Flow Injection Determination of Pesticides

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A general view about the determination of pesticides in the FIA methodology is presented. The review is dealing with the different published analytical procedures divided into three groups: a) homogeneous systems (only solutions) in which the reported procedures are classified according to the used detector; b) heterogeneous systems (solid-liquid or liquid-liquid); and c) a third group is presented with the papers dealing with the FIA assembly as the sample pretreatment for the injection into a chromatograph. A table with the most relevant analytical information of each of reported procedure is also presented.

PESTICIDES. DEFINITION AND FUNDAMENTALS

A pesticide is any chemical used to fight a pest (an animal or vegetable parasite threatening agricultural crops, cattle or human health). Such a broad definition, by

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tacit convention, excludes drugs and veterinary products. Hence, a pesticide can be defined as a chemical used to fight external (animal and vegetable) parasites that attack crops.

The FAO/WHO define the word “pesticide” as any substance or mixture of substances used to prevent and control growth of undesirable vegetable or animal species, including any single or mixed substance used for plant growth control, as a defoliant or as a desiccant. In an explanatory note, the term “pesticide” is said to encompass any substance used to control “pests” during production, storage, transport, marketing and processing of foods for human or animal consumption, as well as any substance that can be administered to animals in order to prevent growth of insects and arachnids on their bodies. Therefore, the word “pesticide” is not used to refer to antibiotics, nor to any other chemicals given to animals for purposes other than those previously stated (e.g. to foster growth or improve reproductive behaviour) or used as fertilizers.

Based on the above definition, pesticides include the following groups: insecticides used against insects, acaricides against mites, nematocides against nematodes, molluscocides against snails and slugs, rodenticides against rodents, fungicides or anticyryptogamic agents against fungal crop parasites, general and specific herbicides against weeds, farming antibiotics against crop bacterioses and soil disinfectants, which fight nematodes, insects, pathogenic fungi and weeds present in cultured soils.

CLASSIFICATION

Pesticides can be classified according to various criteria, namely:

(1) Chemical composition:
   (a) Inorganic pesticides, which include sulphur, arsenic, fluorine, copper and chlorine containing compounds, and various other inorganic substances.
   (b) Natural organic pesticides, typically coumarins, natural pyrethrins and plant extracts.
   (c) Synthetic organic pesticides such as organochlorine and organophosphorus compounds; carbamates and dithiocarbamates; organic cyanides; organic sulphocyanic esters; bipyridyl salts; aromatic nitro compounds; organic acid derivatives; urea, triazine and uracil derivatives as well as other heterocycles; synthetic pyrethroids, etc.

(2) Use:
   According to Spanish Technical Health Regulations, officially enforced by the Royal Decree 3349/1983, pesticides can be used for phytosanitary, cattle-protective, food industrial, environmental, personal hygiene and household purposes.

(3) Hazards:
   Depending on the severity of the risks associated with their inhalation, ingestion and dermal contact, pesticides can be classified as:
   (a) Scarcely hazardous, which pose no appreciable risks.
   (b) Noxious, which may pose moderately serious risks.
(c) **Toxic**, which can lead to severe damage (acute or chronic) or even death.
(d) **Very toxic**, which pose extremely serious hazards of the acute or chronic type, or even result in death.

(4) Formulation:
In their broadest sense, agricultural pesticides are applied to crops in two different forms, viz. as pure chemicals or, more frequently, in formulations (i.e. in mixtures intended to boost their efficiency). Pesticide formulations are either solid (wettable, soluble or sprinkling powder, granules) or liquid (water-soluble or emulsion-forming products, or fluid suspensions).

**TOXICITY AND ENVIRONMENTAL IMPLICATIONS**

Because most pesticides are highly persistent, their extensive use has led to a gradual build-up in the environment, which has started to feel its effects. Since pesticides are usually applied to crops, they are initially found mostly on plants and soil, and in air. Subsequently, rain and irrigation water sweep most of these compounds to waterways, aquifers, etc. The presence of pesticides in the aquatic environment decreases the quality of water, which acquires an odd colour and/or taste, and has various indirect toxic effects on many species.

Many countries have issued maximum allowed concentrations of pesticides over the past few years. EEC directive 76/464 has compiled a black and a red list that include pesticides and other toxic substances for ground water. European laws accept a maximum concentration of 0.1 μg l⁻¹ for any single pesticide and a maximum overall pesticide concentration of 0.5 μg l⁻¹ in drinking water. By contrast, USA laws have established a specific allowable level for each individual pesticide and even for some by-products, which are occasionally more toxic than the parent compounds. This appears more reasonable than the fixed levels established by the EEC since concentrations above 0.1 μg l⁻¹ of some pesticides are harmless and their analysis does not call for extremely sensitive methods [1].

Pesticide residues must be removed from the environment and their effects on it lessened. The so-called "persistent" or "refractory" pesticides cannot be degraded naturally (by biological processes, interaction with soil substances or the action of sunlight), so they require more powerful methods such as treatment with active sludge, chemical oxidation, ozonization, photodegradation, etc. [2]. Pesticide build-up gradually leads to environmental deterioration reflected, for example, in the loss of natural resources such as genetic bio-diversity, drinking water, fertile soil, etc. This in turn decreases living standards. The combined effects of pesticides result in high economic losses through both the decay of resources (e.g. tourist resorts) and the need to invest in repairing the damage.

The persistence and toxicity of pesticides calls for their strict, exhaustive control by use of sensitive methods affording expeditious processing of large numbers of samples. This paper reviews reported FIA analytical methods for the determination of pesticides. Applications are dealt with in two groups according to the type of
chemical system used: homogeneous (single-phase) or heterogeneous (multi-phase). The former are in turn exposed according to the type of detector used.

HOMOGENEOUS SYSTEMS

Detectors based on radiant energy-matter interactions

UV-Visible detection. The pesticide paraquat was determined spectrophotometrically by reduction to a blue semi-quinoid radical of $\lambda_{\text{max}} = 600$ nm using two different methods. In one [3], the pesticide was reduced with alkaline sodium dithionite. The sample was injected into a water stream that was subsequently merged with one of 0.1 mol l$^{-1}$ NaOH containing a 1% concentration of the reductant. The determination of paraquat in mixed formulations is interfered by the presence of diquat, which gives a greenish radical of $\lambda_{\text{max}} = 605$ nm. In order to avoid its adverse effect, diquat is removed from the sample by precipitation with sodium hydroxide before injection. Determining paraquat in waters poses no special problem. In foods (potatoes), it requires preconcentration on a column packed with a cation-exchange resin and elution with an NaCl saturated solution.

The other method for paraquat [4] includes reduction of the pesticide with a solution of ascorbic acid partly oxidized to dehydroascorbic acid with potassium iodate. According to these authors, this solution remains stable for longer (up to 5 days) than one of ascorbic acid or sodium dithionite. The idea of pre-oxidizing the ascorbic acid arose from the finding that the absorbance increased as the solution aged. The authors checked empirically that dehydroascorbic acid plays an important role in the development of the blue colour.

This reaction has also been used with $p$-aminophenol [5-9]. In an alkaline medium containing an oxidant, benzoquinoneimine is produced that can be coupled to a pesticide (e.g. to $m$-aminophenol, the primary metabolite for formethanate) by electrophilic attack on C$_4$ to give the corresponding blue diamine. This reaction has been used in FIA spectrophotometric determinations for other types of compound (e.g. drugs).

The organophosphorus pesticide chlorpyrifos [10] can be determined by spectrophotometric monitoring of its decomposition in an alkaline medium, where the analyte undergoes gradual hydrolysis and a concomitant bathochromic shift in the absorption maximum compared to the spectrum of the starting compound. The hydrolysis reaction is started by injecting the sample into a carrier stream containing sodium hydroxide, hydrogen peroxide and ethanol. The presence of H$_2$O$_2$ appears to increase the rate at which the pesticide is degraded, the process being monitored via the increase in the absorption at 328 nm (new band) and the decrease in the chlorpyrifos band at 290 nm, using the stopped flow technique. The other factor influencing the decomposition rate is temperature (45°C was found to be optimal). The calibration curve is obtained by plotting the rate of the first-order reaction involved against the pesticide concentration. Using kinetic methodology, the method allows the determination of chlorpyrifos in the presence of dimethoate and endosulfan with the need for no prior separation.
**IR absorption detection.** Infrared spectrophotometry is one of the less frequently used detection techniques in FIA. Its inherent shortcomings (e.g. the need to use KBr pellets and its scant application in solution) have somehow deterred usage in continuous-flow assemblies. Its application to organic compounds entails using also organic solvents and hence solvent-proof rather than conventional pump tubing. The alterations required to use this type of detector in FIA include a novel type of flow-cell [11], the “circle cell”, which causes light to be reflected many times between the sample and a ZnSe crystal.

The earliest FIA-IR determination was reported by Curran and Collier in 1985 [12]. They quantified phenyl isocyanate by using a CCl₄ stream and a stainless steel piston pump.

More recent FIA-IR determinations include those of organic drugs and the pesticide carbaryl [13, 14]. A pesticide solution in dichloromethane was injected into a carrier stream containing the same solvent. Readings were made directly at 1747 cm⁻¹, in both continuous manner or in the stopped flow mode. The flow-cell used had a light path length of 1.0 mm. This method offers the typical advantages of IR measurements. The sensitivity of the method was increased by inserting a conventional cartridge to preconcentrate the sample by solid-liquid extraction, followed by elution with the dichloromethane carrier.

**Fluorimetric detection.** One highly sensitive method for determining diquat is based on its reduction with alkaline sodium dithionite to a highly emissive greenish cation radical (see Reaction 1) [15]. The radical is more extensively conjugated than the non-reduced molecule and exhibits an absorption band that is much stronger and appears at a higher wavelength than that for diquat (497 vs 346 nm). The method is also selective enough for determining the herbicide in a wide variety of samples without a prior separation. Thus, paraquat has been quantified in commercially available pesticide formulations, waters, foods (potatoes), soil, flowers, blood serum, and synthetic and true urine samples. Use of a single-channel, FIA assembly (with a sodium dithionite carrier, buffered with borax at pH 8) was precluded by the instability of the reductant solution. Rather a two-channel manifold should be used, where the sample is injected into a borax carrier (pH 8) and subsequently merged with 0.2% reductant solution.

![Reaction 1. Reduction of diquat](image)

The well-known increased quantum yield of various molecules in organized media (micelles) was used for the determination of carbendazim in water samples [16]. This compound is the main metabolite of benomyl and results from its hydrolysis, which takes place in both acid and basic media. Carbendazim is highly stable in an acid or neutral medium, but is further degraded to 2-aminobenzimidazole in
alkaline solution. Water samples containing the pesticide were injected into a buffered carrier that was merged with a surfactant solution. The two surfactants studied, sodium dodecyl sulphate and cetyltrimethylammonium bromide (both in 0.1 mol l⁻¹ HCl), increased the emission intensity by a factor of 2.6 and 11.6, respectively.

**Chemiluminescence detection.** There are very few references to FIA chemiluminescence (CL) methods. In one [17], the emission arises from oxidation of *bis*(2,4,6-trichlorophenyl)oxalate by hydrogen peroxide. The oxidation product transfers its excited state to an "effective" fluorophore (the reaction product formed between the pesticide and a fluorescent reagent). Of the various reagents tested, morin exhibited a high CL intensity, which, however, was accompanied by also high background noise. The flavones tested behaved similarly to morin. Quinoline derivatives proved scarcely fluorescent and highly chemiluminescent, so they were selected. The experimental procedure involves injecting the sample into a carrier-reagent stream containing the fluorescent agent (quinoline), followed by merging with a buffer carrier containing imidazole nitrate at a roughly neutral pH and then with a stream of hydrogen peroxide and *bis*(2,4,6-trichloro-phenyl)oxalate.

\[
\text{PESTICIDE + QUINOLINE} \rightarrow \text{PESTICIDE - QUINOLINE (Fluorophore)}
\]

\[
\begin{array}{c}
\text{Cl-} \quad \text{O-C-C-O-} \quad \text{Cl} + \text{H}_2\text{O}_2 \rightarrow \\
\text{Cl-} \quad \text{O-C-C-O-} \quad \text{Cl} + 2 \text{HO-Cl}
\end{array}
\]

\[
\text{bis(2,4,6-trichlorophenyl)oxalate(TCPO)} \quad 1,2\text{-dioxetane-dione}
\]

\[
\begin{array}{c}
\text{0-0} \\
\text{C-C} \\
\text{O-O}
\end{array} \quad + \text{Fluorophore} \rightarrow \text{Fluorophore* (excited state)} + 2\text{CO}_2
\]

**Reaction 2.** Chemiluminescent procedure transferring the excited state to an effective fluorophore

A method for the determination of paraoxon and aldicarb relies on their inhibitory effect on acetylcholinesterase [18]. The choline formed from the enzyme is oxidized by choline oxidase and the hydrogen peroxide released is determined by the luminol reaction with peroxidase. The chemical process involved is as follows:
Acetylcholine \[ \xrightarrow{\text{acetylcholinesterase}} \] acetate + choline \hspace{1cm} (1)

Choline \[ \xrightarrow{\text{choline oxidase}} \] betaine + H\(_2\)O\(_2\) \hspace{1cm} (2)

Luminol + 2H\(_2\)O\(_2\) + OH\(^-\) \[ \xrightarrow{\text{peroxidase}} \] aminophthalate anion + N\(_2\) + 3H\(_2\)O + light \hspace{1cm} (3)

**Reaction 3.** Chemiluminescent basis for the determination of paraoxon and aldicarb

The resulting decrease in chemiluminescence intensity is related to the amount of pesticide in the sample.

**Atomic absorption detection.** Organoarsenical pesticides are usually determined by oxidation to arsenate ion using a mixed chemical-photochemical reaction [19]. The sample is injected into a distilled water carrier and merged with a stream of potassium sulphate; the resulting solution is irradiated with UV light from a mercury lamp. The following steps involve the conversion of arsenate ion into arsine and its subsequent volatilization with the aid of a helium stream that drives it to the detector (an atomic absorption spectrometer). According to the authors of the method, the conversion of the organoarsenical into arsenate involves the decomposition of persulphate by the UV light; under these conditions, the anion gives highly reactive hydroxyl radicals that can decompose organic compounds to carbon dioxide. The proponents claim that the light-induced decomposition of organoarsenicals to arsenate, water and carbon dioxide is also mediated by hydroxyl radicals.

On-line liquid-liquid separation was used for the FIA-AAS determination of dimethoxydithiophosphate [20] (see under liquid-liquid systems).

**Detectors based on electrical current-matter interactions**

This group includes several amperometric determinations proposed by the same research group. The earliest was reported in 1988 and involved the determination of the pesticides fenthion and fenitrothion [21]. The only channel of the FIA manifold was used to circulate a methanol-water carrier stream buffered with acetic-acetate solution that was deaerated by passage through a zinc column and gaseous nitrogen; the stream also contained the sample. The electrode, glassy carbon, was ground with sandpaper prior to use. The determination was cathodic (at \(+0.9\) V) for fenthion and anodic (at \(+1.3\) V) for fenitrothion. Polarizing the working electrode took 30 min for the anodic process and 15 min for the cathodic process at the potentials used. Hydrodynamic voltammograms exhibited no plateau for fenthion, so the potential leading to the most favourable signal-to-noise ratio was selected. The limiting current
for fenitrothion reached a plateau at $E = -0.9$ V, above which background noise rose considerably. Also, below $-1.0$ V the pesticide signal was not significantly greater.

Paraoxon ($O,O$-diethyl-$O$-4-nitrophenylphosphate) and parathion ($O,O$-diethyl-$O$-4-nitrophenylphosphorothioate) have also been determined electrochemically. They are used jointly in some pesticide formulations, so an effective quantitation method for both does not require prior separation. FIA amperometric method [22] determines paraoxon in the presence of parathion after alkaline hydrolysis of the former and subsequent oxidation of the $p$-nitrophenol formed at a glassy carbon electrode. Parathion is resolved on the basis of its different rate of hydrolysis. The hydrolytic reaction is conducted on-line. For this purpose, the sample, in a water–alcohol solution, is injected into a $0.40 \text{ mol l}^{-1}$ NaOH stream flowing at a rate of $1.5 \text{ ml min}^{-1}$ along a $3 \text{ m}$ line; the reaction yield is greater with a knotted reactor than with a coiled one. The resulting solution is merged with a $0.5 \text{ mol l}^{-1}$ acetic acid solution in such a way that partly hydrolysed paraoxon reaches the detector at pH 5 and the $p$-nitrophenol produced is oxidized at the glassy carbon electrode. This electrode is used in conjunction with an Ag/AgCl reference electrode and a gold counter-electrode. $p$-Nitrophenol is oxidized rather than reduced in order to avoid the interference of non-hydrolyzed parathion, which cannot be oxidized electrochemically, and that of dissolved oxygen, which thus need not be previously removed.

Parathion has also been determined in the presence of paraoxon by previously hydrolysing the analyte and oxidizing the $p$-nitrophenol produced at $E = 1.2$ V [23]. The selective hydrolysis of the analyte is carried out outside the manifold because it is not rapid enough for FIA implementation. It is performed in the presence of Pd(II) or Hg(II), which have a high affinity for the thiophosphate group and thus favour the hydrolysis of parathion over that of paraoxon. The determination can be carried out in the presence of $p$-nitrophenol by subtracting the FIA signals obtained with and without the prior hydrolysis.

A FIA coulometric assembly was used for the oxidation of carbamate and urea pesticides [24]. The ensuing method uses only a few nanolitres of samples and features a relative standard deviation below 1%. The flow-cell accommodates a Pt wire as the working electrode, Ag/AgCl as the working electrode and another Pt wire as the auxiliary electrode. The integration time is not critical since it is long enough for the whole sample to pass through the cell; however, it should not be so long that background current may introduce some error. According to the authors, the coulometric efficiency of the method is close to 100% at low flow-rate and very high potential.

Zürn et al. [25] developed an membrane-immobilized urease electrode. Ion-sensitive field-effect transistors (ISFETs) are effective alternatives to the glass electrode as they can act as enzyme-sensitive transducers (ENFET) when the biochemical reaction involved produces or consumes hydrogen ions. A membrane containing urease was used to extend application of ISFETs to urea and pesticides. Urease catalyses the hydrolysis of urea to ammonium ion and carbon dioxide, which is monitored via pH. The membrane was prepared photolithographically. Urease activity is known to be inhibited by metal ions. This principle was recently exploited for determining carbofuran with a detection limit of $0.1 \mu\text{g l}^{-1}$ by use of the enzyme immobilized on a membrane.
HETEROGEOUS SYSTEMS

Liquid-liquid systems

Dimethoxydithiophosphate (DDTP) can be determined indirectly by formation of a complex with Cu(II) that is subsequently extracted into chloroform. The amount of copper in the complex is determined in an ammonia-ammonium buffer at pH 10 following back-extraction [20]. The method is based on an earlier, batch method that, according to the authors, was somewhat imprecise owing to the low stability of the complex, formed between copper and the pesticide, Cu(DDTP)$_2$. For this reason, they used extraction with an organic solvent followed by mineralization of the extract or back-extraction. In the manifold, the organic solvent (containing the analyte extracted from the sample) was merged with an aqueous stream (back-extractant) and reached the phase separator, where the aqueous phase, free from the organic one, was injected with the aid of a conventional valve into a distilled water carrier that drove it to the detector.

The aqueous and organic phases are separated by passage through a hydrophilic membrane held between two teflon sheets. The results thus obtained are compared with those provided by batch back-extraction and with the mineralization of the organic extracts. This methodology was applied to the determination of malathion in a commercially available formulation.

Solid-liquid systems

Enzymes are the most widely immobilized reagents, and also the best documented in this respect. Their high selectivity and their catalytic action makes them ideal for use in solid-phase reactors.

There are hundreds of reported FIA applications of enzymes, particularly determinations of substrates where the enzyme acts as a catalyst, whether in solution or in immobilized form – the more frequent by far. Less often, the analyte is the enzyme and its activity determined. Also, the inhibition of enzyme activity can be used for analytical purposes; this strategy, however, has scarcely been explored in FIA.
In an application of the latter type [26], a known amount of enzyme was incubated with the sample solution. The resulting activity was inversely proportional to the amount of inhibitor present in the sample. For example, the inhibition of the activity of acetylcholinesterase can be monitored photometrically according to the following reaction (Reaction 4a): or electrochemically according to the following mechanism (Reaction 4b):

\[
\text{Acetylthiocholine} \rightarrow \text{thiocholine + acetate}
\]

\[
\text{Thiocholine + dithiobisnitrobenzoate} \rightarrow \text{thionitrobenzoate (yellow dye)}
\]  

\[
\text{Acetylcholine} \rightarrow \text{choline + acetate}
\]

\[
\text{choline + } 2\text{O}_2 + \text{H}_2\text{O} \rightarrow 2\text{H}_2\text{O}_2 + \text{betaine}
\]

\[
\text{ACHE} = \text{Acetylcholinesterase}
\]

\[
\text{CHO} = \text{Choline oxidase}
\]

**Reaction 4.** Determination based on the inhibitory effect of the activity of acetylcholinesterase

The measured parameter is the residual activity of the immobilized enzyme (following reaction with the sample), which entails immobilization and release of the enzyme for each new sample processed. For this purpose, acetylcholinesterase is immobilized on magnetic particles via its amino groups by using a magnetic reactor consisting of a permanent magnet that can be electrically offset by connection to the integrated circuit. This method was used to determine carbofuran and malaoxon in water samples.

Paraoxon was determined by immobilizing acetylcholinesterase on controlled-pore glass (CPG) beads [27]. The method relies on the inhibition of the enzymatic hydrolysis of α-naphthyl acetate and the subsequent reaction of the α-naphthol formed with p-nitrobenzene-diazonium fluoroborate. For this purpose, the pesticide and substrate are made to reach the detector simultaneously. The FIA manifold used includes two injection valves for the same channel; one is used to inject the sample into a 0.05 mol l\(^{-1}\) phosphate carrier containing NaCl and MgCl\(_2\), while the other is employed to inject the substrate solution into the sample stream 10 s later. The sample then travels through the reactor containing the immobilized enzyme and merges with the reagent channel to form a product that is monitored spectrophotometrically at 500 nm.

In a sequel to the previous application, reaction and detection were integrated in a spectrophotometric flow-cell. In the next method [28], carbofuran, propoxur and carbaryl were determined with no prior separation by hydrolysis to their corresponding
Determination of pesticides

phenols (carbofuran-phenol, 2-isopropoxyphenol and 1-naphthol). They were coupled with sulfanilic acid to give coloured products that were determined jointly on a diode array spectrophotometric detector using nine different wavelengths. Two different manifolds were tested. In one, the analytical reaction was monitored while the sample solution was passed through the detector (i.e. in the usual way). In the other, the flow-cell was packed with C₁₈ bonded phase beads to retain the reaction products, which were then eluted, thus flushing the cell and making it ready for a new sample. The chief conclusion drawn was that, of the two operating modes used (conventional FIA and integrated reaction detection), the integrated one was significantly more sensitive.

In the other method of the sequel [29], paraquat was determined by integrating retention of the analyte on a Dowex-type cation-exchange resin and reaction in a solid-phase reactor that was placed in the flow-cell. The reaction was monitored via the increase of absorbance. Finally, the reaction product was eluted by injecting an appropriate solvent. The analytical reaction employed was the classical reduction of paraquat with alkaline sodium dithionite to give a blue radical. Ammonium chloride proved to be the best reagent for eluting the reaction product retained in the flow-cell. In fact, ammonium ion was found to excel potassium, calcium, magnesium and sodium ions for this purpose. Because these ions can elute paraquat, they can also pose some interferences. As a preventive measure, samples were treated with EDTA at pH 9 to avoid them. The method was used for the determination of paraquat in waters, as well as to study the pesticide adsorption in soils (clays and humic acids). For the latter purpose, a known amount of soil was placed in a beaker and supplied with water and paraquat. Subsequently, periodic measurements of the paraquat content in the supernatant solution were made. The temporal variation of paraquat adsorption by the soils always fitted an exponential curve; adsorption peaked at about 6 h and was very high (ca 90% or even higher in some soils). The organic matter content of the soil had a marked effect on the pesticide adsorption.

Table I provides a global picture of FIA methods for the quantitation of pesticides; applications are grouped according to the particular pesticide determined. Also, Table I shows the chemical methods and detector types used, as well as the analytical figures of merit of each application.

FIA assemblies for the preparation and insertion of samples into a chromatograph

FIA-HPLC. FIA methodology arose and was initially developed as a convenient, expeditious means for transferring samples to a detector, as an interface between sample preparation and analytical instrumental measurement. Therefore a natural trend is reflected in some applications to pesticides.

One determination for the organophosphorus pesticides azinphosmethyl and fenthion [43] involves on-line extraction in an FIA manifold coupled to an HPLC instrument equipped with a spectrophotometric detector. Both pesticides are separated with n-heptane, which effects partial extraction. The sample is inserted into the
Table 1. Analytical survey of FIA methods for determination of pesticides

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Chemical Method</th>
<th>Detector</th>
<th>Linearity range (detection limit)</th>
<th>Reproducibility (%) (Sample throughput, h⁻¹)</th>
<th>Sample</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldicarb(a)</td>
<td>inhibition of acetylcholinesterase (immobilised)</td>
<td>CL</td>
<td>(4 µg l⁻¹)(a) (0.75 µg l⁻¹)(b)</td>
<td>6.6(a) 3.7(b) (15)</td>
<td>soil</td>
<td>18</td>
</tr>
<tr>
<td>Paraoxon(b)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>vegetables</td>
<td></td>
</tr>
<tr>
<td>Atrazine</td>
<td>antibodies immobilised</td>
<td>F 320–404 nm</td>
<td>0.02–0.3 µg l⁻¹</td>
<td></td>
<td>water</td>
<td>37</td>
</tr>
<tr>
<td>Atrazine</td>
<td>automated quasi-continuous immunoanalysis</td>
<td>UV-VIS</td>
<td>30 ng l⁻¹–1 µg l⁻¹</td>
<td>3–20</td>
<td>water</td>
<td>39</td>
</tr>
<tr>
<td>Atrazine</td>
<td>reaction with two antibodies immobilised</td>
<td>F 320–404 nm</td>
<td>1 and 30 ng l⁻¹</td>
<td>2.9–8.3</td>
<td>water, soil</td>
<td>42</td>
</tr>
<tr>
<td>Azinphosmethyl-A</td>
<td>alkaline hydrolysis</td>
<td>amperometric</td>
<td>up 96.6 µmol l⁻¹ (0.41 µmol l⁻¹)</td>
<td>2.2</td>
<td></td>
<td>34</td>
</tr>
<tr>
<td>Carbamates Organophosphorus</td>
<td>inhibition of butylicolinesterase (immobilised)</td>
<td>UV-VIS</td>
<td>(0.5–275 ppb)</td>
<td></td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>Carbamates Organophosphorus</td>
<td>inhibition of acetylcholinesterase (immobilised)</td>
<td>potentiometric electrode pH</td>
<td>(0.5–275 ppb)</td>
<td>(4)</td>
<td>water</td>
<td>31</td>
</tr>
<tr>
<td>Carbaryl(a) 1-naphtol(b) (metabolite)</td>
<td>formation indophenol UV-VIS 596 nm</td>
<td>UV-VIS</td>
<td>1.2–6 mg l⁻¹ (a) (26.5 ng ml⁻¹)(b) 2–8 mg l⁻¹ (a) (11.8 ng ml⁻¹)(b)</td>
<td>0.3(a) (110)</td>
<td>waters</td>
<td>6</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>without reaction, sample in CH₂Cl₂</td>
<td>FTIR</td>
<td>continuous-flow 0.25–10 mg ml⁻¹ stopped-flow 0.125–10 mg ml⁻¹ (11 µg ml⁻¹)</td>
<td>0.8 (53) 0.3 (90)</td>
<td>commercial formulation</td>
<td>13</td>
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<tr>
<td>Parathion</td>
<td>off-line hydrolysis, determination of ( p )-nitrophenol</td>
<td>amperometric glassy carbon electrode</td>
<td>( 1.96 \times 10^{-7} - 7.14 \times 10^{-6} \text{ mol l}^{-1} ) (( 3.4 \times 10^{-8} \text{ mol l}^{-1} ))</td>
<td>2.3</td>
<td>23</td>
<td></td>
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<tr>
<td>Pesticide</td>
<td>membrane with pesticide specific antibody, automated quasi-continuous</td>
<td></td>
<td>F</td>
<td>(atrazine 0.02 ( \mu \text{g l}^{-1} )) (propazine 0.03 ( \mu \text{g l}^{-1} ))</td>
<td>(4)</td>
<td>water</td>
</tr>
<tr>
<td>Propoxur</td>
<td>formation indophenol, on-line hydrolysis</td>
<td>UV-VIS 600 nm</td>
<td>(0.1 mg ( l^{-1} ))</td>
<td>0.1</td>
<td>(80)</td>
<td>natural waters</td>
</tr>
<tr>
<td>Ziram</td>
<td>complex formation with Cu, liquid extraction with MIBK</td>
<td>AAS 324.7 nm</td>
<td>6.0–12 ( \mu \text{mol l}^{-1} ) (0.12 ( \mu \text{mol l}^{-1} ))</td>
<td>2.6</td>
<td></td>
<td>35</td>
</tr>
</tbody>
</table>

1 SDS: sodium dodecyl sulphate.
2 CTAB: cetyltrimethylammonium bromide.
3 Reported results are corresponding to acetylcholinesterase.
4 DDTP: Dimethoxydithiophosphate. A certain number of organophosphorus pesticides belong to the DDTP family (like Malathion).
5 Organotin compounds: di-\( n \)-butyltin dichloride (DBTC); diphenyltin dichloride (DPTC); tri-\( n \)-butyltin chloride (TBTC); triphenyltin chloride (TPTC).
(a), (b), (c) – see miscellaneous applications.
<table>
<thead>
<tr>
<th>Table 1 (continuation)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dichlorvos</strong></td>
</tr>
<tr>
<td><strong>Diquat</strong></td>
</tr>
<tr>
<td><strong>Diquat(a)</strong></td>
</tr>
<tr>
<td><strong>Diquat</strong></td>
</tr>
<tr>
<td><strong>Ethiofencarb</strong></td>
</tr>
<tr>
<td><strong>Fenthion(a)</strong></td>
</tr>
<tr>
<td><strong>Fenitrothion(b)</strong></td>
</tr>
<tr>
<td><strong>Formetanate(a)</strong></td>
</tr>
<tr>
<td><strong>Formetanate</strong></td>
</tr>
<tr>
<td>Organoaarsenicals</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Organotin Compounds (DBTC; DPTC; TBTC and TPTC)</td>
</tr>
<tr>
<td>Paraoxon</td>
</tr>
<tr>
<td>Paraoxon</td>
</tr>
<tr>
<td>Paraoxon</td>
</tr>
<tr>
<td>Paraoquat</td>
</tr>
<tr>
<td>Paraoquat</td>
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</tbody>
</table>

Determination of pesticides
<table>
<thead>
<tr>
<th>Substance</th>
<th>Reaction/Method</th>
<th>Method/Condition</th>
<th>Sensitivity/Range</th>
<th>Medium</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbaryl</td>
<td>without reaction, sample in CH₂Cl₂, solid-liquid extract</td>
<td>FTIR</td>
<td>(15 µg g⁻¹)</td>
<td>water</td>
<td>14</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>reaction with NaNO₂ + sulphanilic acid</td>
<td>UV-VIS 515 nm</td>
<td>0.1–40 ppm 0.5 ppm</td>
<td>water</td>
<td>33</td>
</tr>
<tr>
<td>Carbendazim</td>
<td>micellar medium SDS¹(a) CTAB²(b)</td>
<td>283–368 nm (a) 306–327 nm (b)</td>
<td>(32 µg g⁻¹) (4 µg g⁻¹)</td>
<td>waters</td>
<td>16</td>
</tr>
<tr>
<td>Carbofuran Malaoxon³</td>
<td>inhibition of enzymatic activity</td>
<td>UV-VIS 405 nm electrochemical</td>
<td>(0.5 µg g⁻¹)</td>
<td>water</td>
<td>26</td>
</tr>
<tr>
<td>Carbofuran³</td>
<td>hydrolysis, diazotized</td>
<td>continuous-flow flow-through sensor</td>
<td>3.61(a) 3.5(b) 2.26(c)</td>
<td>water</td>
<td>28</td>
</tr>
<tr>
<td>Carbofuran³</td>
<td>hydrolysis, diazotized</td>
<td>UV-VIS</td>
<td>continuous-flow flow-through sensor</td>
<td>3.61(a) 3.5(b) 2.26(c)</td>
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<td>water</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>kinetic determination, degradation-reaction in medium NaOH + H₂O₂</td>
<td>UV-VIS</td>
<td>(0–2 × 10⁻⁴ mol g⁻¹) (6.6 × 10⁻⁶ mol g⁻¹)</td>
<td>water</td>
<td>10</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>kinetic determination, degradation-reaction in medium NaOH + H₂O₂</td>
<td>UV-VIS</td>
<td>(0–2 × 10⁻⁴ mol g⁻¹) (6.6 × 10⁻⁶ mol g⁻¹)</td>
<td>water</td>
<td>10</td>
</tr>
<tr>
<td>o-Cresol⁴</td>
<td>hydrolysis with NaOH, reaction with KIO₃⁺ p-aminophenol</td>
<td>UV-VIS 614 nm (a) 632 nm (b)</td>
<td>up 4.8 µg ml⁻¹ (a) up 9.6 µg ml⁻¹ (b)</td>
<td>water</td>
<td>38</td>
</tr>
<tr>
<td>m-Cresol⁴</td>
<td>hydrolysis with NaOH, reaction with KIO₃⁺ p-aminophenol</td>
<td>UV-VIS 614 nm (a) 632 nm (b)</td>
<td>up 4.8 µg ml⁻¹ (a) up 9.6 µg ml⁻¹ (b)</td>
<td>water</td>
<td>38</td>
</tr>
<tr>
<td>DDT⁴</td>
<td>complex formation in CHCl₃, back-extraction into NH₃/NH₄⁺ and into the FIA manifold</td>
<td>AAS 324.8 nm</td>
<td>8.5–17 mg g⁻¹ (0.39 mg g⁻¹)</td>
<td>commercial formulation</td>
<td>20</td>
</tr>
<tr>
<td>Carbamate and urea derivatives</td>
<td></td>
<td>coulometric</td>
<td></td>
<td>commercial formulation</td>
<td>24</td>
</tr>
</tbody>
</table>
FIA system in a continuous manner and, once extracted, the organic phase is injected in small portions at preset intervals. The FIA manifold extracts the pesticides and the chromatograph performs the separation and individual detection of the pesticides in the extract. The method was developed for determining pesticides in waste water.

On-line separation as a preliminary step in the insertion of extract into a chromatographic column was performed in the determination of chlorfenvinphos, methyl parathion and their respective degradation products (2,4,5-trichlorophenol and 4-nitrophenol) [44]. The sample was also injected in a continuous mode, using no injection valve, and merged with the organic extractant.

**HPLC-FIA.** The on-line preparation of samples of mono- di- and tributylphényltin [45] involves retaining the analytes by adsorption on bonded silica with octadecyl functional groups and isolating the analytes from the sample matrix. This separation–preconcentration step, which is followed by *in situ* derivatization of the retained pesticides by ethylation with 3% sodium tetraethylborate in water. Once derivatized, the analytes are eluted with methanol and driven to the gas chromatograph for separation and subsequent microwave-induced plasma atomic emission detection. The method was applied to river samples.

The other natural trend in FIA is the development of detection methods for use with post-column derivatization in HPLC. Because the weakest part of the chromatograph – in terms of research developments – is the detector, this could make a suitable target for FIA results. However, this has not been the case so far. Strictly speaking, this is not a HPLC-FIA coupling since the latter uses no injection valve and the sample (*viz.* the effluents from the chromatographic column) is inserted in a continuous manner. The organophosphorus pesticides paraoxon and di-isopropylfluorophosphate, and the carbamates namely isopropyl-N-(3-chloromethyl) carbamate and N-phenyl carbamate were determined in binary mixtures by injecting two pesticides into a chromatographic column (reversed-phase chromatography with tetrahydro-furan–water as the mobile phase). The effluent was merged with a stream of the substrate (α-naphthyl acetate) that was circulated along one of the channels in the unsegmented continuous-flow manifold. It passed through a solid-phase reactor containing acetylcholinesterase immobilized on controlled pore glass (CPG) beads [46]. Beyond the reactor, the mixture was merged with a p-nitrobenzenediazonium fluoroborate solution and driven to the spectrophotometric detector for measurement at 500 nm. Detection relied on inhibition of the enzyme catalytic action.

**Miscellaneous applications**

This section deals with special methods that depart somehow from those described in the preceding sections.

Eight phenoxyacid herbicides (*viz.* MCPA; MCPP; MCPB; 2,4-D; 2,4-DP; 2,4-DB; 2,4,5-T and 2,4,5-TP) plus bentazone were determined in water samples with thermospray tandem mass spectrometry [47]. Experimental parameters were optimized in two steps. In the first one, the analytes were continuously introduced into the system and the influence of the vaporizer temperature, discharge voltage and repeller voltage was studied under a wide range of experimental conditions.
From the different solvent mixtures and source block temperatures (vaporizer temperatures) tested, 90+10 (v/v) ammonium acetate-acetonitrile was found to be the best option. The procedure was fully automated and afforded detection limits as low as 1 mg l\(^{-1}\); also, the calibration graph was linear up to 50 mg l\(^{-1}\).

The determination of water pollutants by immunoassay is a novel research trend aimed at gradually replacing chromatographic procedures. Special emphasis in this respect has been placed on pesticides and, chiefly, herbicides. The reason behind the growing use of immunoassays is the need to detect concentrations below the microgram-per-litre level. Wortberg et al. combined the principles of immunoaffinity chromatography [48] with those of FIA by immobilizing fluorescein-labelled haptens on oxirane acrylic beads that constituted the affinity portion of the assembly. If the haptens are saturated with antibodies of the Eu(III) chelate-labelled type, then some antibodies are displaced by the analyte to an extent dependent on the affinity of the antibodies. The FIA manifold used is very simple: the sample is injected into a stream resulting from the merging of the reagent and a buffer solution. Subsequently, the sample is passed through an affinity column containing the hapten derivative immobilized on oxirane acrylic beads and driven to a fluorimeter. The FIA cycle involves three steps, namely:

(a) packing of the affinity column with the labelled antibodies and subsequent flushing;

(b) injection of the sample and halting of the flow for 20–40 min;

(c) fluorimetric detection. Taking into account that loading the antibodies in the first step can take about 15 min, the overall cycle requires 45–70 min.

The method was applied to s-triazine herbicides. One of the methods for the determination of herbicides relies on the photobleaching of the bacteriochlorophyll dimer (P), which as a result is oxidized [49]. The process can be monitored via the decrease in the absorbance at 860 nm, where the starting dimer absorbs, but its oxidation product does not. In addition to macromolecular complexes present in the solution, electrons can recombine directly with the oxidation product. The recombination rate varies markedly depending on the actual process that takes place. Thus, recombination with the different intermediate states can take from 1 to 100 s (lifetime), depending on the particular bonded groups or substances (ubiquinones or herbicides). The recombination can be triggered by flash excitation and monitored via temporal changes in the absorbance. The irradiation must be controlled in such a way as to maximize differences between the bleaching levels obtained from the replacement with herbicides or ubiquinone. This principle was used to develop biosensors with reaction sites holding *Rodobacter sphaeroides* (from purple bacteria), which allowed the determination of various herbicides in the batch mode [50]. Subsequently, the same strategy was used in a continuous FIA assembly that was irradiated with a He-Ne laser source located between two photodiodes which measured the incoupling light.

REFERENCES

Determination of pesticides

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