ELSEVIER

Journal of Chromatography A, 754 (1996) 3–16

JOURNAL OF CHROMATOGRAPHY A

Review

Recent advances in the residue analysis of N-methylcarbamate pesticides

S.S. Yang*, A.I. Goldsmith, I. Smetena

Research Center, Philip Morris USA, PO Box 26583, Richmond, VA 23261, USA

Abstract

This paper highlights recent advances in the determination of methylcarbamate residues in water, soil and plant tissues. Chromatographic analyses (e.g., HPLC, GC, supercritical fluid chromatography and TLC) with various sample pretreatment procedures and detection methods are reviewed. More generally, some non-chromatographic techniques such as immunoassay, biosensor and spectrophotometry are included.

Keywords: Reviews; Sample preparation; Environmental analysis; Pesticides; Methylcarbamates

Contents

*Corresponding author.

0021-9673/96/$15.00
PII S0021-9673(96)00203-8

1. Introduction

N-Methylcarbamates (NMCs) comprise an important class of pesticide widely used for crop protection. There are approximately 30 commercially available NMCs on the market with some of the most common ones being carbaryl, carbofuran, aldicarb, methomyl and oxamyl. Some of the NMCs are no longer manufactured, or marketed for crop protection use. According to The Pesticide Manual [1], the discontinued NMCs include aminocarb, bufencarb, butacarb, carbanolate, dioxacarb, ethidimuron, mexacarbate and promecarb.

The common structural feature of NMCs is an N-methyl group, that can be easily hydrolyzed to form methylamine in an alkaline media. Structurally, NMCs can be classified into two groups, phenyl N-methyl carbamates and oxime carbamates. As shown in Fig. 1, the first group are carbamate esters derived from substituted phenols. The substituents on the phenyl ring can change the characteristic nature of the parent compound in hydrophobic, electronic and hydrogen bonding, thus affect the ability of complexing with acetylcholinesterase, a determining factor for its cholinesterase inhibition activity [2]. In addition to molecular structure, oxime carbamates also differ from phenyl NMCs in the determining factors of inhibition. The inhibition activity of oxime carbamates is dominated by the rate of reaction with enzyme but not the complexing ability. Most of the oxime carbamates contain an active S-element, which can be easily oxidized to convert the parent compound into its sulfoxide or sulfone metabolites.

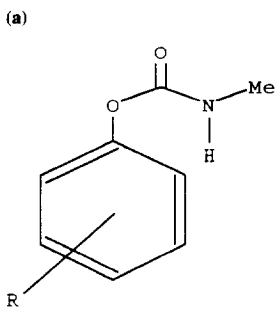

Fig. 1. (a) Phenyl N-methylcarbamate; (b) oxime carbamate.

One of the major advantages of NMC pesticides, is their short persistence on plants. Most of the NMCs are degraded into their metabolites shortly after application. These metabolites quite often are as active or even more active than the parent compound; for example, aldicarb sulfoxide is a more effective cholinesterase inhibitor than aldicarb itself. When monitoring pesticide residues, these metabolites, which are greater in number than the parent NMCs, must be taken into account.

Numerous analytical procedures have been developed for the determination of NMCs and metabolites in various matrices including water, soil, fruits, vegetables and other crops. A review published by McGarvey three years ago covered the applications of high-performance liquid chromatography (HPLC) for NMC analysis with extensive details [3]. The goal of the current review is to highlight the advances in the determination of NMCs in the recent 3 years. Chromatographic methods including HPLC, gas chromatography (GC), supercritical-fluid extraction (SFC) and supercritical fluid chromatography (SFC), thin-layer chromatography (TLC) and capillary electrophoresis (CE) are reviewed. In order to be more inclusive, we also covered a section of non-chromatographic techniques being used for NMC analysis; e.g., immunoassay, biosensor, spectrophotometry and electrochemistry. Some earlier publications are mentioned in the current review to provide background information and the reader is referred to previous reviews for more details [3–5]. The reader may not be surprised that many of the analytical procedures were developed for multiple-residue determination, and so they are applicable to a wider range of pesticides than just NMCs.

2. Sample preparation

2.1. General

McGarvey [3] summarized NMC determinations in water, soil and plant samples in tabular form by chemicals, extraction solvent, cleanup, recovery, detection and sensitivity. NMCs and metabolites were recovered from water samples by several means; for examples: (i); solvent extraction by a water-immicisible organic (e.g., dichloromethane, chloroform); or (ii); enriched in a solid-phase extraction (SPE) cartridge, mostly C_{18} silica or XAD resin, followed by elution with organic solvents. Direct injection of water samples without any concentration procedure was also applied. The use of SPE in multiple-residue pesticide analysis, including NMCs, of water samples was reviewed by Font et al. [5]. The adsorption or partitioning mechanism of various SPE types and the factors affecting extraction efficiency (e.g., nature of the sample, pH and sorbent treatment) were included. Junk and Richard specifically discussed the performance of SPE with C_{18} bonded silica in the analysis of carbaryl and carbofuran among other organics from water [6].

NMC residues in soil and plant tissue, again as covered by McGarvey [3], are traditionally extracted by solvent with the aid of some physical means (i.e., sonication, shaking, homogenizing, Soxhlet process etc), followed by a cleanup procedure to remove interferences prior to the chromatographic analysis. The most commonly used solvents in multiresidue analysis include acetone, mixed acetone/dichloromethane/petroleum ether, acetonitrile, dichloromethane, ethyl acetate and methanol. The choice of cleanup technique closely depends on how the extraction was done; in other words, whatever else enters the extract determines the purification procedure. Liquid–liquid partition (LLP) followed by column chromatography or SPE has been the most common cleanup procedure in NMC analysis. Dichloromethane, petroleum ether, hexane and acetonitrile are frequently used in LLP. Florisil, Celite-Nuchar, silica gel and alumina have been used in column chromatography to remove matrix interferences from samples. SPE cartridges frequently used in NMCs analysis include silica, C_{18} and aminopropyl-bonded silica. On-line cleanup using multi-dimensional HPLC with or without LLP pretreatment has been applied to clean up organic extracts of fruits and vegetables. The first column can provide selectivity needed for removing interferences and make separation of the analytes possible on a second column of different type.

Most methods recover acceptable levels of NMCs, but some of the highly polar ones (e.g., sulfoxides) can present problems. De Kok and Hiemstra studied the recovery of 25 parent NMCs and metabolites on 12 different food product types [7]. The recoveries of polar metabolites (e.g., butocarboxim sulfoxide and aldicarb sulfoxide) could be as low as 20–30% in most of the samples. By monitoring the recovery step by step, it was found that sample loss mainly occurred during liquid–liquid extraction due to the unfavorable partition coefficient of polar compounds in the organic layer. For example, the recoveries of butocarboxim sulfoxide and aldicarb sulfoxide were 19% and 29% in LLP, compared to 90% and 95% in SPE. The recoveries of these two sulfoxides in grain samples (e.g., rice) were above 90%, where the low water content enabled the elimination of LLP step.

2.2. Solid-phase extraction

Over the last 3 years, SPE cartridges gradually replaced traditional column chromatography for sample cleanup and concentration in the analysis of NMCs and metabolites. Here are some applications of SPE using different cartridges. SPE with a C_{18} cartridge was used for pretreatment of water or wine samples prior to chromatographic analysis by HPLC [8], TLC [9], or GC [10]. A Sep-Pak aminopropyl cartridge was used to clean up the organic extracts of grains, fruits, and vegetables for the determination of NMC residues [11]. Analytes were eluted from that cartridge with 1% methanol in dichloromethane, followed by solvent exchange to methanol containing diluted HCl solution and finally analyzed by HPLC. Recoveries of NMCs and their metabolites from these plants ranged from 60–103% at a 20 ppb level. A graphitized carbon black (GCB) extraction cartridge was compared to a C_{18} SPE cartridge and to liquid-liquid extraction for the extraction of polar NMCs and metabolites (e.g., Aldicarb sulfoxide, butocarboxim sulfoxide) from water [12]. Back-

flushing was found to be beneficial in reducing the volume of elution solvent. Analytes were spiked at 1–4 μg/liter levels in 2 l of drinking water, preconcentrated on a 1 g GCB cartridge, eluted by back flushing with minimum amount of solvent and followed by LC analysis. The recoveries of NMCs in drinking water were above 90%, with the exception of 72% for aldicarb sulfone. When ground water or river water was tested, the sample volume was reduced to 0.5–1.0 l to avoid sample loss due to breakthrough. The detection limits of polar NMCs in drinking water were approximately 13–85 ng/l [12].

SPE is a procedure which can be easily automated [13–16]. Two automated trace enrichment devices, OSP-2 and Prospekt, were evaluated with different exchangeable SPE cartridges for their performance in the concentration and cleanup of NMCs in aqueous samples [17]. Quantitative results of NMCs and metabolites were obtained at 0.1 μg/l level with standard deviations in the 2–10% range. Detection limits were between 30–50 ng/l for surface water. The use of an immunoaffinity column for on-line enrichment of carbofuran in food stuffs was reported [18]. The antibodies in the column specifically bind only carbofuran from a complex matrix. When coupled with LC–MS, the detection limit for carbofuran in potato could reach approximately 2.5 ng/g.

An alternative mode of SPE is the use of the "SPE disks". Chiron and Barcelo [13] reported the use of C_{18} Empore extraction disks (4.6 mm) in a preconcentration system coupled with HPLC–UV or HPLC with post-column derivatization and fluorescence detection. Extraction efficiency was dependent upon the preconcentrated volume and the polarity of individual analytes. For polar compounds (e.g., aldicarb sulfoxide, aldicarb sulfone and 3-hydroxy-7-phenolcarbofuran), the breakthrough volume at a flow-rate of 2 ml/min was 3–5 ml. Water volume of 250–400 ml was needed to achieve a limit of detection (LOD) of 0.01–0.03 μg/l in HPLC–UV analysis. A large sample volume injected on the SPE disk caused breakthrough and poor recoveries for polar analytes. With post-column derivatization and fluorescence detection, water volume was reduced to 10 ml for similar LOD. These authors also compared on-line solid-phase disk extraction with liquid–liquid extraction using dichloromethane [19]. A C_8 SPE disk was found to be superior to SPE cartridges and to liquid–liquid extraction for the extraction of carbofuran and other pesticides from soil samples [20]. C_{18} Empore SPE disks were used in a screening method for monitoring carbofuran and other pesticides in river, lake and seawater [21]. Recoveries depended on the sample matrix, i.e., sample loss was more severe in river water and marine water than in distilled or ground water.

Improving the extraction efficiency of SPE is also of interest in the residue analysis of NMCs. Parameters for SPE extraction of NMCs and other pesticides from water were optimized based on orthogonal array design [22]. Efficiency of extracting carbamates from water using a C_8 SPE cartridge was improved by dissolving humic material and NaCl in the sample solution [23].

Walker et al. [24], after reviewing several techniques, recommended matrix solid-phase dispersion (MSPD) extraction as an effective way of reducing labor and solvent in the analysis of contaminants in aquatic species. A typical MSPD procedure entailed the blending of a small sample (0.1–1.0 g) with solid adsorbent (e.g., C_{18}-modified silica), followed by elution using various solvents. In a separate report, such procedure was used for the determination of trace carbofuran in corn followed by HPLC analysis [25].

2.3. Supercritical fluid extraction

Increasingly supercritical fluid extraction (SFE) is being used in pesticide analysis. The unique nature of SFE—low viscosity of the solvent, high diffusion coefficient of the analytes and its overall environmental friendliness have been thoroughly discussed in several papers [26–28]. Successful SFE extraction depends on the optimization of experimental conditions (e.g., pressure, temperature, type of supercritical fluid and modifiers), the chemical and physical nature of the analytes and the matrix, as well as the moisture content of the sample. The primary applications of SFE in NMC analysis are with aqueous samples or samples with high moisture content (e.g., wet soil, fruit, vegetable). Mild heating of the restrictor and solvent trap can be used to

overcome the common problem of ice formation and restrictor plugging in high moisture situations [28].

Lehotay et al. recently reported a study on the experimental considerations in SFE extraction of pesticide from highly moist samples like fruits and vegetables [29]. Measures taken to obtain high recovery and reproducibility included (i); mixing samples with Hydromatrix to control water content, (ii); purging the extraction vessel with carbon dioxide to remove oxygen and (iii); freezing the sample prior to extraction. About 40 pesticides including carbaryl and carbofuran were extracted from potato, orange and peach samples with 90–105% recoveries and 1–6% standard deviation.

An alternative is to adsorb the sample in a solid, porous matrix prior to SFE [30]. That enables extension to water samples or plant tissue samples. The same approach was applied to extract thiocarbamates (i.e., methiocarb and methomyl) from apples [31]. The recoveries of thiocarbamates were increased by adsorbing the aqueous apple extract on diatomaceous earth prior to SFE. Lehotay and Eller [32] extracted carbaryl, carbofuran and other pesticides from plant tissue by SFE at 320 atm (1 atm=101 325 Pa) and 60°C, trapped them on octadecylsilane-modified silica gel, eluted them by acetonitrile, and then analyzed by GC with ion trap mass spectrometry. Recoveries from grapes, carrots, potatoes and broccoli mostly exceeded 80%. A combination of SPE and SFE was also reported [33]. Pesticides including aldicarb in waste water were enriched on a polymeric column, followed by fractional elution using supercritical carbon dioxide at various pressures.

Combined with other extraction methods, SFE becomes an even more versatile technique. Thus, SFE pretreatment removed fat and fiber from a meat sample and largely reduced coextractives in the subsequent acetonitrile extraction for chromatographic analysis [34]. SFE using carbon dioxide was also combined with enzyme immunoassays (EIA) for rapid detection of aldicarb, carbofuran and other pesticides in soil samples [35]. No sample cleanup was required for the extract. Recovery from SFE of soil samples mixed with 10% humic acid was comparable with conventional Soxhlet extraction, and with the combined EIA higher sensitivity and selectivity were achieved. In another study, SFE and EIA were used for pesticide detection in meat samples [36]. Carbofuran and other analytes were extracted from meat (ground beef, bovine liver and lard) by supercritical carbon dioxide. However, interference from co-extractive was observed necessitating a cleanup step.

Other than carbon dioxide, SFE with supercritical methanol has the ability to remove from the matrix bound or otherwise unextractable pesticides. These bound chemicals traditionally were determined by the measurement of ^{14}C-bound residues using various techniques such as combustion, pyrolysis or high temperature distillation (HTD). Those techniques were plagued with difficulties including the loss of information on chemical form, low yield of analytes, or thermal decomposition [37]. At 150 bar and 250°C for 2 h, superior results of 95% recovery of the bound ^{14}C-labelled carbofuran were obtained, as compared to 66% by HTD. Side reactions between the pesticide molecules and methanol under the condition of supercritical extraction (150 bar, 250°C) were observed. The conversion of 3-ketocarbofuran to its methoxyl derivatives was used as an example to explain its absence in radish samples.

3. High-performance liquid chromatography

3.1. HPLC–spectroscopic detection

In general, HPLC analyses of NMCs were carried out under reversed-phase conditions using octadecyl or octyl-silica columns and mixtures of water and organic solvents as the mobile phase. If LC with UV detection was applied to the analysis of NMCs in water, then analytes usually were preconcentrated by an on-line or off-line enrichment device using a C_{18} SPE disk [13], or a C_{18} [38] or C_8 [23] cartridge. Sample volumes for preconcentration ranged from 50–1000 ml. With fluorescence detection, only 10 ml of aqueous samples was necessary. Diode array detection was used for the determination of aldicarb and its metabolites in soil and potato after SPE cleanup [39]. Detection limits for each of the analytes were 40 ppb in soil and 15 ppb in potato. When combined with supercritical fluid extraction LC–UV

was found to give better quantitation for methiocarb and methomyl in apples than GC with flame ionization detection or micro-HPLC with sulfur chemiluminescence detection [31].

Post-column hydrolysis and derivatization of NMCs coupled with fluorescence detection works for samples with complex matrices such as plants [11,40,41], soil [42] and meat [43,44,34], as well as for water [13,17,45]. Post column reactions were first reported by Moye et al. [46], wherein NMCs were hydrolyzed by NaOH to form methylamine, then derivatized by *o*-phthalaldehyde in the presence of 2-mercaptoethanol. Modifications since then included solid-phase reactor, UV photolytic reactors, single stage reaction, etc [3]. Simon et al. [45] described a reagent consisting of NaOH, *o*-phthalaldehyde and N,N-dimethyl-2-mercaptoethylamine hydrochloride (Thiofluor) for a single stage post-column reaction. With the use of Thiofluor, the derivatized samples were found to be more stable than with other reagents (beta-mercaptoethanol, 3-mercaptopropionic acid). In a screening method developed by Yang and Smetena [40] for the determination of aldicarb residues in tobacco using HPLC with post-column derivatization and fluorescence detection, triethanolamine was added to the mobile phase to reduce the interaction from silanols on the silica surface. Under those chromatographic conditions, the major interferences (i.e, primary amino acids and other amines) were eluted as early peaks and were separated from the aldicarb residues. As a result, it was possible to analyze the methanol extract of tobacco for aldicarb, aldicarb sulfoxide and aldicarb sulfone without sample cleanup other than simple filtration.

Selective detection methods, other than by UV or fluorescence, were also pursued. Howard et al. [47,48] reported a procedure for the determination of thiocarbamates (e.g., methiocarb and methomyl) using microcolumn HPLC with flame-based sulfur chemiluminescence detection (SCD). Apple samples spiked with thiocarbamates were extracted by SFE with carbon dioxide containing 2% methanol. Sensitivity of thiocarbamates obtained from micro-HPLC–SCD was affected by the mobile phase composition and the sulfur content of the analytes. With a mobile phase of methanol–water (40:60, v/v), limit of detection could reach sub-picogram levels based on the mass of sulfur element in the analytes.

3.2. HPLC–mass spectrometric detection

Although HPLC with fluorescence or UV detection is widely accepted for the residual analysis of NMCs and metabolites, the need for compound identification cannot be over emphasized. This is particularly necessary for soil and agriculture products in which peak overlapping or unresolved peaks originating from matrix interferences exist. Although the high resolution power and identification capability of capillary GC and GC–MS might provide a solution, it is difficult to analyze polar, thermally labile NMCs under GC conditions without improving their volatility and stability through derivatization or other techniques. The addition of chemical derivatization in the sample pretreatment complicates the analysis and increases the risk of sample loss. Coupling identification capacity onto the HPLC is the logical improvement.

Different interfaces such as particle beam (PB), thermospray (TS), atmospheric pressure ionization (API) and electrospray (ES) have been used for the application of LC–MS in NMC analysis [49]. PB can provide structural information by electron ionization at ng levels. The integrated ion abundance is sufficiently stable for mass spectrum comparison, as well as for quantitative measurement. TS provides soft ionization with little fragmentation but can achieve sensitivity at nanogram levels for full scan or sub-nanogram levels for SIM mode [50]. Nitrogen-containing compounds tend to generate strong thermal spray signal, which is beneficial for NMCs analysis [51]. Volatile organic salts (e.g., ammonium acetate) were often added as the mobile phase modifier to enhance ionization. ES is also a soft ionization technique, but full scan spectra with structural information at pg levels can be obtained through its collisional activation dissociation.

Slobodnik recently reviewed the operating principles, detection limits and mass spectra characteristics of various LC–MS interfaces [52]. The applications of each interface on the identification and quantitation of polar, thermally labile pesticides were emphasized. Another review [53] focused on the determination of carbofuran using LC–MS, and concluded

that the main difficulties encountered in particle beam and thermospray interfaces for carbofuran quantification were caused by the ion source pressure and temperature. In the recently developed atmospheric pressure ionization interfaces, the problems associated with pressure and temperature of ion source were reduced.

Optimization of operational parameters for thermospray and particle beam interfaces is ongoing. Honing et al. [54] studied the effect of chromatographic eluant additives including ammonium formate, ammonium acetate and nicotinic acid, on the ion formation of 19 carbamates and 12 metabolites using a thermospray interface. With these additives, fragmentation was suppressed for some carbamates, but the formation of adduct ions with nicotinic acids or ammonium was enhanced in most of the cases. Quasimolecular ion formation was reduced by nicotinic acid but was increased by the ammonium salts. Some NMCs or metabolites (e.g., methiocarb and its sulfone) suffered thermal degradation at the 90°C temperature in the thermospray interface. Vreeken and co-workers [55] studied the effect of various mobile phase additives on the sensitivity and selectivity of polar pesticides including aldicarb, oxamyl and their degradation products. Triethylammonium formate and tripropylammonium formate possessed higher proton affinity (e.g., the ability of enhancing deprotonation of analytes) than ammonium formate and thus provided better sensitivity and selectivity in the negative ion mode. Volmer et al. [14,56] evaluated the suitability of LC–TS-MS, with and without the addition of volatile salt in the LC mobile phase, for the identification of polar pesticides, including several NMCs, in aqueous samples. Samples were preconcentrated with SPE followed by HPLC analysis. When operated under solvent-mediated chemical ionization without adding ammonium acetate, intense solvent cluster ions derived from methanol in the carrier stream provided additional structural information of the analytes Detection limits at low ng/l range were obtained. Chiron and co-workers [15] applied LC–TS-MS under time-scheduled selected-ion monitoring mode for trace analysis of NMCs and other pesticides in river and ground water. Two selected ions were used for analytes confirmation, $(M+H)^+$ and $(M+NH_4)^+$ or $(M+CH_3CN)^+$ for the positive ion mode, $(M-H)^-$ and $(M+HCOO)^-$ for the negative ion mode. Limits of detection of analytes ranging from 0.01 to 0.4 μg/l were achieved by preconcentrating 100 ml of aqueous samples prior to LC–MS analysis.

The capability of LC–PB-MS for generating classical electron ionization spectra is an obvious advantage, and thus promotes interests of extending its applications to the confirmation of NMC residues. A low voltage (180 V) dc glow discharge device was placed under the pneumatic nebulizer of the PB interface and signal intensity was increased 2–6 fold for several test compounds including carbaryl [57]. A mechanism of improved solute transport efficiency through the interface was proposed by the authors as a possible explanation for the increased signal. However, combining glow discharge with another signal improving technique, ammonium acetate in the mobile phase, did not further increase signal intensity. Quadrupole ion trap mass spectrometry (QITMS) was used in LC–PB-MC analysis of NMCs and other pesticides [58]. Both vaporization and electron ionization occurred in the ion trap. Space charging and chemical ionization originating from residual solvent ions were observed and were overcome by rejecting solvent ions before mass analysis. LC–PB-QITMS demonstrated lower limits of detection than quadrupole mass analyzers and the mass spectra of NMCs obtained are comparable to library EI spectra. Microliter flow-rate particle beam interface was used for the microcolumn HPLC–MS analysis of pesticides including carbaryl, aldicarb and carbofuran [59,60]. The reduced flow-rate, ranging from 1–5 μl/min, improved the signal response.

Atmospheric pressure ionization interfaces are gaining in popularity in LC–MS analysis. Mass spectra of NMCs with protonated molecular ions and fragments were obtained using green pepper samples spiked with NMCs at 0.1 ppm level [61]. The spectra of NMCs obtained from API were comparable with those from GC–MS. Kawasaki et al. [62] used HPLC with atmospheric pressure chemical ionization mass spectrometry for the analysis of 8 methylcarbamates. The addition of ammonium acetate to the mobile phase was tested, but no significant change in the sensitivity was observed. However, high specificity for NMC confirmation was achieved from high intensity of $(M+H)^+$ pseudo-molecular ions. Newcome et al. [63] compared two different de-

tection methods for the HPLC analysis of NMCs in food, namely, atmospheric pressure chemical ionization/mass spectrometry versus post-column derivatization coupled with fluorescence detection. Both detection methods gave similar recoveries at 25 ppb level, but neither could avoid false positive response on a blank sample. Therefore, neither detection method alone can provide definitive confirmation of NMCs in food samples. Electrospray coupled with an ion trap spectrometer was used for the confirmation of aldicarb sulfone and other nonvolatile contaminants in water [64]. Structural information including $(M+H)^+$ ions and collision-induced decomposition spectra were produced. High sample purity was required for collisional activation in the electrospray transport region to avoid interference ions. With extensive instrument setup, MS–MS spectra were also obtainable.

4. Gas chromatography

GC with nitrogen- or sulfur-specific detection [65–67] or MS detection [21,34,68] has been routinely used in the analysis of NMCs. However, the application of gas chromatography to the analysis of polar thermally labile compounds like NMCs requires special precautions to ensure recovery and reproducibility, e.g., modifying GC conditions to reduce thermal decomposition and/or precolumn derivatization to improve volatility.

Cold on-column injection marks many NMC analyses as it tends to reduce thermal degradation in the injection port. Matten et al. [69] analyzed carbaryl, carbofuran and other pesticides in plant tissues using capillary GC with cold on-column injection and a chemical ionization ion trap detector. The use of electronic pressure programming (EPP) at the GC inlet enabled rapid sweeping of the injected sample into the column to reduce thermal decomposition on the hot, active inlet surface [70]. Inlet pressure was temporally increased during injection and was subsequently reduced to normal operating range for GC analysis with capillary columns. By using EPP with splitless injection, the thermal decomposition of carbaryl at the GC inlet declined from 16.8 to 9.9%.

Suzuki and co-workers [71] reported the use of a large volume injection technique in GC analysis. Propoxur and other pesticides were determined in water. An aliquot of 25–150 μl of hexane extract of the water samples was injected in a splitless mode into a cold-trap column, which was simultaneously connected to a 30 m analytical column and a 2 m solvent diversion column. After the trapped hexane was eluted out of the oven through the diverted column, the trapped pesticides were introduced into the analytical column.

Trey et al. [72] designed a chemical ionization GC–MS based procedure for the analysis of thermolabile compounds by combining on-column injection with the use of a short, thin-film bonded phase GC column (J&W DB-5 column, 1–2 m×0.25 mm I.D., 0.1 μm film). Injection port temperature and the GC–MS interface temperature were set at 130 and 135°C, respectively. GC column temperature was programmed from 40–100°C. Rapid chromatographic analysis decreased resolution, but that was partially offset by the added selectivity from MS or MS–MS. With a short column, concentration of the analyte reaching the mass spectrometer is higher, hence the enhancement of the mass sensitivity. Aldicarb, aldicarb sulfoxide and aldicarb sulfone were successfully chromatographed, and the mass spectrum of each compound was obtained. Rapid GC analysis using a short, narrow bore capillary column followed by mass spectrometric detection later was applied to screening several NMCs and other pesticides in a variety of crops [73].

Other element-selective detection methods such as atomic emission detection (AED) also have been used [74,75]. GC with AED was compared to GC–nitrogen–phosphorous detection and GC–electron-capture detection for the analysis of pesticides including NMCs from plant foodstuffs using Deutsche Forschungsgemeinschaft multiresidue method S19. GC–AED was found to be more selective and suitable for monitoring pesticide residue, in foodstuffs. Its wide linear dynamic range also makes GC–AED more reliable for quantitative measurement.

Derivatization of NMCs for GC analysis has been reviewed [76,77]. Recently, Ballesteros et al. developed an on-line extraction-derivatization procedure for the determination of six NMCs in milk [77]. Samples were submitted to liquid–liquid extraction

and evaporation, reconstituted with a mixture of acetonitrile and diluted NaOH and then injected into a continuous liquid–liquid extraction/derivatization module. NMCs were hydrolyzed and derivatized with pentafluoropropionic anhydride during this process. The fluoro derivatives of NMCs were analyzed by GC with electron-capture detection. The recoveries ranged from 93.7–100.8% for the six NMCs covered, benthiocarb, propoxur, carbofuran aminocarb, carbaryl and methiocarb. Detection limits of NMCs were from 2–20 ng/ml. These authors also used a similar protocol for the determination of the same group of NMCs in aqueous samples [78]. NMCs were hydrolyzed to the corresponding phenols, extracted with or without ethyl acetate derivatization by a continuous liquid–liquid extractor, and analyzed by GC with flame ionization detection. Derivatized NMCs showed higher thermal stability and were well separated by GC with better sensitivity.

Another on-line derivatization technique using trimethylsulfonium hydroxide (TMSH) was developed for the determination of NMCs in water [79]. The sample was preconcentrated with a C_{18} SPE cartridge and a mixture of the SPE eluate and TMSH was injected into a programmed temperature vaporizer at elevated temperatures, where the reaction took place. NMCs were hydrolyzed to phenolates first then methylated with TMSH at 250°C, and the derivatives were subsequently analyzed by GC–MS. Some of the NMCs did not react under these conditions and could not be quantitated. The detection limits for the derivatized NMCs were in the range from 25–50 ng/ml in water depending on the analytes. Benzyl derivatization also was used for the determination of 12 carbamates in 309 agriculture products [80]. A methanol extract was purified by precipitation with zinc sulfate and sodium tetraborate, and analyzed using GC–MS after benzyl derivatization.

5. Other chromatographic techniques

5.1. Supercritical fluid chromatography

Supercritical fluid chromatography (SFC) coupled with MS detection provides an alternate tool for the determination of thermally labile compounds like NMCs. Murugaverl and co-workers [81] designed a capillary SFC–MS unit using a benchtop mass spectrometer and a modified GC–MS interface. The temperature of the probe tip and ion source was adjusted to 290°C to overcome the sensitivity problems for solid analytes like aldicarb benthiocarb, bendiocarb and carbaryl. A capillary frit restrictor replaced the integral restrictor to prevent clogging. Electron ionization mass spectra, comparable with reference EI spectra, were obtained for NMCs in the nanogram range. Limits of detection for these analytes at picogram levels was achievable. Jedrzejewski and Taylor [82] reported the use of a particle beam interface in the packed column SFC–MS analysis for NMCs and other analytes. Supercritical carbon dioxide with or without methanol was used as the mobile phase. Electron impact spectra comparable to online library spectra were obtained.

5.2. Thin-layer chromatography

Because of its low cost and simplicity, TLC is frequently used for the determination of NMCs, mainly carbaryl and carbofuran. Recent developments are described below.

Chromatographic behavior of NMCs (e.g., carbaryl, propoxur, carbofuran) on different TLC plates and various solvent systems was examined [83]. Several new TLC spraying reagents were introduced for the selective detection of carbaryl in variety of matrices, e.g., phenylhydrazine hydrochloride [84], 6-amino-l-naphthol-3 sulfonic acid [85] and ammonium cerium(IV) nitrate [86]. A liquid crystal, 4-(*trans*-4′-*n*-hexlcyclohexyl)benzene isothiocyanate, was also evaluated for quantitative detection of carbaryl and other pesticides [87]. Carbofuran and its environmental byproducts, hydroxycarbofuran and 3-ketocarbofuran, were analyzed using high-performance TLC with F_{254} indicator [88]. Applications in simultaneous determination of several NMCs were also reported. NMCs including carbaryl, aldicarb, oxamyl, butocarboxim and butoxycarboxim as well as several other pesticides in drinking water were detected at picogram levels by coupling TLC with an enzymic inhibition test designed for cholinesterase-inhibiting insecticides [89]. In another report, several NMCs in water were preconcentrated by C_{18} SPE

cartridge, separated on a silica gel TLC plate, and then reacted with *p*-nitrobenzenediazonium fluoroborate prior to quantitation by densitometric scanning [9]. Recoveries of 96.8% at 0.5 to 5 ppm levels were obtained for carbaryl, carbofuran, methiocarb and propoxur.

5.3. Micellar electrokinetic capillary chromatography

Micellar electrokinetic capillary chromatography (MECC) involves the use of a micellar phase, formed by a surfactant above its critical micellar concentration, in the capillary electrophoresis for neutral analytes. Sodium dodecyl sulfate (SDS) is frequently used to form micelle for MECC analysis. Carbofuran, propoxur and 7 other pesticides were separated by MECC using a borate buffer solution (pH=8) containing SDS. Detection limits for the test analytes ranged up to 0.1 mg/ml [90].

6. Non-chromatographic techniques

6.1. Immunoassays

Enzyme-linked immunosorbent assay (ELISA) provides reactions specific to a compound or a group of compounds, which could reduce matrix effect and the need for sample cleanup and concentration. A number of ELISA kits are available for NMC analysis, mainly for aldicarb, carbaryl, carbofuran and their metabolites.

A commercial ELISA kit was used for the determination of carbofuran and aldicarb sulfone in meat and liver, and the direct assay of aldicarb sulfone in bovine milk, blood and urine [91]. A patented immunoassay [92] for total aldicarb in aqueous samples claimed oxidizing aldicarb residues to aldicarb sulfone, which is then measured by immunoassay using aldicarb-horseradish peroxidase conjugate and monoclonal antibodies. A paramagnetic particle-based ELISA, where antibodies are attached to the paramagnetic particles, was used for rapid quantitation of carbaryl in water without sample preparation [93]. A magnetic particle-based ELISA was used for the determination of carbofuran with a detection limit of 0.056 ppb in water and 5.6 ppb in soil [94]. Magnetic particle-based and microtiter plate ELISA were compared for their performance in the determination of carbaryl and 1-naphthol in ground water [95]. The results obtained from ELISA kits were comparable to those from EPA method 531.1, HPLC analysis with post-column derivatization and fluorescence detection. A magnetic bead-based enzyme immunoassay was used for the determination of carbofuran and other pesticides in meat after the sample was pretreated with supercritical fluid extraction [36]. A combination of SFE and enzyme immunoassay for the analysis of aldicarb, carbofuran and other analytes in soil samples also was reported [35].

6.2. Biosensors

Most biosensors are based on the monitoring of acetylcholinesterase activity and its inhibition by one or a group of analytes (e.g., pesticides). A number of applications were reported using biosensors with assorted base materials configurations, substrates and means of measurement. Of late, amperometric acetylcholinesterase-based biosensors have become common for the determination of some NMCs (e.g., carbaryl, carbofuran) in various matrices. In this type of biosensor, a compound formed in the enzymic hydrolysis of the substrate (e.g., 4-aminophenol produced from the hydrolysis of 4-aminophenylacetate) is oxidised on the electrode surface to give a steady-state current. When the activity of acetylcholinesterase (enzyme) is inhibited in the presence of pesticides, the amount of hydrolysis product is decreased and is reflected as a change in the current flow.

Recent applications of amperometric acetylcholinesterase-based biosensors included the use of several different substrates. Thiobutyrylcholine was used as the substrate with a phthalocyanine-modified graphite composite electrode covered with a layer of cholinesterase for the determination of carbaryl and other insecticides [96]. Thiocholine was formed by the reaction between the substrate and acetylcholinesterase and was monitored as an indication of enzyme inhibition. A similar type of thiocholine sensor was used for the determination of aldicarb, carbofuran and other pesticides [97]. 4-Aminophenyl acetate was also used in amperometric acetylcho-

linesterase-based biosensors for the determination of carbaryl in water [98,99]. The detection limit for carbaryl in water using this biosensor reached 13 nmol/l with a preincubation time of 3 min [98]. No interference was observed from other electroactive species, e.g., ascorbic or uric acids [99]. The main interferences were caused by compounds like bovine serum albumin, which strongly adsorb on the electrode surface and reduce the enzyme activity.

In a different configuration, enzyme acetylcholinesterase was mixed with epoxy-graphite in a biocomposite and was used as the base material for an amperometric transducer [100]. Acetylthiocholine was used as the substrate and its hydrolysis produced thiocholine. In a light addressable potentiometric sensor (LAPS), the acetylcholinesterase was immobilized on a biotinylated nitrocellulose membrane [101]. When the enzyme was inhibited by the NMCs in samples flowing through the membrane, the hydrolysis of acetylcholine and the potentiometric signal were reduced. LAPS provided fast sample throughput by allowing 8 samples to be assayed simultaneously. Detection limits were at 10 n*M* for bendicarb, but were not as good for aldicarb or methomyl.

Enzyme inhibition can also be monitored by pH measurement, when the pH value changes as a function of enzyme activity; i.e., as the amount of acetic acid produced [102]. Enzymes such as butyrylcholinesterase or acetylcholinesterase were immobilised on a solid substrate (e.g., a nylon net or a membrane). The inhibition of enzyme activity by carbofuran or carbaryl in the sample was reflected in decreasing acetic acid produced, and was determined by pH measurement using a pH glass electrode. In a flow-injection analysis, the enzyme was immobilized in a single bead string reactor coupled with a pH electrode and a wall-jet entry. pH change was measured by injecting acetylcholine (the substrate) before and after the test sample flows through. Detection limits at ppb levels were observed for carbaryl and carbofuran.

Another type of biosensor involved the use of a chemiluminescence flow technique [103]. Acetylcholinesterase with choline oxidase and peroxidase were immobilized on methacrylate beads. Choline, produced by acetylcholinesterase, reacted with choline oxidase and the H_2O_2 generated was measured via the luminol–peroxidase luminescent reaction. The detection limits for aldicarb reached 4 μg/l.

6.3. Spectrophotometry

Since there is no separation process in the spectrophotometric analysis, the specificity of a color reaction is often a key factor for the quantitation of the target analyte. Most recent applications of spectrophotometric measurement were for the analysis of carbaryl and were carried out with various sample pretreatments, reactions with different coupling reagents and measurements at different wavelengths. In water samples, carbaryl was hydrolyzed to 1-naphthol, fixed on a solid adsorbent and measured by spectrofluorimetry with a solid-surface attachment [104]. In another case, carbaryl was preconcentrated with SPE and measured spectrophotometrically after elution and solvent exchange [105]. Total carbaryl in samples can be determined by extracting carbaryl and its hydrolysis product, 1-naphthol, in a NaOH solution as 1-naphtholate, and measured at 596 nm after reacting with *p*-aminophenol [106]. The detection limit was 26.5 ng/ml for carbaryl with a sample frequency of 110 injections/h. Micelle-stabilized room-temperature phosphorimetry combined with a stopped-flow mixing technique was used to measure the phosphorescence of carbaryl in micelles of SDS [107]. No solid substrate was needed and the results of phosphorescence measurement were obtained in seconds with a detection limit in the range of 10–14 ng/ml. In another report, micelle-stabilized room temperature phosphorescence with Na_2SO_3 as an oxygen scavenger was used for the determination of carbaryl in waste water [108]. The detection limit for carbaryl was 0.2 μM. Fourier transform infrared (FT-IR) was also used for the determination of carbaryl in water [109]. Water samples were preconcentrated on a SPE cartridge, then eluted on-line for FT-IR measurement at 1746 cm^{-1}. The detection limit was approximately 200 μg/l for carbaryl. For soil samples, carbaryl was coupled with diazotized 2-aminonaphthalenesulfonic acid, extracted with *n*-butanol, then measured at 490 nm [110]. Carbaryl in air was collected, hydrolyzed, coupled with diazotized *p*-aminoacetophenone and measured at 580 nm [111].

Propoxur in grain, vegetable and water was hydrolyzed to *o*-propoxyphenol first, then coupled with *p*-aminobenzoic acid [112] or 4-aminoantipyrine [113] followed by spectrophotometric measurement. Ethiofencarb in water was hydrolyzed to phenol sulfone, reacted with *p*-aminophenol and measured at 638 nm in an alkaline solution [114]. Bendicarb in grain and water was hydrolyzed to its phenol, coupled with diazotized *p*-aminobenzenesulfonamide and measured at 448 nm [115].

6.4. Electrochemistry

An indirect electrochemical method was used for the determination of carbaryl in water and soil [116]. Carbaryl was oxidized to 1,4-naphthoquinone, which was then reduced at a dropping mercury electrode and measured by differential pulse polarography or by adsorptive stripping voltammetry. The detection limit for carbaryl was approximately 5 ppm. A differential pulse voltammetric method was used for the simultaneous determination of carbaryl and carbofuran in river water [117]. NMCs were hydrolyzed to their phenols prior to the electrochemical measurement. The overlapped peaks of carbaryl and carbofuran were resolved using a partial least squares calibration.

7. Conclusion

Determination of NMCs in various sample matrices continued to be the interest of fundamental research and applications. Newer analytical tools, including SPE cartridge or disk, SFE, immunoaffinity columns, etc, were increasingly used for sample pretreatment in NMC analysis. The application of SFE is particularly helpful to the effort to reduce waste chemicals. Recent development in interfaces for HPLC–MS make significant improvements in compound identification, as well as selectivity and sensitivity, of polar thermally labile NMC pesticides. In addition to HPLC, GC and TLC, non-chromatographic methods (e.g., immunoassay, biosensors, etc.) provide a different approach for the residue analysis of NMCs with less sophisticated instrumentation.

References

[1] The Pesticide Manual, British Crop Protection Council and the Royal Society of Chemistry, 10th ed., 1994.
[2] J.R. Corbett, K. Wright and A.C. Baillie, The Biochemical Mode of Action of Pesticides, Academic Press, New York, 2nd ed., 1984, pp. 128–131.
[3] B.D. McGarvey, J. Chromatogr., 642 (1993) 89.
[4] B.D. McGarvey, J. Chromatogr. B, 659 (1994) 243.
[5] G. Font, J. Manes, J.C. Molto and Y. Pico, J. Chromatogr., 642 (1993) 135.
[6] G.A. Junk and J.J. Richard, Anal. Chem., 60 (1988) 451.
[7] A. de Kok and M. Hiemstra, J. AOAC Int., 75 (1992) 1063.
[8] P. Parrilla, J.L. Martinez Vidal, G. Martinez and A.G. Frenich, Fresenius J. Anal. Chem., 350 (1994) 633.
[9] S.C. McGinnis and J. Sherma, J. Liq. Chromatogr., 17 (1994) 151.
[10] P.T. Holland, D.E. McNaughton and C.P. Malcolm, J. AOAC Int., 77 (1994) 79.
[11] Y. Tsumura, K. Ujita, Y. Nakamura, Y. Tonogai and Y. Ito, J. Food Prot., 571 (1994) 1001.
[12] A. Di Corcia, R. Samperi, A. Marcomini and S. Stelluto, Anal. Chem., 65 (1993) 907.
[13] S. Chiron and D. Barcelo, J. Chromatogr., 645 (1993) 125.
[14] D. Volmer, K. Levsen and W. Engewald, Vom Wasser, 82 (1994) 335.
[15] S. Chiron, S. Dupas, P. Scribe and D. Barcelo, J. Chromatogr. A, 665 (1994) 295.
[16] V. Pichon and M.C. Hennion, J. Chromatogr. A, 665 (1994) 269.
[17] M. Hiemstra and A. de Kok, J. Chromatogr. A, 667 (1994) 155.
[18] G.S. Rule, A.V. Mordehai and J. Henion, Anal. Chem., 66 (1994) 230.
[19] S. Chiron, A. Fernandez and D. Barcelo, Environ. Sci. Technol., 27 (1993) 2352.
[20] M.J. Redondo, M.J. Ruiz, R. Boluda and G. Font, Chromatographia, 36 (1993) 187.
[21] T.A. Albanis and D.G. Hela, J. Chromatogr. A, 707 (1995) 283.
[22] H.B. Wan, W.G. Lan, M.K. Wong, C.Y. Mok and Y.H. Poh, J. Chromatogr. A, 677 (1994) 255.
[23] B. Jimenez, J.C. Molto and G. Font, Chromatographia, 41 (1995) 318.
[24] C.C. Walker, H.M. Lott and S.A. Barker, J. Chromatogr., 642 (1993) 225.
[25] R. Yang and C. Fu, Fenxi Ceshi Xuebao, 13 (1994) 72.
[26] V. Camel, A. Tambute and M. Caude, J. Chromatogr., 642 (1993) 263.
[27] V. Janda, K.D. Bartle and A.A. Clifford, J. Chromatogr., 642 (1993) 283.
[28] S.B. Hawthorne, J.D. Miller, M.D. Burford, J.L. Langenfeld, S. Eckert-Tilotta and P.K. Louie, J. Chromatogr., 642 (1993) 301.
[29] S.J. Lehotay, N. Anaronson, E. Pfeil and M.A. Ibrahim, J. AOAC Int., 78 (1995) 831.

[30] F.I. Onuska, J. High Resolut. Chromatogr. Chromatogr. Commun., 7 (1984) 660.
[31] A.L. Howard, C. Braue and L.T. Taylor, J. Chromatogr. Sci., 31 (1993) 323.
[32] S.J. Lehotay and K.I. Eller, J. AOAC Int., 78 (1995) 821.
[33] K. Wuchner and R. Grob, Analusis, 23 (1995) 227.
[34] R.J. Argauer, K.I. Eller, M.A. Ibrahim and R.T. Brown, J. Agri. Food Chem., 43 (1995) 2774.
[35] V. Lopez-Avila, C. Charan and W.F. Beckert, Trends Anal. Chem., 13 (1994) 118.
[36] K.S. Nam and J.W. King, J. Agri. Food Chem., 42 (1994) 1469.
[37] P. Capriel, A. Haisch and S.U. Khan, Anal. Chem., 34 (1988) 70.
[38] M.R. Driss, M.C. Hennion and M.L. Bouguerra, J. Chromatogr., 639 (1993) 352.
[39] J.I. Mora, M.A. Goicolea, R.J. Barrio and Z.G. De Balugera, J. Liq. Chromatogr., 18 (1995) 3243.
[40] S.S. Yang and I. Smetena, J. Chromatogr. A, 664 (1994) 289.
[41] Y. Tsumura, K. Ujita, Y. Nakamura, Y. Tonogai and Y. Ito, J. Food Prot., 58 (1995) 217.
[42] K.M.S. Sundaram and J. Curry, J. Chromatogr. A, 672 (1994) 117.
[43] M.S. Ali, J.D. White, R.S. Bakowski, E.T. Phillippo and R.L. Ellis, J. AOAC Int., 76 (1993) 1309.
[44] 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 Int., 76 (1993) 907.
[45] V.A. Simon, K.S. Pearson and A. Taylar, J. Chromatogr., 643 (1993) 317.
[46] H.A. Moye, S.J. Scherer and P.A. St. John, Anal. Lett., 10 (1977) 1049.
[47] A.L. Howard, C.L.B. Thomas and L.T. Taylor, Anal. Chem., 66 (1994) 1432.
[48] A.L. Howard, C. Braue and L.T. Taylor, Natl. Meet, Am. Chem. Soc. Div. Environ. Chem., 33 (1993) 316.
[49] R.D. Voyksner, Proc. Int Congr. Pestic. Chem., 7 (1991) 383.
[50] R. Eisert, K. Levsen and G. Wuensch, Int. J. Environ. Anal. Chem., 58 (1995) 103.
[51] T.A. Bellar and W.L. Budde, Anal. Chem., 60 (1988) 2076.
[52] J. Slobodnik, B.L.M. van Baar and U.A.T. Brinkman, J. Chromatogr. A, 703 (1995) 81.
[53] M. Honing, D. Barcelo, B.L.M. van Baar and U.A.T. Brinkman, Trends Anal. Chem., 14 (1995) 496.
[54] M. Honin D. Barcelo B.L.M. van Baar R.T. Ghijsen and U.A.T. Brinkman, J. Am. Soc. Mass Spectrom, 5 (1994) 913.
[55] R.J. Vreeken, W.D. van Dongen, R.T. Ghijsen and U.A.Th. Brinkman, Int. J. Environ. Anal. Chem., 54 (1994) 119.
[56] D. Volmer, K. Levsen and G. Wuensch, J. Chromatogr. A, 660 (1994) 231.
[57] T.A. Bellar, W.L. Budde and D.D. Kryak, J. Am. Soc. Mass Spectom., 5 (1994) 908.
[58] B.L. Kleintop, D.M. Eades and R.A. Yost, Anal. Chem., 65 (1993) 1295.
[59] A. Cappiello, G. Famiglini and F. Bruner. Anal. Chem. 66 (1994) 1416.
[60] A. Berloni, A. Cappiello, G. Famiglini and P. Palma, Chromatographia, 39 (1994) 279
[61] S. Pleasance, J.F. Anacleto, M.R. Bailey and D.H. Narth, J. Am. Soc. Mass Spectrom., 3 (1992) 378.
[62] S. Kawasaki, F. Nagumo, H. Ueda, Y. Tajima, M. Sano and J. Tadano, J. Chromatogr., 620 (1993) 61
[63] W.H. Newcome, B.P.-Y. Lau, D. Ducharme and D. Lewis, J. AOAC Int., 78 (1995) 1312.
[64] H.Y. Lin and R.D. Voyksner, Anal. Chem., 65 (1993) 451.
[65] C.S. Barrio, J.S. Asensio and J.G. Bernal, Chromatographia, 39 (1994) 320.
[66] Y. Nakamura, Y. Tonogai, Y. Sekiguchi, Y. Tsumura, N. Nishida, K. Takakura, M. Isechi, K. Yuasa and M. Nakamura, J. Agri. Food Chem., 42 (1994) 2508.
[67] C. Albelda, Y. Pico, G. Font and J. Manes, J. AOAC Int., 77 (1994) 74.
[68] T. Cairns, K.S. Chiu, D. Navarro and E. Siegmund, Rapid Commun. Mass Spectrom., 7 (1993) 971.
[69] G.C. Mattern, G.M. Singer, J. Louis, M. Robso and J.D. Rosen, J. Agric. Food Chem., 38 (1990) 402.
[70] P.L. Wylie, K.J. Klein, M.Q. Thompson and B.W. Henmann, J. High Resolut. Chromatogr., 15 (1992) 763.
[71] T. Suzuki, K. Yaguchi, K. Ohnishi and T. Yamagishi, J. Chromatogr. A, 662 (1994) 139.
[72] M.L. Trehy, R.A. Yost and J.G. Dorsey, Anal. Chem., 58 (1986) 14.
[73] W. Liao, T. Joe and W.G. Cusik, J. Assoc. Off. Anal. Chem., 74 (1991) 554.
[74] H.J. Stans and M. Linkerhaegner, J. High Resolut. Chromatogr., 16 (1993) 539.
[75] M. Linkerhaegner and H.J. Stans, Z. Lebensm.-Unters.-Forsch., 198 (1994) 473.
[76] K.G. Furton and J. Rein, Anal. Chim. Acta, 236 (1990) 99.
[77] E. Ballesteros, M. Gallego and M. Valcarcel, Anal Chem., 65 (1993) 1773.
[78] E. Ballesteros, M. Gallego and M. Valcarcel, J. Chromatogr., 633 (1993) 169.
[79] H. Faerber and H.F. Schoeler, J. Agric. Food Chem., 41 (1993) 217.
[80] Y. Akiyama, N. Takeda and K. Adachi, Shokuhin Eiseigaku Zasshi, 36 (1995) 42.
[81] B. Murugavel, K.J. Voorhees and S.J. DeLuca, J. Chromatogr., 633 (1993) 195.
[82] P.T. Jedrzejewski and L.T. Taylor, J. Chromatogr. A, 703 (1995) 489.
[83] H.S. Rathore and T. Begum, J. Chromatogr., 643 (1993) 321.
[84] V.B. Patil and M.S. Shingare, J. Chromatogr. A, 653 (1993) 181.
[85] D. Bose, P. Shivhare and V.K. Gupta, J. Planar Chromatogr., Mod. TLC, 7 (1994) 415.
[86] V.B. Patil and M.S. Shingare, Analyst, 119 (1994) 415.
[87] J. Bladek, J. Planar Chromatogr., Mod. TLC, 6 (1993) 487.
[88] D.N. Judge, E. Mullin and R.W. Young, J. Planar Chromatogr., Mod. TLC, 6 (1993) 300.
[89] C. Weins and H. Jork, Vom Wasser, 83 (1994) 279.
[90] H. Susse and H. Muller, Fresenius' J. Anal. Chem., 352 (1995) 470.

[91] S.J. Lehotay and R.J. Argauer, J. Agric. Food Chem., 41 (1993) 2006.
[92] S.B. Friedman and T.N. Stewart, US Pat., 6 pp, USX, 5 200 346, 930 406.
[93] J.A. Itak, E.G. Olson, J.R. Fleeker and D.P. Herzog, Bull. Environ. Contam. Toxicol., 51 (1993) 260.
[94] S.C. Jourdan, A.M. Scutellaro, J.R. Fleeker, D. Herzog and F.M. Rubio, J. Agri. Food Chem., 43. (1995) 2784.
[95] M-P. Marco, S. Chiron, J. Gascon, B. Hammock, D. Bruce and D. Barcelo, Anal. Chim. Acta, 311 (1995) 319.
[96] P. Skladal, L. Macholan, M. Petrivalsky, I. Pluhacek and L. Stancic, GIT Fachz. Lab., 37 (1993) 606.
[97] J.L. Marty, N. Mionetto, T. Noguer, F. Ortega and C. Roux, Biosens. Bioelectron., 8 (1993) 273.
[98] C. La Rosa, F. Pariente, L. Hernandez and E. Lorenzo, Anal Chim. Acta, 295 (1994) 273.
[99] C. La Rosa, F. Pariente, L. Hernandez and E. Lorenzo, Anal. Chim. Acta, 308 (1995) 129.
[100] D. Martorell, F. Cespedes, E. Martinez-Fabregas and S. Alegret, Anal. Chim. Acta, 290 (1994) 343.
[101] J.C. Fernando, K.R. Rogers, N.A. Anis, J.J. Valdes, R.G. Thompson, A.T. Eldefrawi and M.E. Eldefrawi, J. Agri. Food Chem., 41 (1993) 511.
[102] C. Tran-Minh, Anal Proc., 30 (1993) 73.
[103] A. Roda, P. Rauch, E. Ferri, S. Girotti, S. Ghini, G. Carrea and R. Bovara, Anal. Chim. Acta, 294 (1994) 35.
[104] L.F. Capitan-Vallvey, J. Rohand, A. Navalon, R. Aviad and J.L. Vilchez, Talanta, 40 (1993) 1695.
[105] H.S. Rathore, I. Ali and T. Begum, Microchem. J., 51 (1995) 393.
[106] K.D. Khalaf, A. Morales-Rubio and M. de la Guardia, Anal. Chim. Acta, 280 (1993) 231.
[107] S. Panadero, A. Gomez-Hens and D. Perez-Bendito, Anal. Chem., 66 (1994) 919.
[108] Y. Wei, W. Jin, R. Zhu, C. Liu and S. Zhang, Talanta., 41 (1994) 1617.
[109] S. Gamgues, M.T. Vidal, M. Gallignani and M. de la Guardia, Analyst, 119 (1994) 659.
[110] L. Mathew, M.L.P. Reddy, T.P. Rao, C.S.P. Iyer and A.D. Damodaran, Analyst, 120 (1995) 1799.
[111] J.V. Das, K.N. Ramachandran and V.K. Gupta, Fresenius' J. Anal. Chem., 348 (1994) 480.
[112] V. Agrawal and V.K. Gupta, Chem. Anal., 38 (1993) 197.
[113] B. Venkateswarlu and K. Seshaiah, Talanta, 42 (1995) 73.
[114] K.D. Khalaf, J. Sancenon and M. de la Guardia, Fresenius' J. Anal. Chem., 347 (1993) 52.
[115] N.V.S. Naidu and P.R. Naidu, Asian J. Chem., 6 (1994) 859.
[116] J.A. Perez-Lopez, A. Zapardiel, E. Bermejo, E. Arauzo and L. Hernandez, Fresenius' J. Anal. Chem., 350 (1994) 620.
[117] A. Guiberteau, T. Galeano Diaz, F. Salinas and J.M. Ortiz, Anal. Chim. Acta, 305 (1995) 219.