump. Following reaction in a stainless-steel column packed with 40-μm glass beads, the derivatives were monitored using fluorescence detection. Detection limits for most enantiomers were in the range of 50–100 ng.

6. Conclusions

A wide variety of derivatization reactions have been employed for pesticide analysis by HPLC. In most cases the purpose of derivatization was to increase sensitivity or selectivity. It has also been used for the purpose of confirmation and, *e.g.* in the case of warfarin, for separation of enantiomers. Detection of derivatives was by absorption in the UV or visible range or by fluorescence, with use of fluorescence predominating over use of absorption by a factor of 3:1. This is likely due to the superior sensitivity and selectivity of fluorescence detection over absorption detection.

The single most widely used derivatization reagent for pesticide determination by HPLC would appear to be OPA/MERC, which has been used in post-column reaction systems for fluorescence detection of N-methylcarbamates, nabam (an EBDC), glyphosate, and phenylurea

herbicides, accounting for almost half the references included in this review.

In recent years there has been a trend favouring post-column derivatization over pre-column derivatization, as evidenced by the fact that only 25% of the reviewed methods reported up to 1982, but 70% of those reported since 1982, employed post-column derivatization. This may be because post-column derivatization is performed automatically on-line and does not require additional analyst's time for preparation of samples. Nor does post-column derivatization affect the chromatographic separation of analytes. This is important in cases where derivatization eliminates differences between compounds of the same class; N-methylcarbamates, for example, must be separated prior to being derivatized.

The utility of derivatization in pesticide determination by HPLC for improving sensitivity and selectivity is evident by the large body of literature on this subject. Work in this area is continuing and further applications of derivatization to the determination of pesticides by HPLC are likely.

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References

[1] Y.-C. Li, D. Strupp, A. Koßmann and W. Ebing, *Fresenius' Z. Anal. Chem.*, 316 (1983) 290.
[2] D.R. Lauren and M.P. Agnew, *J. Chromatogr.*, 292 (1984) 439.
[3] M.C. Pietrogrande, G. Blo and C. Bighi, *J. Chromatogr.*, 349 (1985) 63.
[4] M.T. Tena, M.D. Luque de Castro and M. Valcárcel, *J. Chromatogr. Sci.*, 30 (1992) 276.
[5] M.T. Tena, M.D. Luque de Castro and M. Valcárcel, *J. Liq. Chromatogr.*, 15 (1992) 2373.
[6] R.W. Frci and J.F. Lawrence, *J. Chromatogr.*, 83 (1973) 321.
[7] R.W. Frei, J.F. Lawrence, J. Hope and R.M. Cassidy, *J. Chromatogr. Sci.*, 12 (1974) 40.
[8] J.F. Lawrence and R. Leduc, *J. Assoc. Off. Anal. Chem.*, 61 (1978) 872.
[9] F.J. Bezuidenhout and L.P. van Dyk, *Bull. Environ. Contam. Toxicol.*, 26 (1981) 789.
[10] J.F. Lawrence and R. Leduc, *J. Chromatogr.*, 152 (1978) 507.
[11] H.A. Moye, S.J. Scherer and P.A. St. John, *Anal. Lett.*, 10 (1977) 1049.
[12] S.S. Simons, Jr. and D.F. Johnson, *J. Am. Chem. Soc.*, 98 (1976) 7098.
[13] R.T. Krause, *J. Chromatogr. Sci.*, 16 (1978) 281.
[14] R.T. Krause, *J. Chromatogr.*, 185 (1979) 615.
[15] R.T. Krause, *J. Assoc. Off. Anal. Chem.*, 63 (1980) 1114.
[16] R.T. Krause, *J. Assoc. Off. Anal. Chem.*, 68 (1985) 726.
[17] R.T. Krause, *J. Assoc. Off. Anal. Chem.*, 68 (1985) 734.
[18] A. Dekker and N.W.H. Houx, *J. Environ. Sci. Health*, B18 (1983) 379.
[19] S. Lesage, *LC-GC*, 7 (1989) 268.
[20] K.M. Hill, R.H. Hollowell and L.A. Dal Corvito, *Anal. Chem.*, 56 (1984) 2465.
[21] K.W. Edgell, L.A. Biederman and J.E. Longbottom, *J. Assoc. Off. Anal. Chem.*, 74 (1991) 309.
[22] D.L. Foerst and H.A. Moye, U.S. Environ. Prot. Agency, Off. Res. Dev., Cincinnati, OH, EPA/600/D-85/051, 1985.
[23] D. Chaput, *J. Assoc. Off. Anal. Chem.*, 69 (1986) 985.
[24] W. Blaß, *Fresenius J. Anal. Chem.*, 339 (1991) 340.
[25] N. Aharonson, L. Muszkat and M. Klein, *Phytoparasitica*, 13 (1985) 129.
[26] A. de Kok, M. Hiemstra and C.P. Vreeker, *Chromatographia*, 24 (1987) 469.
[27] K.-C. Ting, P.K. Kho, A.S. Musselman, G.A. Root and G.R. Tichelaar, *Bull. Environ. Contam. Toxicol.*, 33 (1984) 538.
[28] K.-C. Ting and P.K. Kho, *Bull. Environ. Contam. Toxicol.*, 37 (1986) 192.
[29] H. Engelhardt and B. Lillig, *Chromatographia*, 21 (1986) 136.
[30] C.E. Goewie and E.A. Hogendoorn, *J. Chromatogr.*, 404 (1987) 352.
[31] D. Chaput, *J. Assoc. Off. Anal. Chem.*, 71 (1988) 542.
[32] G.L. Muth and F. Erro, *Bull. Environ. Contam. Toxicol.*, 24 (1980) 759.
[33] M.W. Reeve, L.P. O'Connell, S. Bissell and J. Ross, *Bull. Environ. Contam. Toxicol.*, 49 (1992) 105.
[34] C.J. Miles and H.A. Moye, *Chromatographia*, 23 (1987) 109.
[35] M.J. Page and M. French, *J. AOAC Intern.*, 75 (1992) 1073.
[36] M.S. Ali, *J. Assoc. Off. Anal. Chem.*, 72 (1989) 586.
[37] M.S. Ali, J.D. White, R.S. Bakowski, N.K. Stapleton, K.A. Williams, R.C. Johnson, E.T. Phillippo, R.W. Woods and R.L. Ellis, *J. AOAC Intern.*, 76 (1993) 907.

[38] Method 531.1, Measurement of N-methylcarbomoyloximes and N-methylcarbamates in water by direct aqueous injection HPLC with post-column derivatization, U.S. Environment Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, OH, 1989.
[39] L. Nondek, R.W. Frei and U.A.Th. Brinkman, *J. Chromatogr.*, 282 (1983) 141.
[40] L. Nondek, U.A.Th. Brinkman and R.W. Frei, *Anal. Chem.*, 55 (1983) 1466.
[41] A. de Kok, M. Hiemstra and U.A.Th. Brinkman, *J. Chromatogr.*, 623 (1992) 265.
[42] L.K. She, U.A.Th. Brinkman and R.W. Frei, *Anal. Lett.*, 17 (1984) 915.
[43] A. De Kok, M. Hiemstra and C.P. Vreeker, *J. Chromatogr.*, 507 (1990) 459.
[44] H. Jansen, U.A.Th. Brinkman and R.W. Frei, *Chromatographia*, 20 (1985) 453.
[45] A. de Kok and M. Hiemstra, *J. AOAC Intern.*, 75 (1992) 1063.
[46] C.J. Miles and H.A. Moye, *Chromatographia*, 24 (1987) 628.
[47] C.J. Miles and H.A. Moye, *Anal. Chem.*, 60 (1988) 220.
[48] B.D. McGarvey, *J. Chromatogr.*, 481 (1989) 445.
[49] B.D. McGarvey, Th.H.A. Olthof and J.L. Townshend, *J. Agric. Food Chem.*, 38 (1990) 1608.
[50] S.C. Stafford and W. Lin, *J. Agric. Food Chem.*, 40 (1992) 1026.
[51] V.A. Simon, K.S. Pearson and A. Taylor, *J. Chromatogr.*, 643 (1993) 317.
[52] J.F. Lawrence, C. Renault and R.W. Frei, *J. Chromatogr.*, 121 (1976) 343.
[53] V. Bardarov and M. Mitewa, *J. Chromatogr.*, 462 (1989) 233.
[54] J.W. Tolan, P. Eskola, D.W. Fink, H. Mrozik and L.A. Zimmerman, *J. Chromatogr.*, 190 (1980) 367.
[55] P.C. Tway, J.S. Wood, Jr. and G.V. Downing, *J. Agric. Food Chem.*, 29 (1981) 1059.
[56] G.V. Downing, in W.C. Campbell (Editor), *Ivermectin and Abamectin*, Appendix I, Springer-Verlag, New York, 1989, pp. 324–335.
[57] I. Nordlander and H. Johnsson, *Food Additives and Contaminants*, 7 (1990) 79.
[58] S.V. Prabhu, T.A. Wehner and P.C. Tway, *J. Agric. Food Chem.*, 39 (1991) 1468.
[59] G.V. Downing, in W.C. Campbell (Editor), *Ivermectin and Abamectin*, Appendix II, Springer-Verlag, New York, 1989, pp. 336–343.
[60] P. de Montigny, J.-S.K. Shim and J.V. Pivnichny, *J. Pharm. Biomed. Anal.*, 8 (1990) 507.
[61] S.V. Prabhu, T.A. Wehner, R.S. Egan and P.C. Tway, *J. Agric. Food Chem.*, 39 (1991) 2226.
[62] S.V. Prabhu, R.J. Varsolona, T.A. Wehner, R.S. Egan and P.C. Tway, *J. Agric. Food Chem.*, 40 (1992) 622.
[63] M.J.M. Jongen, R. Engel and L.H. Leenheers, *Am. Ind. Hyg. Assoc. J.*, 52 (1991) 433.
[64] R.J. Demchak and J.G. MacConnell, *J. Chromatogr.*, 511 (1990) 353.
[65] K.H. Gustafsson and R.A. Thompson, *J. Agric. Food Chem.*, 29 (1981) 729.
[66] K.H. Gustafsson and C.H. Fahlgren, *J. Agric. Food Chem.*, 31 (1983) 461.
[67] M.J.M. Jongen, J.C. Ravensberg, R. Engel and L.H. Leenheers, *J. Chromatogr. Sci.*, 29 (1991) 292.
[68] C.J. Miles and M. Zhou, *J. Assoc. Off. Anal. Chem.*, 74 (1991) 384.
[69] H. Irth, G.J. de Jong, U.A.Th. Brinkman and R.W. Frei, *J. Chromatogr.*, 370 (1986) 439.
[70] H. Irth, G.J. de Jong, R.W. Frei and U.A.Th. Brinkman, *Intern. J. Environ. Anal. Chem.*, 39 (1990) 129.
[71] W.H. Newsome, *J. Agric. Food Chem.*, 26 (1978) 1325.
[72] W.H. Newsome and L.G. Panopio, *J. Agric. Food Chem.*, 26 (1978) 638.
[73] R.M. Smith, K.C. Madahar, W.G. Salt and N.A. Smart, *Chromatographia*, 19 (1984) 411.
[74] F. Tafuri, C. Marucchini, M. Patumi and M. Businelli, *J. Agric. Food Chem.*, 28 (1980) 1150.
[75] M. Chiba and D.F. Veres, *J. Assoc. Off. Anal. Chem.*, 63 (1980) 1291.
[76] M. Kojima, N. Sekigawa, N. Shiga, O. Matano and S. Goto, *Bunseki Kagaku*, 29 (1980) 738.
[77] P.G. Baker and P.G. Clarke, *Analyst*, 109 (1984) 81.
[78] M. Inoue and T. Hagimoto, *J. Pesticide Sci.*, 8 (1983) 321.
[79] M. Inoue and T. Hagimoto, in J. Miyamoto and P.C. Kearney (Editors), *Pesticide Chemistry: Human Welfare and the Environment*, Vol. 4, Pergamon, Oxford, 1982, pp. 83–88.
[80] Z. Lin, G. Lin and Y. Su, *Chinese J. Biol. Control*, 7 (1991) 67.
[81] H.A. Moye and A.J. Boning, Jr., *Anal. Lett.*, 12 (1979) 25.
[82] H.A. Moye and P.A. St. John, in J. Harvey, Jr. and G. Zweig (Editors), *Pesticide Analytical Methodology*, ACS Symp. Ser. No. 136, American Chemical Society, Washington, D.C., 1980, pp. 89–102.
[83] H. Roseboom and C.J. Berkhoff, *Anal. Chim. Acta*, 135 (1982) 373.
[84] R.L. Glass, *J. Agric. Food Chem.*, 31 (1983) 280.
[85] C.J. Miles, L.R. Wallace and H.A. Moye, *J. Assoc. Off. Anal. Chem.*, 69 (1986) 458.
[86] F.A. Antón, L.M. Cuadra, P. Gutierrez, E. Laborda and P. Laborda, *Bull. Environ. Contam. Toxicol.*, 51 (1993) 881.
[87] H.A. Moye, C.J. Miles and S.J. Scherer, *J. Agric. Food Chem.*, 31 (1983) 69.
[88] J.E. Cowell, J.L. Kunstman, P.J. Nord, J.R. Steinmetz and G.R. Wilson, *J. Agric. Food Chem.*, 34 (1986) 955.
[89] M.E. Oppenhuizen and J.E. Cowell, *J. Assoc. Off. Anal. Chem.*, 74 (1991) 317.
[90] C.J. Miles and G. Leong, *LC · GC*, 10 (1992) 452.
[91] T.E. Archer and J.D. Stokes, *J. Agric. Food Chem.*, 32 (1984) 586.

[92] L.G.M.Th. Tuinstra and P.G.M. Kienhuis, *Chromatographia*, 24 (1987) 696.
[93] Y.Y. Wigfield and M. Lanouette, *Anal. Chim. Acta*, 233 (1990) 311.
[94] Y.Y. Wigfield and M. Lanouette, *Pestic. Sci.*, 33 (1991) 491.
[95] Y.Y. Wigfield and M. Lanouette, *J. Assoc. Off. Anal. Chem.*, 74 (1991) 842.
[96] Method 547, Determination of glyphosate in drinking water by direct aqueous injection HPLC, post-column derivatization, and fluorescence detection, U.S. Environment Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, OH, 1990.
[97] L.N. Lundgren, *J. Agric. Food Chem.*, 34 (1986) 535.
[98] D.G. Thompson, J.E. Cowell, R.J. Daniels, B. Staznik and L.M. MacDonald, *J. Assoc. Off. Anal. Chem.*, 72 (1989) 355.
[99] S. Kawai, B. Uno and M. Tomita, *J. Chromatogr.*, 540 (1991) 411.
[100] M. Tomita, T. Okuyama, S. Watanabe, B. Uno and S. Kawai, *J. Chromatogr.*, 566 (1991) 239.
[101] M.J. Lovdahl and D.J. Pietrzyk, *J. Chromatogr.*, 602 (1992) 197.
[102] H. Roseboom, H.A. Herbold and C.J. Berkhoff, *J. Chromatogr.*, 249 (1982) 323.
[103] H. Roseboom and P.A. Greve, in J. Miyamoto and P.C. Kearney (Editors), *Pesticide Chemistry: Human Welfare and the Environment*, Vol. 4, Pergamon, Oxford, 1982, pp. 111–116.
[104] B. Blessington and N. Crabb, *J. Chromatogr.*, 454 (1988) 450.
[105] T. Suzuki and S. Watanabe, *J. Chromatogr.*, 541 (1991) 359.
[106] T. Suzuki and S. Watanabe, *J. AOAC Intern.*, 75 (1992) 720.
[107] R.G. Luchtefeld, *J. Chromatogr. Sci.*, 23 (1985) 516.
[108] R.G. Luchtefeld, *J. Assoc. Off. Anal. Chem.*, 70 (1987) 740.
[109] V.A. Simon and A. Taylor, *J. Chromatogr.*, 479 (1989) 153.
[110] H. Løkke, *J. Chromatogr.*, 200 (1980) 234.
[111] C.R. Banfield and M. Rowland, *J. Pharm. Sci.*, 72 (1983) 921.
[112] C. Banfield and M. Rowland, *J. Pharm. Sci.*, 73 (1984) 1392.
[113] K. Hunter, in J. Sherma (Editor), *Analytical Methods for Pesticides and Plant Growth Regulators*, Vol. XVI, Academic, New York, 1988, pp. 119–177.