resin fractions, which demonstrates the great complexity of crude oil composition.

The choice of a procedure primarily depends on the information required. Combining different separation steps and/or short-cuts to achieve a specific purpose is possible.

See also: **II/Chromatography: Liquid:** Mechanisms: Ion Chromatography. **Chromatography: Thin-layer (Planar):** Modes of Development: Conventional. **III/Crude Oil: Liquid Chromatography. Flame Ionization Detection: Thin-Layer (Planar) Chromatography. Flash Chromatography. Metal Complexes. Petroleum Products:** Liquid Chromatography.

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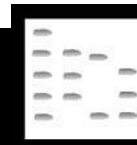
CARBAMATE INSECTICIDES IN FOODSTUFFS: CHROMATOGRAPHY AND IMMUNOASSAY



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Introduction

Pesticides have received special attention over the years, due mainly to the problems of environmental and food contamination. Analytical methods for determining pesticide residues have their main application in the control of food for human consumption,

especially in the control of fruit and vegetables produced using direct applications of pesticides. Moreover, the determination of pesticide residues in food is fundamental in monitoring and regulatory programmes. Pesticide residue levels higher than the maximum residue level (MRL) are monitored through two different but complementary approaches: regulatory monitoring focusing on raw agricultural commodities that measure the levels in individual lots for compliance with legal tolerances, and total diet study, in which dietary intakes of pesticides are determined by analysis of fruit and vegetables.

Carbamates constitute a family of pesticides registered for use on several crops in South America, Europe and the USA. Their use for pest control has progressively increased in recent years, along with organophosphates (OPs), as alternatives to organochlorine (OC) insecticides. Owing to their broad spectrum of biological activity, carbamates can be used as insecticides, miticides, fungicides, nematicides and molluscicides. **Table 1** shows the structures of the most extensively used carbamates, including some *N*-methylcarbamate (NMC) insecticides. Carbamate residues are of concern because of the acute toxicity of some compounds – aldicarb and carbofuran exhibit an LD_{50} (the dose of a compound that causes death in 50% of the organisms to which it has been administered) in rats of 1 and 8 mg kg^{-1}, respectively. Some carbamate residues are suspected carcinogens and mutagens. Such insecticides act as inhibitors of the acetylcholinesterase enzymes, and a number of adverse effects have been reported in the literature.

Several analytical methods have been proposed for the separation and quantification of carbamate residues in food samples. The carbamate pesticides are thermally labile and not readily amenable to gas chromatography (GC), making the use of the liquid chromatographic (LC) techniques preferable. In general, the time and expenses involved in classical analytical methods (i.e. sampling, sample preparation and laboratory analysis) have substantially limited the number of samples that can be analysed in food research and surveys. In addition, the quantity of chemicals and toxic solvents that are used sometimes offer a risk factor considerably greater than that of the pesticide residue to be determined. These disadvantages have emphasized the need for developing fast, easy, robust, sensitive and cost-effective methods capable of being used in the field. Instrumental techniques that combine these characteristics are slowly starting to appear in pesticide residue analysis. They include the immunoassay (IA) techniques, immunoaffinity chromatography and electrochemical/optical biosensors. To avoid the general drawbacks of the classical methods, significant developments have taken place in extraction/clean-up procedures, and in the final determination of pesticide residues in foodstuffs.

This article examines recent progress, focusing primarily on simplified and miniaturized analytical methods for determining carbamate insecticides in foodstuffs, including fast and simple extraction/clean up strategies, and the use of IA techniques for detection prior to laboratory analysis.

Chromatographic Methods for Carbamate Analysis

LC Methods

Standard LC methods for carbamate determination based on Krause's method generally consist of reversed-phase HPLC with gradient elution followed by two post-column reactions to yield fluorescent species (**Figure 1**). The carbamates are hydrolysed with an alkaline solution (usually sodium hydroxide) to a methylamine that is sequentially derivatized in the presence of *o*-phthaldehyde (OPA) and mercaptoethanol (MERC) to create the fluorescent product. Two high pressure pumps are used to introduce the post-column reagents. To separate the main NMCs and their metabolites, a cycle time of 45–60 min is required. Limits of detection (LODs) in water analysis are in the range of 1 to 4 ng, which is equivalent to 2.5 to 10 p.p.b. in the water injected. However, if a preconcentration step is carried out before chromatographic separation, the LOD value is lower.

The sensitivity and selectivity of Krause's method have allowed its use for the determination of residues of NMCs in fruits and vegetables with great accuracy. Unfortunately, such methods involves extraction with methanol, and large amounts of sample and solvent are used. The clean-up, starting with successive liquid–liquid partition (LLP) steps, and finishing with elution of the target compounds on a Celite®/charcoal adsorbent column, is mainly responsible for the slowness and tediousness of the method, making the analysis of a large number of samples impractical. Usually, it is satisfactorily used as a reference method in collaborative studies and also to carry out validation of new methods. Different procedures for clean-up of crop extracts by employing either glass adsorbent columns and commercially available solid-phase extraction (SPE) cartridges have been compared and a specific solid-phase elution protocol employing a solvent polarity gradient proposed. A schematic outline of the method is illustrated in **Figure 2**. The extraction is carried out with methanol, and a LLP step is only necessary if a UV detector is to be used. In most cases, recoveries of the more polar compounds

Table 1 Carbamates commonly used in crop protection and some of their breakdown products

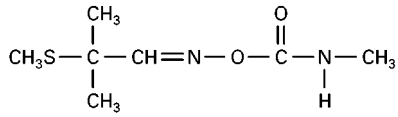
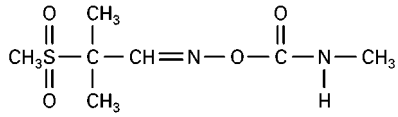
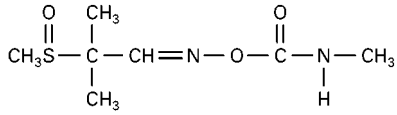
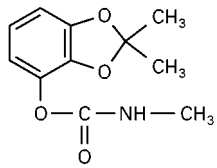
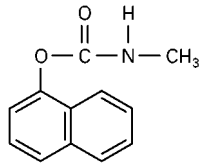
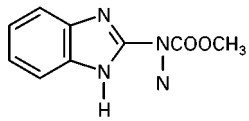
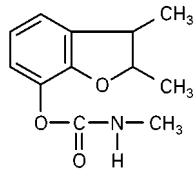
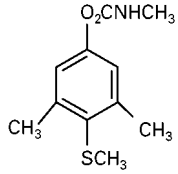
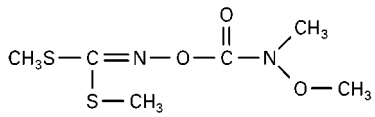
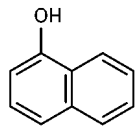
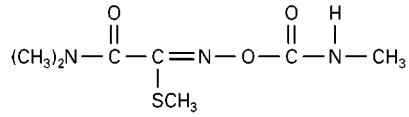
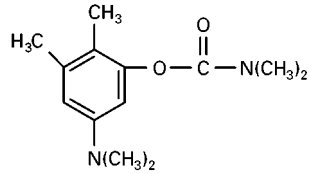
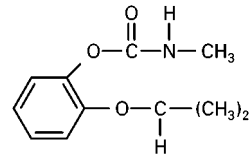
Compound name	*Chemical structure*	*Class*	*IUPAC name*
Aldicarb		Insecticide	2-Methyl-2-(methylthio)propionaldehyde *o*-methylcarbamoyloxime
Aldicarb sulfone		Insecticide (metabolite of aldicarb)	2-Methyl-2-(methylsulfonyl)propanal *o*-[(methylamino)carbonyl]-oxime
Aldicarb sulfoxide		Insecticide (metabolite of aldicarb)	2-Methyl-2-(methylsulfinyl)propanal *o*-[(methylamino)carbonyl]-oxime
Bendiocarb		Insecticide	2,3-Isopropylidenedioxyphenyl methylcarbamate
Carbaryl		Insecticide	1-Naphthyl methylcarbamate
Carbendazim		Insecticide	2-Methyl benzimidazol-2-ylcarbamate
Carbofuran		Insecticide	2,3-Dihydro-2,2-dimethylbenzofuran-7-yl-methylcarbamate
Methiocarb		Insecticide, acaricide, molluscicide	4-Methylthio-3,5-xylyl methylcarbamate
Methomyl		Insecticide	*S*-Methyl *N*(methylcarbomoyloxy) thioacetimidate
1-Naphthol		Insecticide (metabolite of carbaryl)	1-Naphthalenol

Table 1 *Continued*

Compound Name	*Chemical structure*	*Class*	*IUPAC name*
Oxamyl		Insecticide, nematicide, acaricide	*N,N*-Dimethyl-2-methylcarbamoyloxime-2-(methylthio)acetamide
Pirimicarb		Insecticide	2-Dimethylamine-5,6-dimethylpirimidine-4-dimethylcarbamate
Propoxur		Insecticide	2-Isopropoxyphenyl-*N*-methylcarbamate

are higher if the partitioning step is omitted and fluorimetric detection is employed.

A series of tests to assess the feasibility of using activated carbon membranes in the clean-up of vegetables (green peppers) for the analysis of NMC pesticides by HPLC with post-column derivatization and fluorescence detection have been carried out. These tests showed that the membranes were effective in retaining sample interferences in both offline (with a 22 cm diameter activated carbon membrane) and online (extract injected directly in the HPLC system) methods. Solid-phase extraction with bonded-silica adsorbents, including octyl (C_8), ethyl (C_2), octadecyl (C_{18}) and cyclohexyl (CH), has also been successfully developed as an alternative to LLP clean-up. Elution patterns and recovery from various adsorbent materials were determined for 17 OPs, nine carbamates and three other pesticides in a sample matrix of rice grain. The method performance was comparable to conventional clean-up procedures.

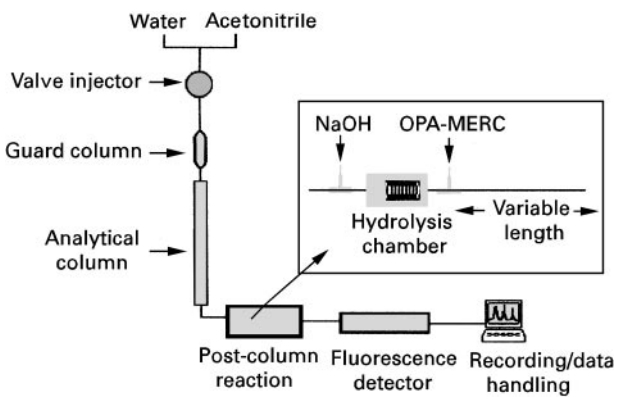


Figure 1 Liquid chromatography system with post-column reactions and fluorimetric detection for determination of *N*-methylcarbamate pesticides.

Following the general tendency toward miniaturization, a new method for NMC determination in fruits and vegetables has been proposed. This method eliminates the need for delivery pumps to introduce the hydrolysis/derivatization reagents, since they are already present in the mobile phase. For this purpose, a Kromasil-100 C_{18} column (which tolerates basic conditions) and a borate buffer mobile phase were chosen. A simplified LC–fluorescence (FS) method for the determination of traces of carbamates in grains, fruits and vegetables has been described. Here, the sample size and solvent volumes were considerably reduced, and a clean-up on aminopropyl-bonded Sep Pak® cartridge was performed in order to separate the target compounds from the coextractives. Recovery of the pesticides and limits of detection in different food matrices varied from 60 to 103% and from 1 to 4 ppb. A complete analytical methodology for the determination of some NMCs in vegetable, fruit and feed crop samples has been developed in which the pesticides are extracted, cleaned on an aminopropyl-bonded SPE column, and then determined by LC-FS.

Depending on the detection technique, not only the method selectivity, but also the method sensitivity, can be changed considerably. For example, by using LC with UV detection, the presence of coextractives can severely limit the analyte determination, resulting in poor method sensitivity when analysing real samples compared with standard solutions. Despite this, and considering that the maximum residue levels for foodstuffs are higher than those for water samples,

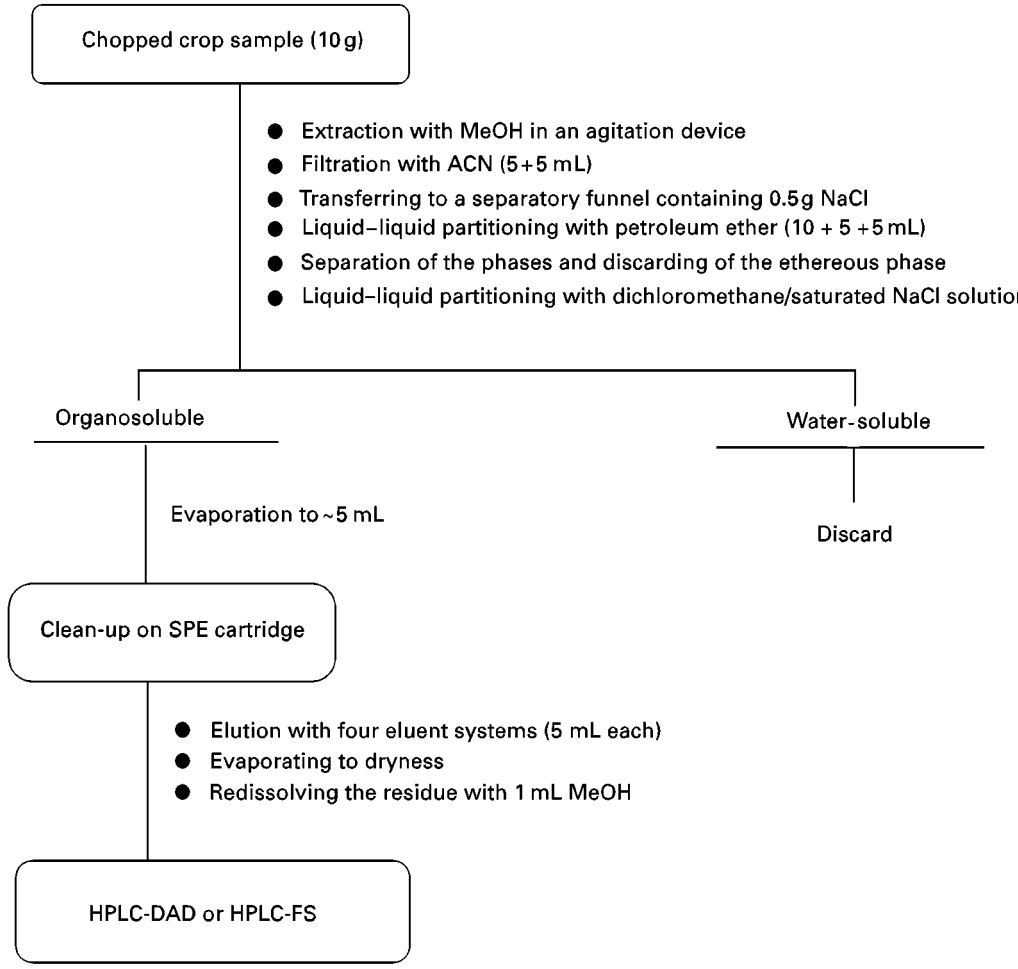


Figure 2 Block diagram of a scheme for extract preparation for HPLC analysis *N*-methylcarbamate pesticides in fruit and vegetables. If fluorimetric detection is performed, the LLP step can be omitted. Elution through an SPE column was carried out according to the method described by Nunes GS, Ribeiro ML, Polese L and Barceló D (1998) Comparison of different clean up procedures for the determination of *N*-methylcarbamate insecticides in vegetable matrices by high-performance liquid chromatography with UV detection. *Journal of Chromatography* 795: 43–51.

a simple and rapid method has been proposed to analyse eight carbamate insecticides and 10 of their main metabolites in apples, pears and lettuce. The clean-up procedure is based on that of the De Kok method, which uses solid-phase cartridges, and the amounts of solvents and sample are substantially reduced. It was found that matrix interferences could be minimized by diluting the final extract, but the method sensitivity is also reduced.

GC Methods

The relationship between the mode of detection and the required extraction procedure for this mode has resulted in the emergence of numerous sample preparation procedures. Using GC–mass spectrometry (MS) and LC-FS techniques, the extraction of 199 pesticides from fruits and vegetables with acetonitrile, and removal of the coextractives with a miniature charcoal–Celite clean-up column have been carried out. Good recovery data obtained by spiking pears, carrots and bananas at the 0.1–0.5 p.p.m. demonstrated the excellent performance of this method.

Various types of detectors have been evaluated for the analysis for the carbamate insecticides by GC. Among these, the nitrogen-phosphorus detector (NPD), the flame photometric detector (FPD) with either a phosphorus or sulfur filter, the electron-capture detector (ECD) and the mass spectrometric detector (MS), either in electron impact mode (EI) or in positive chemical ionization (PCI) mode, have been tested. Sensitivity, linearity and selectivity of the different detectors have been detailed and application to real samples, including foodstuffs, has also been presented. Although the thermolability of the carbamates is considered the major limitation as regards to the use of GC techniques, it has been shown that

thermal degradation of some carbamates and metabolites does not occur under certain conditions, as was demonstrated by the fragmentation patterns of the studied compounds. GC-NPD methods for the monitoring of residues of some carbamates in real samples have been also described. A comparison has been made of GC and LC techniques for the analysis of the most popular (in terms of amount produced and applied) pesticide classes, such as carbamates, phenylureas, triazines, phenoxy acid herbicides and chlorinated phenols. Sample concentration and detection have been discussed in relation to their influence on the performance of the particular separation technique. GC methods, when applicable, have the advantages of greater separation efficiency, higher speed of analysis and the availability of a wide range of highly sensitive detectors. On the other hand, LC is often the choice when polar, nonvolatile and/or thermolabile compounds need to be analysed, as it is in the case of carbamates.

Supercritical Fluid Chromatography/Extraction

Supercritical fluid chromatography (SFC) is a technique that in many ways is a hybrid of GC and HPLC. It is recognized as a valuable technique for the analysis of thermally labile compounds such as carbamates. A few applications have been reported for SFC in the field of pesticide determination in food. The versatility in separation, the possibility of using different detectors (LC or GC detectors), and the prospect of directly coupling with supercritical fluid extraction (SFE), make this analytical technique very attractive. SFE using CO_2 has been examined for separating carbamates from interfering coextractives prior to analysis either by LC-FS or by GC with ion trap mass spectrometry (ITMS) detection. Pre-extraction of ground meat with acetonitrile before SFE left behind over 99% of the fat and fibre. SFE of the acetonitrile extracts with pelletized diatomaceous earth further reduced the amount of coextractives 10-fold, removing the interferences that would have appeared in the fluorescence mode.

Simplification of Multiresidue Methods

Official laboratories that investigate and analyse pesticide residues usually utilize the established multiresidue methods (MRM) of analysis. One of the most commonly used MRM for pesticide analysis in fruit and vegetables samples is the AOAC (Association of Official Analytical Chemists) method. It involves an aqueous acetone extraction and laborious clean-up employing LLP procedures using organic solvent of limited water capacity, to achieve the removal of the coextractives present in the sample extract and/or solid-phase clean-up with silica or Florisil. Finally, analyte determination is performed by GC or HPLC with selective detectors.

More than 300 pesticides and pesticide-related compounds can be determined by the well-known MRMs described in the official literature, such as the AOAC method, the German MRM S19 (Deutsche Forschungsgemeinschaft, DFG), and the method adopted by the National Food Administration of Sweden. They all have several disadvantages, such as their inefficiency as screening methods, since they are too time-consuming and labour-intensive, the large amount of solvents used, and, in addition, newly developed groups of pesticides are becoming more polar and/or thermodegradable, making difficult their analysis by conventional chromatographic techniques.

During the past two decades, research has gone in the direction of reduction of organic solvent toxicity, elimination of the partition step and elimination of the column clean-up. A rapid and efficient multiresidue extraction procedure has been reported using ethyl acetate and sodium sulfate, followed by gel permeation chromatography (GPC) on an SX-3 column. Its effectiveness to analyse OC and OP insecticides was confirmed. Several other methods based on it have arisen using specific detectors that have decreased the number of interfering chromatographic peaks.

LC-MS Techniques

Confirmation of the presence of carbamates in real samples has been performed by HPLC-MS with various interfaces, but in general most of these studies have been performed with standard solutions. **Table 2** summarizes the development of LC-MS techniques for the analysis of different pesticides, including carbamate insecticides. Up to the present, few food analysis applications have been reported. Atmospheric pressure chemical ionization (APCI) techniques for the mass spectral analysis of several NMCs have been evaluated and the results compared with those obtained using ionspray (ISP) and thermospray (TSP) interfaces. The results were also compared with EI ionization and methane CI spectra obtained with a particle beam (PB) interface. These methods were applied to the confirmatory analysis of three representative carbamate pesticides, spiked at the 0.1 ppm level in green peppers.

Multiresidue confirmations of pesticides from different classes using APCI techniques have also been performed. It was observed that the fragmentation of the carbamates studied was highly voltage-dependent. An increase in the potential of the sampling

Table 2 MS interfaces most commonly used for LC analysis of pesticides, including carbamate insecticides

Spectrometry interface	*Pesticides studied*	*Sample type*	*LOD (ng)*
APCI, ISP, TSP and CI	NMCs	Aqueous solutions Green peppers	—
APCI (in positive- and negative-ion modes), TSP, and PB-MS	17 pesticides in five chemical classes (triazines, phenylureas, carbamates, organophosphorus and miscellaneous)	Ground water	Full-scan mode: 0.8–10 SIM mode: 0.01–1
TSP	Anilides, carbamates, *N*-heterocyclic, organophosphorus, and phenylureas)	Aqueous solutions	—
TSP (with online and offline SPE procedures)	Nitrogen- and phosphorus-containing pesticides	Aqueous solutions	SIM mode: 0.04–0.6
TSP	3 *N*-methylcarbamates, 3 *N*-methylcarbamoyl-oximes, 2 substituted urea pesticides, and 1 ester of a substituted carbamic acid	Aqueous solutions	—
TSP (with either filament- and discharge-assisted ionization modes	19 carbamates and 12 of their known degradation products	Aqueous solutions	—
TSP	16 carbamates from four chemical subclasses (oxime-NMCs, aryl-NMCs, *N*-phenylcarbamates and methyl esters of substituted carbamic acids)	Aqueous solutions	—
FIA-PB-PCI	14 carbamates	Aqueous solutions	—
FIA-PB-EII-CI (with either negative- and positive-ion modes)	33 carbamates and 14 of their degradation products	Surface water	—
APCI	11 carbamates	Food samples	0.3–10
ES	20 carbamates	Fruits and vegetables	—
ES	*N*-Heterocyclic compounds, phenylureas and carbamates	Fruits and vegetables	—

APCI, atmospheric pressure chemical ionization; CI, chemical ionization; DCI, desorption chemical ionization; EII, electron impact ionization; ES, electrospray interface; FIA-PB-PCI, flow injection analysis-particle beam-positive chemical ionization; ISP, pneumatically assisted electrospray ionization; LOD, limit of detection; SIM, selected-ion monitoring; SPE, solid-phase extraction; TSP, thermospray interface.

cone strongly affected the formation of diagnostic daughter ions. The dependence of the ion abundances in the TSP mass spectra of several pesticides has been studied with regard to the vaporizer and the gas-phase temperatures and under collision-activated dissociation conditions. Fragmentation pathways under certain experimental conditions were investigated for some of the carbamate pesticides. Both vaporizer and source jet temperatures were monitored. APCI, ESP, fast-atom bombardment, Cf-252 plasma desorption and collision-activated dissociation spectra were then performed for the pesticides to confirm proposed pathways and to gain additional information.

Through an interlaboratory study involving nine laboratories, it was concluded that, in TSP-LC-MS systems, the thermospray tip temperature plays a major role in adduct formation and ion fragmentation of thermally labile carbamate pesticides. As a result, this temperature needs to be carefully controlled. This effect has been confirmed by investigating the influence of three LC eluent additives (ammonium acetate, ammonium formate and nicotinic acid) and the vaporizer temperature on ion formation. The performances of different thermospray interfaces (which exhibit wide differences in source geometry) used in carbamate analysis have also been compared, and the so-called suppression effects on the ion formation have been studied in coeluted compounds. Thermally labile carbamates gave unsatisfactory results with regard to spectral compatibility between the interfaces. These differences are due to thermally assisted hydrolysis reactions that occur in the various vaporizer designs. Different approaches using desorption chemical ionization (DCI) and flow injection (FIA)–particle beam (PB)–ammonia PCI–MS were further evaluated. The mass spectra using FIA-PB-PCI-MS exhibit higher relative abundances for fragment ions, and the ion intensities are strongly dependent on the ion source pressure. Another study, employing EI ionization and ammonia/methane with positive/negative chemical ionization, has shown ammonia to be the best reagent gas. Using ammonia gave less fragmentation and better quantitative results than methane for the analysis of 33 carbamates and 14 of their degradation products.

LC-APCI-MS and LC-post-column fluorimetry for the determination of carbamates in foodstuffs were compared. It was observed that, despite its great potential in detection and confirmation, in general LC-APCI-MS is less sensitive for quantitation

purposes, which agrees with previous reports. The feasibility of using reversed-phase LC-MS with an electrospray interface for measuring traces of NMC insecticides in 10 different types of fruit and vegetables has been evaluated. Extraction with methanol, followed by clean-up on a Carbograph 1 extraction cartridge, provided an average recovery higher than 80% for all compounds. It was noticed that changing from methanol to acetonitrile as the organic modifier resulted in a significant decrease in ion signal for the carbamates. The presence of coextractives derived from the crop samples in the electrosprayed solution did not interfere significantly with the ionization process of the compounds studied. The photochemical behaviour of pesticides in a photolysis reactor coupled online with an LC-ESP-MS system has been investigated this system has been used successfully to trace confirmatory analysis of carbamates in foodstuffs.

Recently an analytical method for confirmation of aldicarb and its metabolites in fruit and vegetable samples has been optimized. Aldicarb is one of the NMCs most commonly used for insect control in tropical countries in different agricultural fields. **Figure 3** illustrates schematically the metabolic pathway of aldicarb under environmental conditions, and shows the formation of the mass fragments used to identify the parent and metabolic compounds. Initial metabolic attack is rapid and complete oxidative conversion to aldicarb sulfoxide is followed by a much slower oxidation to the sulfone. It is interesting to consider the rapid degradation of aldicarb after its application, because in some cases the parent compound is not present in the studied matrix. Since that the toxicity could be effectively more acute if the metabolites were present in more elevated concentrations, their confirmation in real samples by LC-MS is of crucial importance. Such compounds have been analysed by LC-APCI-MS using the selected ion monitoring (SIM) mode based on five channels. **Figure 4** shows a typical total ion chromatogram and the five selected ions used to identify the compounds in orange extracts. Here, the fragmentation was assisted by the addition of ammonium formate in the mobile phase (acetonitrile/water) and ion adducts did not appear in the mass spectra, resulting in a sensitive method free from interferences.

Thin Layer Chromatography Methods

Thin-layer chromatography (TLC) is used for the qualitative and quantitative analysis of a wide variety of compounds, including pesticides. Among the carbamate insecticides analysed by TLC can be found those most commonly used in crop protection, such as carbaryl, carbofuran, methomyl, bendiocarb, propoxur, aldicarb and carbendazim. Since the 1980s, only a few papers concerning carbamate analysis by TLC have been published. Thin-layer chromatographic procedures have been developed for the separation of several carbamates, such as

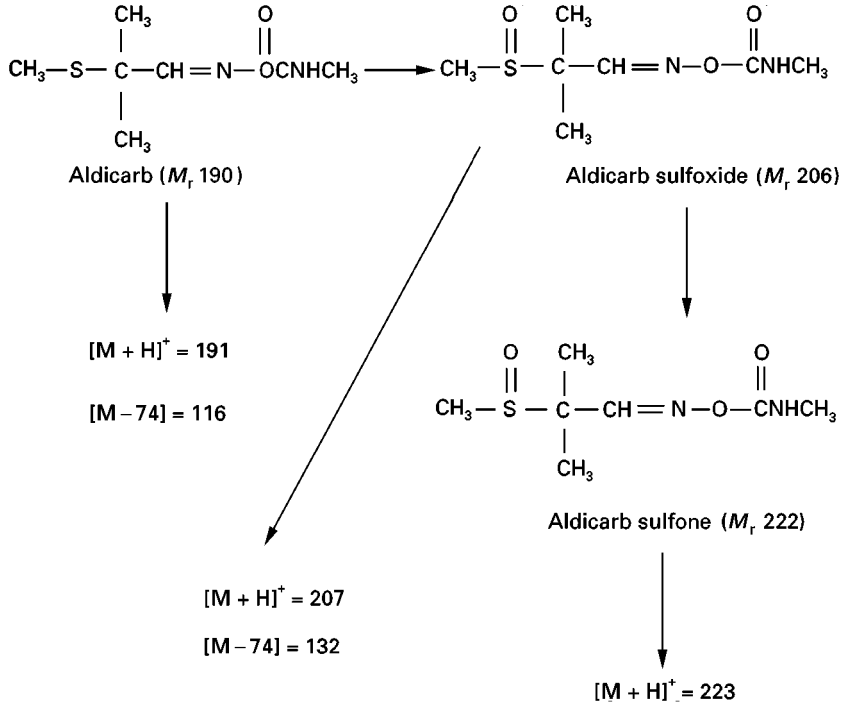


Figure 3 Metabolic pathway of aldicarb to its degradation products under environmental conditions, and mass fragments used for the LC-MS confirmation of the compounds.

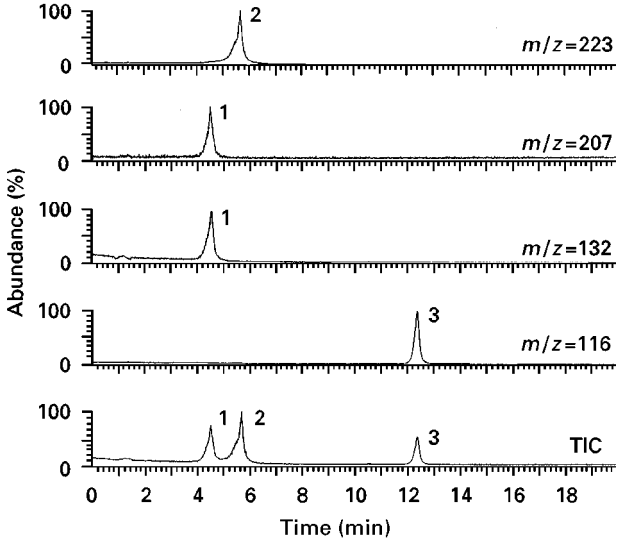


Figure 4 Mass spectra for (1) aldicarb sulfoxide (2), aldicarb sulfone and (3) aldicarb present in a spiked orange extract. Peak confirmation by SIM mode of five selected channels on the total ion chromatogram (TIC). Final extract 20-fold concentrated (see sample preparation in Figure 2). Chromatrographic separation of the compounds was carried out using a C_{18} RP-LC column and a water/acetonitrile mixture, both containing 0.1% ammonium formate as mobile phase.

carbaryl, bendiocarb, carbofuran, 2-isopropylphenyl-*N*-methylcarbamate (MIPC) and 2-*sec*-butyl-*N*-methylcarbamate (BPMC). Suitable schemes using plates coated with silica gel containing 1% zinc acetate as support, and benzene/ethyl acetate (50:10) as solvent have been developed for carbamate analysis in various matrices, such as aqueous biological fluids and food samples, mainly fruit juices. Residues of carbofuran and its two metabolites have been extracted with HCl, partitioned into CH_2Cl_2, chromatographed on silica gel and detected with KOH-*p*-nitrobenzenediazonium fluoroborate. Sequential TLC has been used for the detection and determination of carbaryl in water samples. More recently, the chromatographic behaviour of carbamate pesticides and related compounds has been examined on thin layers of alumina, barium, sulfate, calcium carbonate, calcium phosphate, calcium sulfate, cellulose and silica Gel G®.

Immunoassay (IA) Methods for Carbamate Determination

In the last few years, the number of enzyme-linked immunosorbent assay (ELISA) methods for the determination of pesticides has increased, but there is still a lack of IA methods for pesticide residue determination in food and crop samples. IA techniques can provide complementary and/or alternative approaches in reducing the use of expensive equipment and analysis time while still maintaining reliability and sensitivity. Moreover, IA can be used as a screening method in order to detect food contamination. The relatively short analysis time allows for the screening of a large number of samples which is a major advantage.

IA has been shown to have potential as a screening method for the detection of pesticides in food samples. A rapid bioluminescence method was used for screening several OP and NMC insecticides in processed baby food. Among the 155 samples tested, there were 23 suspected positives (14.2%) that were further analysed by HPLC; this resulted in confirmation of the presence of carbaryl in 18 of the samples.

The utility and applicability of an analytical method depends in great part on the absence of matrix interferences. In this regard, ELISA is not different from other detection techniques, and the sample preparation prior to analysis is still a critical point for pesticide residue determination by IA methods. A competitive ELISA has been developed for quantification of methyl 2-benzimidazolecarbamate in fruit juices. Matrix effects are minimized by diluting the samples before IA. Rapid methods based on water or acetonitrile extraction have been evaluated for screening carbofuran and aldicarb sulfone in mean and liver using commercial ELISA kits. The final extracts are diluted in order to eliminate the effect of the solvent, which is more pronounced than the natural compound effects in some cases. Attempts have been made to minimize the matrix and organic solvent effects in ELISA for carbaryl by diluting the crop extracts in the assay buffer, but it was observed that the effect of the solvent residue in the diluted extracts on the assay was still higher than the matrix effect. The direct application of IA to vegetable/fruit juices diluted in the assay buffer is possible, without large losses in the method sensitivity. As an example, **Figure 5** shows some calibration curves for carbaryl in assay buffer and in lemon juices diluted in assay buffer. In this case the absence of matrix effect, after sample dilution and pH adjustment, indicates good IA performance. Unfortunately, in most cases the recoveries of IA-based methods have proved to be lower than those of methods that do not employ any prior sample treatment.

Different experimental approaches to ELISA quantification of NMC insecticides in fruit juices, without any sample pre-treatment other than dilution, have also been developed. For carbaryl and carbofuran residue determination in fruit juices, high affinity monoclonal antibodies (MABs) have been used. Off-line SFE and ELISA have been used for the determination of nine pesticides, including four carbamates, in foodstuffs consisting of baby food and Food and

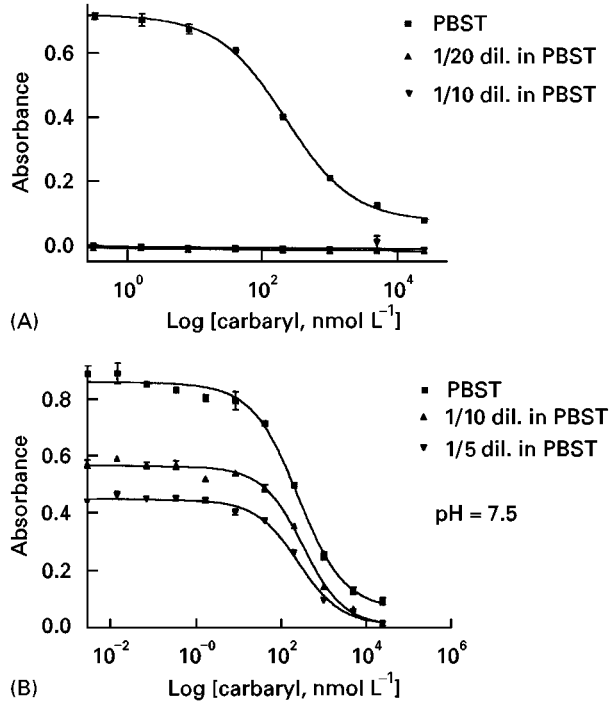


Figure 5 Matrix effect of lemon juice on the enzyme-linked immunosorbent assay (ELISA) for carbaryl analysis. Calibration curves were constructed with PBST buffer and PBST-diluted lemon juices. (A) Natural pH of the diluted samples; (B) pH adjusted with an alkaline solution. PBST, (Reproduced with permission from Nunes GS (1999) Analysis of *N*-methylcarbamate insecticides by chromatographic techniques, immunoassay (ELISA) and amperometric biosensors. Institute of Chemistry IUNESP, Anaraquara, São Paulo, 230 pp [doctorate thesis].

Drug Administration (FDA) Total Diet Study (TDS) samples. The benefits of SFE-ELISA included replacement of harmful organic extraction solvents, rapid extractions with a relatively inexpensive extractant, and a reduced number of steps in the determination of the target compounds.

In contrast to conventional chromatographic techniques for pesticide analysis in foods, IA methods have not yet been extensively characterized. The validation of the proposed ELISA against another validated method has been the primary objective in only a few published papers. As for any analytical method, quality control and assessment of material and equipment stability are required. In addition, IA evaluation involves defining working ranges, sensitivity, precision, accuracy, linearity, specificity and matrix effects.

Immunoaffinity Chromatography

Immunoaffinity chromatography (IC) has been widely used for the determination of various analytes in the medical field; however, the use of antibodies immobilized on an appropriate support to pre-concentrate pesticides from environmental samples is only a recent development. In the analysis of pesticides in food matrices, the use of IC is still more limited. IC is based on the highly selective interaction of antigens with their antibodies, which are immobilized on a support material called an immunosorbent. The production of antibodies against pesticides is based on the conjugation of hapten to a large immunogenic carrier molecule (typically bovine serum albumin or keyhole limpet haemocyanin), and subsequently the complex is injected into a suitable vertebrate (rabbit, mouse, rat, sheep). Since antibody–antigen interactions occur over short distances, steric effects are involved in the coupling reaction. These steric effects are what make antibody–antigen interactions so selective, and only the antigen that produced the immune response, or very closely related molecules, will be able to blind to the antibody. Thus, theoretically, when the sample is run through the immunosorbent the analytes are selectively retained and subsequently eluted free of the coextratives (**Figure 6**). Once the analytes have been separated from interferences, they can be determined by conventional chromatographic techniques. The use of IC as a separation tool before pesticide analysis is thus extremely attractive.

Among the carbamate pesticides, only carbofuran has been separated from natural components of crop samples by IC and a highly sensitive online IC with coupled-column LC-MS was used to analyse some fruit and vegetable samples.

Current Trends and Conclusions

A tremendous amount of work has been done and much more is under way in the field of pesticide analysis is food samples. In the chromatographic field several new techniques for pesticide analysis, such as immunoaffinity chromatography and LC-MS with various interfaces, have appeared. These have undoubtedly contributed to increased separation efficiency and improved sensitivity. It is expected that the bioanalytical techniques for pesticide analysis will become a common analytical alternative because of their demonstrated advantages, especially in the analysis of carbamates in food samples. The capacity that these compounds show in inhibiting a certain class of enzymes (the cholinesterases) must be further explored. Development of approaches based on the coupling of the chromatographic separation with biodetection systems is a promising alternative.

Sample handling is the bottleneck in the analysis of carbamate insecticides in foodstuffs, since in many cases a complex clean-up step is needed before chromatographic separation. The use of biological techniques, such as immunoassays and biosensors,

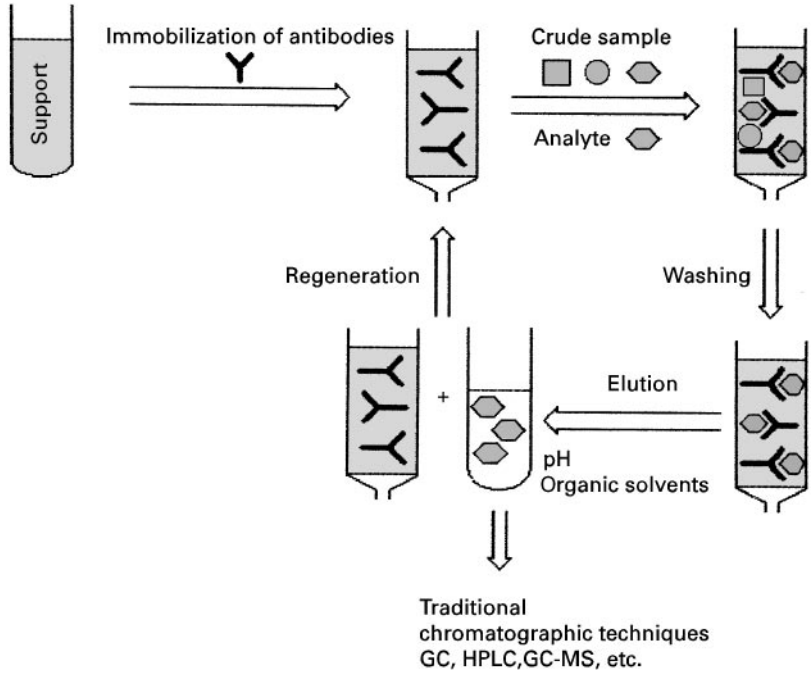


Figure 6 Schematic separation of analytes by immunoaffinity chromatrography for pesticide determination in food matrices.

can overcome some of these limitations. In the LC-MS techniques, the use of an atmospheric pressure chemical ionization interface is at present the best alternative since it offers high selectivity and sensitivity for the trace determination of carbamates.

See also: **II/Affinity Separation:** Immunoaffinity Chromatography. **Chromatography: Gas:** Detectors: Mass Spectrometry. **Chromatography: Liquid:** Detectors: Mass Spectrometry. **Extraction:** Supercritical Fluid Extraction. **III/Immunoaffinity Extraction. Multiresidue Methods: Extraction. Pesticides:** Extraction from Water; Gas Chromatography; Supercritical Fluid Chromatography; Thin-Layer (Planar) Chromatography.

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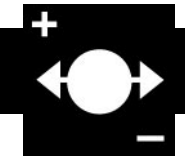
CARBOHYDRATES



Electrophoresis

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Introduction

Electrophoresis has been an important tool for carbohydrate analysis since its early stages of development. Moving with time from paper electrophoresis to polyacrylamide slab gel electrophoresis and then to the sophisticated high performance capillary