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# Determination of organophosphorus pesticide residues in human tissues by capillary gas chromatography–negative chemical ionization mass spectrometry analysis

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**Abstract**

We describe an analytical method that allows the determination of organophosphorus pesticides (OPs) in different human tissues. It involves an extraction procedure with ethanol–ethyl acetate, followed by gel permeation chromatography clean-up step and analysis by capillary gas chromatography–negative chemical ionization mass spectrometry in the selected ion monitoring mode. The method was tested for 37 OPs and the recoveries obtained vary between 60 and 106% with standard deviations ranging between ±2 and ±10. These values are independent of the analyzed tissue. Peak area repeatability as RSD for some OPs was ≤4.8% while a good linear relationship in the range 1.0–500 pg $\mu l^{-1}$ with $r^2 \geq 0.9878$ was obtained. The limit of detection for the 37 OPs falls between 0.01 and 0.09 ng $g^{-1}$ with an RSD≤9.5%. The analytical set up in this paper has been used to analyze different samples of human tissues (liver, healthy kidney, cancer kidney and adipose tissue) of 24 patients. The number of the identified OPs in the tissue samples is different (max. 20) according to the sample while their concentration ranges between the limit of detection and 28.0 ng $g^{-1}$. The highest concentrations have been determined in liver samples without any pathology (0.4–28.0 ng $g^{-1}$) while the lowest concentrations have been determined in healthy kidney samples (0.01–1.50 ng $g^{-1}$). In the cancer kidney samples OP concentrations vary between 0.03 and 4.6 ng $g^{-1}$: these concentrations are more elevated than those determined in healthy kidney samples. The comparison between the concentration of OPs determined in the healthy part, when possible, and those determined in the cancer part of the same kidney sample are very interesting: in fact, in the latter the OP concentration is generally 1–2-times higher than that in the former, an index of lower enzymatic activity in the cancer tissue.


*Keywords:* Organophosphorus pesticide residues

## 1. Introduction

Organophosphorus pesticides (OPs) are widely used in agriculture and their properties provide numerous benefits in terms of production and quality increase. As a result, consumers are indirectly ex-

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posed to pesticides, usually in minute quantities, through several food groups including meat, dairy products, fruits, vegetables, dried foods, most processed foods, and many other household products [1]. The OPs exhibit a large degree of persistence in the environment, which causes various health and safety problems [2]. One of the most important OP reactions is water hydrolysis. This reaction can take place at the P atom or in the alkylic chain and in general induces a loss of pesticide action. The oxidation of P=S to P=O is another common and important reaction that takes place under biotic and abiotic conditions. The phosphorodithionate conversion into their O-homologues, i.e., the phosphates, strongly increases the toxicity for insects and mammals. The typical reaction with O-phosphorothioates is the thiol–thiol isomerization induced by light and temperature. In this way, the O-phosphorothioates are transformed into S-phosphorothioates, which are more toxic than the parent compounds. The OPs inhibit the phosphorylate esterases, particularly the enzyme acetylcholinesterase, thus causing an accumulation of the neurotransmitter acetylcoline [3]. The cholinesterase inactivation by OPs involves a reaction in which one substituent group, i.e., the leaving group, produces a dialkylphosphoryl enzyme. The vast majority of insecticides produce a dimethylphosphorylate enzyme or a diethylphosphorylate enzyme, and the reactivation kinetics are the same for each derivative regardless of the OP leaving group structure. The dimethylphosphorylate enzyme reactivation is (considerably) quicker than that one of the diethyl equivalent and it occurs in few hours [4]. Acute intoxication with OPs can cause major effects such as convulsions, respiratory failure, and cardiac arrhythmias, all of which can result in anoxia. It is hardly surprising that major intoxication is sometimes associated with long-term central nervous system changes. A number of studies on this problem have shown behavioral and psychological alterations, and others have failed to demonstrate any effect [5]. It should never be forgotten that OPs may have other properties entirely independent of their anticholinesterase effects, including mutagenicity and carcinogenicity as well as specific organ toxicity to heart, liver, kidney, and other organs [6]. Even if the OP analysis is a well-known task and has been applied to different matrices, like biota, animal tissues, food (meat, fish, potatoes, fruits, vegetables), water and wine [7–15], at present no references on OP determination in human tissues are reported in the literature. Concerning the analytical method, OP analysis is normally performed by gas chromatography (GC) coupled to selective detection methods such as electron-capture detection (ECD), nitrogen–phosphorous detection (NPD), photometric detection (FPD) and by both electronic impact (EI) and positive (PCI) and negative chemical ionization (NCI) mass spectrometry (MS), and high-performance liquid chromatography with diode array detection (HPLC–DAD) [16–26].

The present paper describes a multiresidue OP determination method for complex matrices of different tissues. The method involves an extraction with ethanol–ethyl acetate (2:98, v/v), followed by a gel permeation chromatography (GPC) clean up step and analysis by capillary gas chromatography–negative chemical ionization mass spectrometry (GC–NCI-MS) in the selected ion monitoring (SIM) mode. The method was tested for 37 organophosphorus insecticides by means of a recovery study from different human tissues.

## 2. Experimental

### *2.1. Equipment and apparatus*

A Hewlett-Packard (HP, Palo Alto, CA, USA) Model 5890 series II gas chromatograph, equipped with a programmed temperature vaporizing (PTV, Dani, Monza, Italy) injector and connected to a mass spectrometer Model 5889A, and the data system ''Chemstation 59940A'' were used for GC–MS analysis in the SCAN and NCI modes.

An ultra-turrax T8 homogenizer (Janke&Kunkel, UK), a CWS4235, an ultrasonic clear Uniset-Ac., a Büchi 011 rotavapor (Büchi, Switzerland), a Varian Model 5400 pump (Varian Instruments, San Ferdinando, CA, USA) and a lambda 5 UV–Vis spectrophotometer (Perkin-Elmer, Milan, Italy) were used. The silica gel solid-phase extraction column, containing 500 mg of adsorbent, was from J.T. Baker (Phillipsburg, NJ, USA).

### 2.2. Chemicals

Acetone, hexane, ethyl acetate, ethanol and methanol (pesticide grade), anhydrous sodium sulfate (ACS reagent grade) were obtained from Carlo Erba (Milan, Italy). Decanol (99%) from Aldrich (Milan, Italy). Bio-beads SX3 (mesh size 200–400) were from Bio-Rad (Richmond, CA, USA).

Pesticide standards: acephate, omethoate, phorate-oxon, phorate, dimethoate, propetamphos, terbufos, diazinon, paraoxon-methyl, disulfoton, parathion-methyl, malaoxon, paraoxon, ronnel, fenitrothion, pirymiphos, malathion, fenthion, chlorpyriphos, parathion-ethyl, ethion, carbophenothion, ENP, oxo-azinphos-methyl, phosalone, azinphos-methyl, azinphos-ethyl, Co-ral-o and Co-ral (Coumaphos) were obtained from Società Italiana Chimici (Milan, Italy). Standard solutions (1 g $l^{-1}$) were prepared by dissolving the pesticides in acetone. These solutions were further diluted with *n*-hexane to prepare final solutions for spiking (1.25 ng $\mu l^{-1}$ and 12.5 ng $\mu l^{-1}$ for disulfoton, paraoxon-methyl, parathion-methyl and malaoxon).

### 2.3. Preparation and calibration of the GPC column

A 40-g amount of stationary phase (Bio-Beads SX3, mesh size 200–400) was used for the packed glass column (25 cm×2.5 cm) with PTFE tap, porous septum and conic adapter (Marbaglass, Rome, Italy) for the connection ''in series'' with the SPE silica gel column. The GPC column was conditioned with 150 ml (50 ml×3) of a mixture 30% of ethyl acetate in *n*-hexane. The GPC column was calibrated using 5 ml of a standard mixture of pesticides (bromophos ethyl, acephate and parathion methyl, 1 mg $l^{-1}$ each), eluted with 300 ml of a solution 30% of ethyl acetate in *n*-hexane. The eluate (volumes of 4 ml) were analyzed by spectrophotometry at 288 nm. The collection pesticide volume was between 76 and 202 ml (126 ml).

### 2.4. Human tissue samples

The human tissue samples (liver, kidney, adipose) were supplied from ''Policlinico Umberto I'' of the University ''La Sapienza'' of Rome and stored at −20 °C in an $N_2$ atmosphere. They were obtained from autopsied tissues of patients with various causes of death. The samples from patients of the Urological Clinic of the University of Rome ''La Sapienza'' were given to us with the agreement of the ethical committee.

### 2.5. Tissue sample analysis (liver and kidney)

Human tissue samples were used to study the clean-up technique recovery and efficiency. Liver and kidney samples were freeze–dried; about 2–3 g of sample was spiked with a standard pesticide mixture to 15 and 50 ng $g^{-1}$ concentrations of freeze–dried material. The sample was homogenized for 1 min, anhydrous sodium sulfate (1 g tissue+5 g $Na_2SO_4$) and a mixture 2% ethanol in ethyl acetate (1 g tissue+10 ml mixture) were added. It was placed in an ultrasound bath for 5 min at 25 °C and was centrifuged at 3000 rpm for 2 min. After adding a few drops of solution 5% of decanol in acetone as a ''keeper'', the liquid phase was evaporated in a rotary evaporator until 3 ml volume. It was kept in a freezer for 90 min; after centrifugation at 3000 rpm for 40 s, we obtained an extract. The liquid phase was diluted with 7 ml of *n*-hexane and 5 ml of the liquid sample underwent the following clean-up method. The same procedure was applied to the analysis of the blank and real tissue samples.

### 2.6. GPC clean-up

GPC was used to remove the lipid material. Prior to GPC clean-up the organic phase was filtered through a Gelman glass-fiber pad (pore size 5–10 μm) to remove any suspended particles. For GPC analysis, a laboratory-prepared Bio-Beads S-X3 (mesh size 200–400) glass column was used. The extract was introduced onto the column and eluted with 250 ml of ethyl acetate–*n*-hexane (3:7, v/v) at a flow-rate of 5 ml $min^{-1}$. The first 76 ml was discharged and the silica Sep-Pak cartridge, previously conditioned with 6 ml of ethyl acetate–*n*-hexane (3:7, v/v), was quickly connected ''in series''. After collection of 132 ml eluate, the mobile phase flow was stopped and the cartridge was

disconnected. It was eluted with 15 ml of acetone–ethyl acetate (1:4, v/v) at a flow-rate of 5 ml $min^{-1}$. Both elutes were combined and evaporated in a rotary evaporator at 45 °C and the residue was dissolved in 0.2 ml of acetone–ethyl acetate (1:4, v/v) prior to injection into the GC–MS system. The volume injected onto the GC column was 2 μl.

### 2.7. Validation study

Liver and kidney human tissues were fortified at 50 and 15 ng $g^{-1}$ with an OP standard mixture. Three replicates including extraction, concentration, GPC clean-up and analysis procedures, of each fortification level for both matrices, were prepared.

### 2.8. GC–MS analysis

A gas chromatograph series II Model 5890 connected to a mass spectrometer Model 5889A and interfaced to a data system ''Chemstation 59940A'' (HP) was used. A 2 m×520 μm I.D. uncoated column, retention gap, connected ''in series'' with a 30 m×250 μm I.D. fused-silica capillary column, coated with 0.25 μm of chemically immobilized SE-54 (HP) and directly introduced into the mass spectrometer ion source, was used. Helium was the carrier gas at a flow-rate of 1 ml $min^{-1}$. The column was kept at 60 °C for 2.7 min, and then was programmed from 60 to 173 °C at 25 °C $min^{-1}$; after 2 min it was programmed from 173 to 195 °C at 2 °C $min^{-1}$, and successively from 195 to 270 °C at 25 °C $min^{-1}$. The PTV injector was performed in the total-sample-injection mode. Five seconds after injection the vaporizer was heated from 60 to 300 °C at 800 °C $min^{-1}$ and cooled after 120 s; the splitter valve was closed for 70 s. The transfer line, ion source and analyzer were held at 280, 200 and 230 °C, respectively.

Scan acquisition was from $m/z$ 45 up to 600 u at 1.68 scan $s^{-1}$. The NCI mode was used for the analysis of the different OP compounds. Methane was used as reagent gas at $1.7 \cdot 10^{-4}$ Torr (1 Torr= 133.322 Pa).

### 2.9. Quantitation of organophosphorus compounds

The quantitative OP analysis in the human tissue samples has been performed by means of a comparison of a standard pesticide mixture using the ''bracketing'' method (analysis of standard, analysis of sample and analysis of standard; all the samples are analyzed under the same experimental conditions).

## 3. Results and discussion

### 3.1. Optimization of the analytical method

From systematic studies [27–30] it was obvious that the solvent polarity and solvent strength have a dominant influence on the extraction and on the elution behavior of analytes. Different solvents, cyclohexane or mixtures like dichloromethane+ cyclohexane, ethyl acetate or mixtures of toluene–ethyl acetate, etc., were studied [31–34]. For aspects of toxicity and of environmental problems we eliminated any solvents like benzene (carcinogenic effect) and dichloromethane (carcinogen). With regard to the boiling point of solvents, especially toluene (b.p.= 110.8 °C) should not be used. Because of these considerations preliminary studies were performed by using ethyl acetate as extracting solvent mixed with acetone or ethanol or methanol in different ratios. The 2% mixture of ethanol in ethyl acetate was found to be the most suitable to extract OPs from human tissue samples (recovery>96%). Moreover, it is insoluble in water, interacts poorly with matrix and quantitatively extracts OPs.

The eluting mixture ethyl acetate–hexane (3:7) in comparison with all the others, is polar enough to elute the OPs in a small volume range (132 ml) and to quantitatively separate the matrix components from pesticides. GPC is excellent for the removal of lipids and other large molecules from sample extracts. When coupled in series with a silica gel SPE column to remove polar matrix co-extractives, a clean-up extract can be obtained. A filtered liver extract representing a 2 g sample has a dry mass of 39.5 mg before clean-up. This extract following clean-up has a dry mass of 2.1 mg.

To optimize the experimental analysis conditions in GC–NCI-MS, different tests of the considered analytes were performed. In Table 1 the retention times, the molecular mass, the typical fragment ions

Table 1
Peak, molecular mass, CAS registry number, retention time, typical fragment ion (abundance 100%), ion mass and limit of detection and RSD of each organophosphorus pesticide in GC–NCI-MS

| Peak | Molecular mass | Compound | CAS registry number | $t_R$ (min) | Typical fragment | Ion mass | LOD (ppb) | RSD (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | 183 | Acephate | [30560-19-1] | 9.42 | $[(CH_3)_2OPSNHCO]^-$ | 168 | 0.05 | 9.1 |
| 2 | 213 | Omethoate | [1113-02-6] | 11.13 | $[(CH_3O)_2PSO]^-$ | 141 | 0.07 | 5.6 |
| 3 | 244 | Phorate oxon | [2600-69-3] | 11.42 | $[(C_2H_5O)_2POS]^-$ | 169 | 0.06 | 7.0 |
| 4 | 260 | Phorate | [298-02-2] | 12.69 | $[(C_2H_5O)_2PS_2]^-$ | 185 | 0.04 | 9.0 |
| 5 | 229 | Dimethoate | [60-51-5] | 13.33 | $[(CH_3O)_2PS]^-$ | 157 | 0.03 | 9.5 |
| 6 | 281 | Propetamphos | [31218-83-4] | 14.32 | $[(CH_3)_2CH_2NHO_2PS]^-$ | 154 | 0.05 | 8.9 |
| 7 | 288 | Terbufos | [13071-79-9] | 14.35 | $[(C_2H_5O)_2PS_2]^-$ | 185 | 0.06 | 7.5 |
| 8 | 246 | Fonofos | [944-22-9] | 14.50 | $[(C_2H_5OC_2H_5PS_2]^-$ | 169 | 0.02 | 8.7 |
| 9 | 304 | Diazinon | [333-41-5] | 14.86 | $[(C_2H_5O)_2PSO]^-$ | 169 | 0.02 | 9.1 |
| 10 | 247 | Paraoxon-methyl | [950-35-6] | 15.05 | $[M]^-$ | 247 | 0.05 | 8.5 |
| 11 | 274 | Disulfoton | [298-04-4] | 15.07 | $[(C_2H_5O)_2PS_2]^-$ | 185 | 0.05 | 4.2 |
| 12 | 263 | Parathion-methyl | [298-00-0] | 17.19 | $[M]^-$ | 263 | 0.02 | 8.1 |
| 13 | 314 | Malaoxon | [1634-78-2] | 17.24 | $[(CH_3O)_2POS]^-$ | 172 | 0.04 | 6.7 |
| 14 | 375 | Paraoxon-ethyl | [311-45-5] | 17.81 | $[M]^-$ | 375 | 0.04 | 9.1 |
| 15 | 321 | Ronnel | [299-84-3] | 17.90 | $[Cl_2C_6H_5O_2P]^-$ | 211 | 0.01 | 7.8 |
| 16 | 277 | Fenitrothion | [122-14-5] | 18.76 | $[M]^-$ | 277 | 0.04 | 8.0 |
| 17 | 333 | Pirimiphos-ethyl | [23505-41-1] | 18.83 | $[(C_2H_5)_2N_3C_2HO]^-$ | 141 | 0.03 | 8.9 |
| 18 | 330 | Malathion | [121-75-5] | 19.35 | $[(CH_3O)_2PS_2]^-$ | 157 | 0.03 | 9.1 |
| 19 | 278 | Fenthion | [55-39-9] | 19.93 | $[(CH_3O)_2PSO]^-$ | 141 | 0.02 | 9.0 |
| 20 | 350 | Chlorpyriphos | [2921-88-2] | 19.93 | $[M\text{-}HCl]^-$ | 313 | 0.02 | 8.5 |
| 21 | 291 | Parathion-ethyl | [56-38-2] | 19.96 | $[M]^-$ | 291 | 0.02 | 8.0 |
| 22 | 360 | Chlorfenvinphos | [470-90-6] | 21.66 | $[(C_2H_5O)_2PO_2]^-$ | 153 | 0.02 | 5.0 |
| 23 | 345 | Isofenphos | [25311-71-1] | 21.68 | $[C_6H_5O_2PSC_2H_5NHC_2H_4]^-$ | 244 | 0.04 | 6.9 |
| 24 | 302 | Methidathion | [950-37-8] | 22.05 | $[(CH_3O)_2PS_2]^-$ | 157 | 0.05 | 5.6 |
| 25 | 394 | Bromophos-ethyl | [4824-78-6] | 22.14 | $[M\text{-}HCl)^-$ | 358 | 0.04 | 9.0 |
| 26 | 374 | Profenophos | [41198-08-7] | 22.75 | $[(C_6H_3BrPO_3S]^-$ | 267 | 0.05 | 9.2 |
| 27 | 314 | DEF | [78-48-8] | 22.81 | $[(n\text{-}But)_2S_3PO]^-$ | 257 | 0.01 | 9.4 |
| 28 | 215 | Carbophenothion-methyl | [953-17-3] | 23.33 | $[(CH_3O)_2PS_2]^-$ | 157 | 0.05 | 9.5 |
| 29 | 384 | Ethion | [563-12-2] | 23.60 | $[(C_2H_5O)_2PS_2]^-$ | 185 | 0.01 | 5.9 |
| 30 | 343 | Carbophenothion | [786-19-6] | 23.94 | $[(C_2H_5O)_2PS_2]^-$ | 185 | 0.02 | 6.0 |
| 31 | 323 | EPN | [2104-64-5] | 25.04 | $[M]^-$ | 323 | 0.05 | 9.0 |
| 32 | 301 | Oxo azinphos-methyl | | 25.69 | $[C_2H_6H_4CH_2N_3H]^-$ | 133 | 0.04 | 8.1 |
| 33 | 368 | Phosalone | [2310-17-0] | 25.75 | $[(C_2H_5O)_2PS_2]^-$ | 185 | 0.01 | 8.2 |
| 34 | 317 | Azinphos-methyl | [86-50-0] | 25.77 | $[(CH_3O)_2PS_2]^-$ | 157 | 0.03 | 7.0 |
| 35 | 345 | Azinphos-ethyl | [2642-71-9] | 26.56 | $[(C_2H_5O)_2PS_2]^-$ | 185 | 0.04 | 7.1 |
| 36 | 346 | Co-ral-O-analog | [321-54-0] | 26.74 | $[M]^-$ | 346 | 0.05 | 6.5 |
| 37 | 362 | Co-ral (Coumaphos) | [56-72-4] | 27.54 | $[M]^-$ | 362 | 0.04 | 6.4 |

(abundance 100%) and the limit of detection (LOD) of the 37 OPs studied are reported. The limit of detections of the OPs analyzed, using the typical fragment ion each, are between 0.01 and 0.09 ng $ml^{-1}$ with RSDs≤9.5%. These values were determining according to Knoll's definition [35], i.e., an analyte concentration that produces a chromatographic peak equal to three times the standard deviation of the baseline noise. Furthermore, it should be underlined that the LOD of each pesticide is comparable with those obtained by other authors in different studies [7,8,10,25]. Based on Table 1 various comments can be done. The formation of the typical fragment ion reported in Table 1 will depend on the type of molecule. In this sense it is possible that two or more compounds will have an ion with the same $m/z$ value but with different structures. This is the case for OPs of different classes: e.g., $m/z$ 185 corresponds to $[(C_2H_5O)_2PS_2]^-$, i.e., ethion, phosalone, carbophenothion, terbufos, disulfoton,

anziphos-ethyl and phorate, $m/z$ 157 corresponds to $[(CH_3O)_2PS_2]^-$, i.e., malathion, methidathion, carbophenothion-methyl, dimethoate and azinphos-methyl, $m/z$ 169 corresponds to $[(C_2H_5O)_2PSO]^-$, i.e., phorate oxon and diazinon, $m/z$ 141 corresponds to $[(CH_3O)_2PSO]^-$, i.e., omethoate and fenthion. The diagnostic ions formed under NCI are indicated in Table 1; in many instances the base peaks of the different compounds and also, in a few instances, the only spectral information were obtained. This is a typical characteristic of GC–MS-NCI of OPs, so their unequivocal identification should always be carried out in combination with the retention time data. The formation of $[M]^-$ has a 100% relative abundance for the organophosphorus compounds of the so-called parathion group and compounds having an aromatic moiety (with exception of chlorinated organophosphorus pesticides). This is due to the fact that $[M]^-$ is fairly stable under NCI conditions when an aromatic moiety exists in the molecule, and is even more important when a nitro group is present. The chlorinated OPs with an aromatic moiety yield intense or base peaks corresponding to losses of Cl owing to the facility of such processes under NCI conditions. This is the case with chlorpyriphos and bromophos-ethyl. The formation of thiophenolate versus phenolate ions for compounds such as parathion-methyl, fenitrothion, parathion-ethyl, EPN and Co-ral is due to the strong acidity of thiophenolate versus phenolate in the gas phase and the oxygen atom of the aromatic moiety is replaced by a sulfur atom. The fragment ion formation at $m/z$ values of 168 (fenitrothion), 225 (Co-ral) and 154 (parathion-methyl, EPN and parathion-ethyl) gives the formula $[X\text{-}ArS]^-$. In these compounds the thiophenolate anion is favored with respect to the formation of a phenolate anion so that the preferred phenyl transfer followed by cleavage of the P–S bond occurs.

Table 2
Ion mass, linearity range and correlation coefficient of the OP standard mixture in GC–NCI-MS (SIM)

| Compound | Ion mass | Linearity range (pg $\mu l^{-1}$) | $r^2$ |
|---|---|---|---|
| Acephate | 168 | 1–500 | 0.9958 |
| Omethoate | 141 | 1–500 | 0.9972 |
| Phorate oxon | 169 | 2–500 | 0.9864 |
| Phorate | 185 | 1–500 | 0.9997 |
| Dimethoate | 157 | 2–500 | 0.9998 |
| Propetamphos | 154 | 3–500 | 0.9997 |
| Terbufos | 185 | 1–500 | 0.9967 |
| Fonofos | 169 | 1–500 | 0.9921 |
| Diazinon | 169 | 1–500 | 0.9986 |
| Paraoxon-methyl | 247 | 3–500 | 0.9854 |
| Disulfoton | 185 | 2–500 | 0.9915 |
| Parathion-methyl | 263 | 3–500 | 0.9934 |
| Malaoxon | 172 | 1–500 | 0.9997 |
| Paraoxon-ethyl | 375 | 2–500 | 0.9924 |
| Ronnel | 211 | 2–500 | 0.9962 |
| Fenitrothion | 277 | 1–500 | 0.9921 |
| Pirimiphos-ethyl | 141 | 2–500 | 0.9864 |
| Malathion | 157 | 1–500 | 0.9994 |
| Fenthion | 141 | 1–500 | 0.9996 |
| Chlorpyriphos | 313 | 3–500 | 0.9964 |
| Parathion-ethyl | 291 | 2–500 | 0.9972 |
| Chlorfenvinphos | 153 | 1–500 | 0.9825 |
| Isofenphos | 244 | 2–500 | 0.9989 |
| Methidathion | 157 | 1–500 | 0.9993 |
| Bromophos-ethyl | 358 | 2–500 | 0.9991 |
| Profenophos | 267 | 1–500 | 0.9924 |
| DEF | 257 | 1–500 | 0.9990 |
| Carbophenothion-methyl | 157 | 2–500 | 0.9938 |
| Ethion | 185 | 1–500 | 0.9878 |
| Carbophenothion | 185 | 1–500 | 0.9991 |
| EPN | 323 | 3–500 | 0.9975 |
| Oxo azinphos-methyl | 133 | 3–500 | 0.9879 |
| Phosalone | 185 | 1–500 | 0.9926 |
| Azinphos-methyl | 157 | 2–500 | 0.9931 |
| Azinphos-ethyl | 185 | 2–500 | 0.9912 |
| Co-ral-O-analog | 346 | 1–500 | 0.9956 |
| Co-ral (Coumaphos) | 362 | 2–500 | 0.9996 |

The calibration data obtained for OP standard mixture are reported in Table 2. The linearity of the response was good for the used concentration ranges with a correlation coefficient between 0.9825 and 0.9997. In order to evaluate the reliability of the GC–NCI-MS (SIM) analysis a standard solution of some OPs (10 ng $ml^{-1}$ each) being representative of the different analyzed compounds, was analyzed and the RSD was calculated: Table 3 shows the results obtained for five OP replicate analyses where RSD results are $\leq$4.8%. In Table 4 the percentage recoveries of the 37 investigated pesticides at different concentrations are reported. The values were obtained by clean-up of the liver and kidney samples spiked with OPs (15 and 50 ng $g^{-1}$). The recoveries ranged between 60 and 104% for the kidney and 63–106% for the liver samples with a relative standard deviation varying between 2 and 10%: therefore, in this case the recoveries are independent on the matrix. These values take into account the

Table 3
Repeatability ($n=5$) of a standard solution containing nine OPs analyzed by GC–NCI-MS (SIM) (10 ng $ml^{-1}$ each, 1 μl injected)

| Compound | Selected ion | Average area | SD | RSD (%) |
|---|---|---|---|---|
| Fonofos | 169 | 197 090 | 9510 | 4.8 |
| Ronnel | 211 | 301 820 | 11 140 | 3.7 |
| Chlorfenvinphos | 153 | 81 211 | 2253 | 2.8 |
| Bromophos-ethyl | 358 | 55 520 | 2060 | 3.7 |
| DEF | 257 | 85 718 | 3818 | 4.4 |
| Ethion | 185 | 142 029 | 3741 | 2.6 |
| Carbophenethion | 185 | 169 077 | 4353 | 2.6 |
| EPN | 323 | 16 513 | 729 | 4.4 |
| Phosalone | 185 | 130 720 | 3241 | 2.5 |

entire analytical procedure (i.e., OP extraction, clean-up, and analysis steps).

### 3.2. Analysis of real samples

The method described in this paper, several times tested with different steps, was applied to determine OP residues in different human organ samples. Twenty-five samples of human tissue, 11 of liver and 14 of kidney (and for 10 of them also the cancerous portion of the organ was able to be examined) were analyzed. Fig. 1a and b reports the chromatograms of a blank run and of a healthy kidney sample containing more OPs.

In Table 5 results on kidney samples of five different patients without any pathology are reported. Twelve OPs were determined at a concentration ranging between 0.01 and 1.5 ng $g^{-1}$. The pesticides more present in the tissue samples are ronnel, ethion, carbophenothion, Co-ral and phosalone: the latter is recorded in all the analyzed samples at a concentration ranging between 0.03 and 0.90 ng $g^{-1}$. In kidney sample 3 and from the newborn the presence of DEF, respectively, at 0.50 and 0.10 ng $g^{-1}$, was found, thus being the metabolite of carbophenothion.

In Table 6 results obtained on nine samples of kidney tissue with cancer pathologies are reported. For some patients both the healthy kidney tissue (K) and the cancer tissue (K*) were analyzed while for others adipose tissue was analyzed. It can be observed in Table 6 that 18 OPs were identified at concentrations ranging between 0.01 and 4.60 ng $g^{-1}$. The more common OPs were ronnel, chlorphenvinphos, DEF, ethion, carbophenothion, phosalone and Co-ral. Even if the highest concentration level was observed for malaoxon (4.60 and 4.60 ng $g^{-1}$), for isofenphos (3.40 and 3.40 ng $g^{-1}$) and for profenophos (4.30 and 4.40 ng $g^{-1}$). These three OPs were found in the K* sample of kidney of patients 6 and 8. In this table it is also evidenced that in the K* kidney sample the same pesticide is present at concentration 10-times higher than in the healthy portion (Co-ral, anziphos-ethyl, ronnel and fonophos in patient 5; ethion and ronnel in patient 6). In patients whose adipose tissue was analyzed, it was found that it contained, even if at a lower concentration, the same OPs present in the kidney (healthy and K*) tissues.

In Table 7 the results obtained for 11 samples of liver tissue without any pathologies are reported: all present an accumulation of OPs. In the liver tissue samples 20 OPs were determined at concentrations ranging between 0.40 and 28.00 ng $g^{-1}$. It can be also observed that some of these pesticides are present in all the analyzed samples: they are ronnel, ethion and phosalone. Parathion-methyl and chlorphenvinphos are absent only in samples 5 and 1, respectively. In sample 2 the highest number of OPs, i.e., 14, was recorded, followed by sample 3 with 11 OPs and sample 4 with 10 OPs. The RSDs of the results reported in Tables 5–7, are $\leq$9.5%.

On observing the results in Table 6 it can be noted that for the patients for whom both healthy and cancerous samples of their kidney were analyzed, it is evidenced that in the latter the concentration is generally higher, thus indicating that the cancerous organ is unable to completely perform the metabolising function. Comparing Tables 5 and 6 other considerations can be made: (1) the OP concen-

Table 4
Percent recoveries ($n=3$) and standard deviations (SDs) of each OP obtained from liver and kidney samples spiked with 80 and 15 ng $g^{-1}$ of analyzed compounds

| Compound | Recovery (%) | | | |
|---|---|---|---|---|
| | Kidney | | Liver | |
| | 15 ppb±SD | 50 ppb±SD | 15 ppb±SD | 50 ppb±SD |
| Acephate | 93±3 | 91±4 | 92±4 | 90±4 |
| Omethoate | 80±4 | 79±4 | 86±3 | 85±3 |
| Phorate-oxo | 61±4 | 60±4 | 72±5 | 70±7 |
| Phorate | 64±5 | 62±5 | 68±6 | 66±9 |
| Dimethoate | 81±3 | 80±3 | 104±4 | 106±4 |
| Propetamphos | 78±4 | 76±4 | 86±5 | 82±9 |
| Terbufos | 70±5 | 68±5 | 71±5 | 68±2 |
| Fonofos | 72±5 | 71±5 | 84±4 | 83±6 |
| Diazinon | 101±2 | 104±3 | 89±3 | 86±9 |
| Paraoxon-methyl | 100±3 | 102±2 | 100±2 | 98±5 |
| Disulfoton | 76±5 | 74±5 | 67±6 | 63±5 |
| Parathion-methyl | 89±4 | 87±4 | 88±4 | 83±3 |
| Malaoxon | 88±3 | 86±4 | 90±5 | 85±10 |
| Paraoxon-ethyl | 94±3 | 91±4 | 75±6 | 68±9 |
| Ronnel | 97±2 | 95±3 | 95±3 | 92±5 |
| Fenitrothion | 90±3 | 89±4 | 93±3 | 90±6 |
| Pirymiphos | 78±4 | 77±4 | 90±4 | 88±9 |
| Malathion | 98±2 | 96±3 | 98±3 | 96±5 |
| Fenthion | 95±3 | 94±4 | 86±4 | 81±6 |
| Chlorpyriphos | 84±4 | 80±5 | 85±4 | 80±7 |
| Parathion-ethyl | 95±2 | 91±5 | 88±4 | 83±4 |
| Chlorfenvinphos | 91±3 | 89±4 | 97±3 | 95±5 |
| Isofenfos | 101±3 | 103±3 | 99±2 | 98±4 |
| Methidathion | 101±3 | 102±2 | 91±4 | 87±5 |
| Bromophos-ethyl | 90±3 | 88±4 | 90±3 | 86±3 |
| Profenofos | 80±5 | 78±5 | 67±7 | 64±5 |
| DEF | 85±4 | 82±5 | 95±3 | 92±2 |
| Carbophenothion-methyl | 78±5 | 76±5 | 87±4 | 82±5 |
| Ethion | 82±4 | 80±5 | 89±4 | 84±6 |
| Carbophenethion | 79±5 | 79±5 | 91±3 | 87±5 |
| EPN | 85±4 | 86±3 | 101±2 | 98±5 |
| Phosalone | 93±3 | 94±3 | 104±3 | 103±4 |
| Oxo-azinphos-methyl | 99±2 | 98±3 | 100±2 | 101±3 |
| Azinphos-methyl | 94±3 | 93±3 | 98±3 | 96±5 |
| Azinphos-ethyl | 95±3 | 94±4 | 75±5 | 65±9 |
| Co-ral-O-analog | 90±3 | 91±3 | 96±3 | 91±4 |
| Co-ral | 81±5 | 84±4 | 93±4 | 88±7 |

trations in healthy kidney are of the same order as those ones in the healthy portion of a cancerous kidney; (2) the OP concentrations in the cancerous part are much higher by about one order than those recorded in a healthy kidney. In the case of liver samples the OP concentrations are higher than those in the kidney, probably due to the different function of the two organs.

Further, a few significant evaluations can be preliminarily suggested even if the data are few: analyzing by two different statistic tests, i.e., SAS software with the GLM procedure and analysis of variance of two factors (class of compounds and healthy and non-healthy patients) and $t$ test, the resulting values are statistically significant, $P<0.01$ and $P<0.05$, respectively.

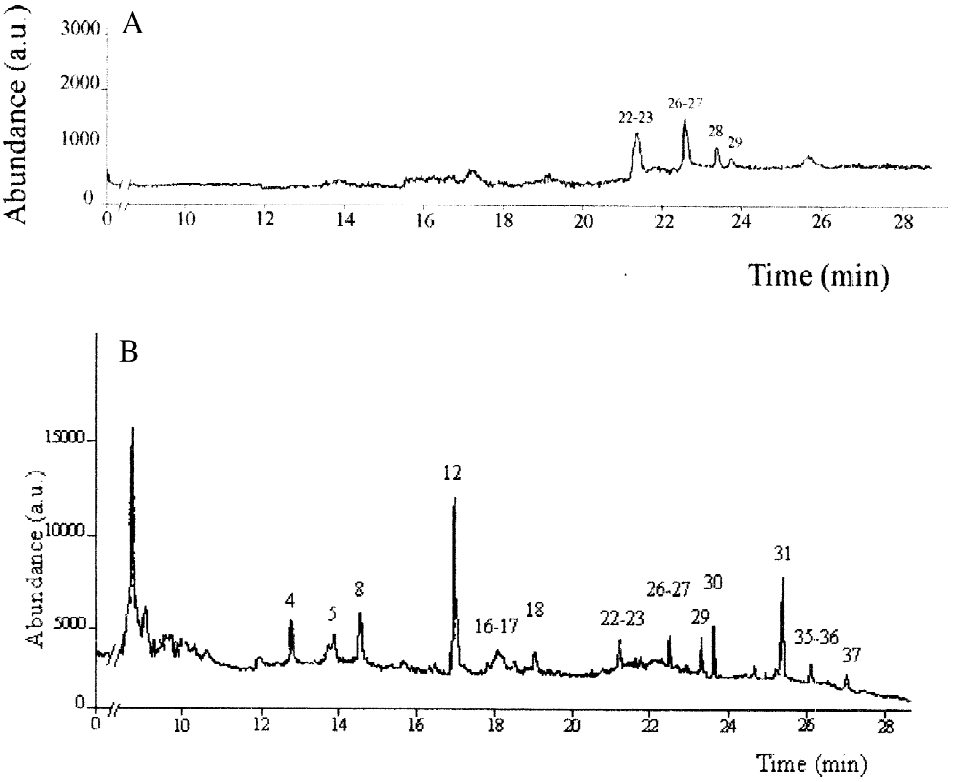


Fig. 1. TICs of a blank run (a) and of a healthy kidney sample (b). See text for experimental conditions and Table 1 for peaks.

Table 5
Organophosphorus pesticides (ng $g^{-1}$) determined in kidney samples of healthy patients

| Compound | Patient 1 kidney | Patient 2 kidney | Patient 3 kidney | Patient 4 kidney | Newborn kidney |
|---|---|---|---|---|---|
| Dimethoate | 0.2 | | | | |
| Propetamphos | | | 0.1 | | |
| Fonofos | | | | 0.5 | |
| Diazinon | | 0.9 | | | |
| Ronnel | 0.02 | 0.9 | 0.1 | 0.5 | |
| Malathion | | 1.0 | | 0.9 | |
| Chlorpyriphos | | | 0.1 | | |
| Chlorfenvinphos | | | 0.4 | | |
| Isofenfos | | 1.4 | | 0.1 | |
| DEF | | | 0.5 | | 0.1 |
| Ethion | 0.02 | 0.4 | 0.2 | 0.2 | |
| Carbophenothion | 0.03 | 0.3 | | 0.2 | |
| EPN | | | | 0.02 | |
| Phosalone | 0.03 | 0.9 | 0.3 | 0.4 | 0.04 |
| Co-ral | | 1.5 | 0.6 | 0.8 | |

Table 6
OPs (ng $g^{-1}$) determined both in healthy kidney tissues (K) and kidney tissues affected by cancer (K*) and in adipose tissues (adip)

| Compound | Patient 5 | | Patient 6 | | Patient 7 | | Patient 8 | | Patient 9 | | Patient 10 | | Patient 11 | | | Patient 12 | | | Patient 13 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | K | K* | K | K* | K | K* | K | K* | K | adip | K* | adip | K | K* | adip | K | K* | adip | K | K* | adip |
| Dimethoate | 0.3 | 0.5 | | | | | | | 0.4 | 0.4 | | | 0.7 | 0.4 | 0.2 | 0.6 | 0.05 | 0.3 | | | 0.4 |
| Propetamphos | | | | | | 0.2 | | | | | | | | | | | | | | | |
| Fonofos | 0.03 | 0.2 | 0.4 | | | | | | | | 0.5 | | 0.1 | 0.1 | | | | | | | |
| Diazinon | | | | | | | 0.4 | | | | | | | | | | | | | | |
| Malaoxon | | | | 4.6 | | | | 4.6 | | | | | | | | | | | | | |
| Ronnel | 0.04 | 0.1 | 0.04 | 0.3 | 0.03 | 0.1 | 0.4 | 0.3 | 0.02 | | 0.4 | | 0.1 | 0.1 | 0.01 | 0.1 | | | 0.1 | 0.04 | |
| Malathion | | | | | | | | | | | 0.8 | | | | | | | | | | |
| Chlorpyriphos | 0.03 | | | | | 0.1 | | | | | | | | | | | | | | | |
| Chlorfenvinphos | | 0.3 | 0.5 | 0.5 | 0.1 | 0.3 | 0.5 | 0.5 | 0.05 | | 1.0 | | 0.3 | 0.1 | | | 0.03 | 0.05 | | | 0.2 |
| Isofenphos | | | | 3.4 | | | 0.1 | 3.4 | | | | | | | | | 0.05 | | | | |
| Methidathion | | | | 0.7 | | 0.2 | | 0.7 | | | 0.5 | | | | | | | | | | |
| Bromophos-ethyl | | 0.3 | 0.2 | | | | | | | | | | | | | | | | | | |
| Profenophos | | | | 4.3 | | | 0.1 | 4.4 | | | | | | | | | | | | | |
| DEF | 0.04 | | 0.4 | 0.4 | 0.1 | 0.2 | 0.4 | 0.4 | | 0.04 | 0.2 | | 0.5 | 0.1 | 0.03 | | | | | | 0.2 |
| Ethion | 0.04 | 0.3 | 0.1 | 1.1 | 0.04 | 0.1 | 0.1 | 1.1 | 0.04 | 0.03 | 0.2 | | 0.3 | 0.2 | 0.02 | 0.1 | 0.02 | 0.04 | | | 0.1 |
| Carbophenothion | 0.04 | 0.4 | 0.1 | 0.1 | | | 0.1 | 0.1 | 0.04 | 0.03 | 0.1 | | 0.3 | 0.2 | 0.02 | 0.1 | 0.03 | 0.05 | | | |
| Phosalone | 0.1 | 0.7 | 0.5 | 0.2 | 0.1 | 0.2 | 0.5 | 0.2 | 0.1 | 0.02 | 0.7 | | 0.5 | 0.5 | 0.03 | 0.1 | 0.1 | | | | 0.1 |
| Azinphos-ethyl | 0.1 | 1.3 | | | | | 1.3 | 2.5 | 0.2 | | | | 0.1 | 0.1 | | | 0.1 | | | | |
| Co-ral | 0.1 | 0.9 | 1.6 | 1.1 | 0.1 | 0.3 | 1.6 | 1.1 | 0.1 | | 0.9 | | 0.6 | 1.0 | | | 0.1 | | | | 0.3 |

Table 7
OPs (ng $g^{-1}$) determined in liver tissue samples of patients not affected by epathic pathologies

| Compound | Patient 14 | Patient 15 | Patient 16 | Patient 17 | Patient 18 | Patient 19 | Patient 20 | Patient 21 | Patient 22 | Patient 23 | Patient 24 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Phorate | | 3.4 | | | | | | | | | |
| Dimetoate | | | 2.1 | | | | | | | | |
| Fonofos | | 2.7 | 2.2 | 2.4 | | 1.6 | 2.4 | 3.7 | | | |
| Paraoxon-methyl | | | | | 11.7 | | | | | | |
| Parathion-methyl | 14.5 | 28.2 | 13.4 | 13.6 | | 11.6 | 17.3 | 25.6 | 18.60 | 15.5 | 11.9 |
| Malaoxon | | 24.1 | | | | | | | | | |
| Ronnel | 0.4 | 0.4 | 4.0 | 4.7 | 5.3 | 3.8 | 5.2 | 7.0 | 5.10 | 4.7 | 4.3 |
| Fenitrothion | 7.9 | 21.7 | | | | | | | | | |
| Malathion | | | 4.0 | 4.8 | 4.3 | 3.5 | 0.5 | 0.7 | | | |
| Chlorpyriphos | | 3.2 | | | | | | | | | |
| Chlorfenvinphos | | 4.0 | 3.7 | 3.8 | 4.6 | 2.4 | 3.3 | 4.3 | 3.70 | 2.8 | 1.9 |
| Methidathion | | 8.2 | | | | | | | | | |
| Profenofos | | | | 10.6 | 13.9 | | | | | | |
| DEF | | 2.3 | 0.9 | 0.9 | 1.1 | | | | | | |
| Ethion | 0.7 | 6.6 | 5.3 | 5.4 | 5.7 | 4.6 | 5.8 | 8.1 | 5.03 | 6.1 | 3.6 |
| Carbophenothion | 0.7 | 6.2 | 0.5 | | | | | | | | |
| EPN | | | 6.0 | 2.6 | | 2.9 | 4.3 | 8.6 | | | |
| Phosalone | 1.0 | 7.8 | 6.6 | 4.2 | 4.4 | 4.0 | 5.5 | 8.2 | 6.75 | 5.1 | 3.8 |
| Azinphos-ethyl | 1.8 | | | | | | | | | | |
| Coral | | 7.1 | | | | | | | | | |

## Conclusions

This paper describes an analytical, simple and reproducible method for determining residues of organophosphorus pesticides in different human tissues. The recoveries obtained using human tissues spiked with a standard OP mixture, range between 60 and 106% with a standard deviation between $\pm 2$ and $\pm 10$: the recoveries seem to be independent of the analyzed tissue. For the pesticides involved in this study the limit of detection and the linearity range are between 0.01 and 0.09 ng $g^{-1}$ with an RSD$\leq$9.5% and between 1.0 and 500 ng $g^{-1}$ with an RSD$\leq$4.8%. The OP concentrations determined in different human tissues, vary over a wide range (between 0.01 and 28.0 ng $g^{-1}$). Meaningful differences in the OP concentrations have been found in tissues affected by cancer: in fact, in these samples the concentration results were more elevated by some order of magnitude than in the healthy tissues.

## References

[1] A.M. Fan, R.J. Jackson, Regul. Toxicol. Pharmacol. 9 (1989) 158.
[2] C.F. Jelinek, J. AOAC Int. 68 (1985) 1064.
[3] T.C. Marrs, in: B. Ballantine, T. Marrs, P. Turner (Eds.), General and Applied Toxicology, Stockton, New York, 1993, p. 1329.
[4] E.P. Krenzelok, Penn. Pharm. 65 (1984) 141.
[5] A. Ferrer, J.P. Cabral, Food Addit. Contam. 6 (1989) S95.
[6] J.M. Concon, in: J.M. Concon (Ed.), Food Toxicology, Marcel Dekker, New York, 1988, p. 1133.
[7] M. Mansour, D. Barceló, J. Albaiges, Sci. Total Environ. 123 (1992) 45.
[8] S. Lacorte, C. Molina, D. Barceló, Anal. Chim. Acta 281 (1993) 71.
[9] S.M. Dogheim, S.A. Gad Alla, S.M.A. El-Syes, M.M. Almaz, E.Y. Salama, J. AOAC Int. 79 (1996) 949.
[10] K. Shimizu, H. Shiono, T. Fukushima, M. Sasaki, H. Akutsu, M. Sakata, Forensic Sci. Int. 83 (1996) 61.
[11] R.K. Juhler, J. Chromatogr. A 786 (1997) 145.
[12] T. Hyötyläinen, K. Jauho, M.-L. Riekkola, J. Chromatogr. A 813 (1998) 113.
[13] S.M. Dogheim, S.A. Gad Alla, A.M. El-Marsafy, J. AOAC Int. 84 (2001) 519.
[14] F.A. Tarbah, H. Mahler, O. Temme, T. Daldrup, Forensic Sci. Int. 121 (2001) 126.
[15] E. van Wyk, H. Bouwman, H. van der Bank, G.H. Verdoorn, D. Hofmann, Comp. Biochem. Physiol. C 129 (2001) 243.
[16] G. Durand, R. Forteza, D. Barceló, Chromatographia 28 (1989) 597.
[17] D. Barceló, C. Porte, J. Cid, J. Albaiges, Int. J. Environ. Anal. Chem. 38 (1990) 199.
[18] G. Durand, D. Barceló, Anal. Chim. Acta 243 (1991) 259.
[19] D. Barceló, M. Solè, G. Durand, J. Albaiges, Fresenius' J. Anal. Chem. 339 (1991) 676.
[20] C. Porte, D. Barceló, J. Albaiges, Chemosphere 24 (1992) 735.
[21] V. Leoni, J. AOAC Int. 75 (1992) 511.
[22] C. Molina, M. Horning, D. Barceló, Anal. Chem. 66 (1994) 4444.
[23] S. Lacorte, D. Barceló, Anal. Chem. 68 (1996) 2464.
[24] A. Laganà, G. D'Ascenzo, G. Fago, A. Marino, Chromatographia 46 (1997) 256.
[25] T.F. Moate, J.J. Jenkis, J. Chromatogr. A 775 (1997) 307.
[26] L. Amendola, F. Botrè, A.S. Carollo, D. Longo, L. Zoccolillo, Anal. Chim. Acta 461 (2002) 97.
[27] H. Steinwandter, Fresenius Z. Anal. Chem. 313 (1982) 536.
[28] L.R. Snyder, J.J. Kirkland, in: Introduction To Modern Liquid Chromatography, Wiley-Interscience, New York, 1974, p. 348.
[29] D.L. Stalling, R.C. Tindle, J.L. Johnson, J. Assoc. Off. Anal. Chem. 55 (1972) 32.
[30] D.W. Kuehl, E.N. Leonhard, Anal. Chem. 50 (1978) 182.
[31] R.C. Tindle, D.L. Stalling, Anal. Chem. 44 (1972) 1768.
[32] J.D. Tessari, L. Griffin, M.J. Aaronsen, Bull. Environ. Contam. Toxicol. 25 (1980) 59.
[33] J.A. Ault, C.M. Schofield, L.D. Johnson, R.H. Waltz, Agric. Food Chem. 27 (1979) 825.
[34] G. Fuchsbichler, Landwirtsch. Forsch. 32 (1979) 341.
[35] J.E. Knoll, J. Chromatogr. Sci. 23 (1985) 422.