ANALYTICAL METHODS FOR TEA PESTICIDE RESIDUES

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ABSTRACT

Guidance methods are provided for analysis of residues of pesticides in tea and tea brew. These methods cover most of the pesticides used in tea. Method for analysis of organochlorines, organophosphates and pyrethroids in tea involves extraction of the prehydrated tea samples with acetone. The acetone extract is evaporated, dissolved in water and re-extracted into n-hexane. Further clean-up is done on adsorption column with neutral alumina (activity grade V) as adsorbent and hexane-acetone mixtures as eluting solvents. Pesticide concentrations in the cleaned-up tea extracts are determined using gas chromatograph with either Electron Capture Detector (ECD) or Nitrogen Phosphorous Detector (NPD). Method for Triazole residues in tea is slightly different from the one proposed for organochlorines, organophosphorous & pyrethroids. In this method after acetone extraction, re-extraction is into dichloromethane instead of n-hexane and in adsorption column chromatographic clean-up, the eluting solvent is a mixture of dichloromethane and hexane. Method for pesticide residue analysis in tea brew follows the same steps by starting from re-extraction (liquid-liquid partitioning) step. The proposed methods are derived out of experience on pesticide residue analysis in tea and need to be optimized and validated prior to implementation.

1. INTRODUCTION

"Pesticide" is a general term for all plant protection and pest control agents and for protecting stored products. Pesticide Residue is any specified substance in food, agricultural commodities, or animal feed resulting from the application of pesticides. The term includes any derivatives of a pesticide, such as conversion products, metabolites, reaction products and impurities considered to be of toxicological significance. Residues of pesticides, more specifically, of all

chemicals applied to agricultural crop, their intermediary substances, toxins and metabolites produced is causing concern to the producers and consumers of food items since the introduction of chemical pesticides. These pesticides and their metabolites find their way into the human body through the food and the water cycle. Tea like other tropical crops supports a large population of various kinds of insects, mites, rodents, fungi and nematodes. It is interesting to note that tea is mostly subjected to autochthnus pest recruitment in its new area cultivation. Though a number of chemicals are available for pest control of tea, the choice for widespread application is limited to a very few chemicals for which maximum residue limits (MRL) have been declared by several countries and international organizations like EPA, FAO, EEC and Codex Alimentarius Commission. It is indeed desirable that tea is kept free from pesticide residues but this proposition would be unrealistic in view of the serious pest and disease problems and the economy of tea production.

Pesticide residues are present in trace amounts in relatively large number of biological materials. Hence, important part in pesticide residue analysis is to extract the pesticide components from the substrate and isolate them from interfering co-extractives. Next step involves estimation of residues employing chromatographic techniques like, Gas Chromatography, High Performance Liquid Chromatography, etc., with highly sensitive and selective detectors.

Residues may be expressed as,

a. The parent compound {(i) when there are no significant metabolites; (ii) or when significant metabolites are present and the analytical method measures the total residue as a single compound which may be numerically expressed as the parent compound; (iii) or when analytical method measures the parent compound and metabolites separately and the total residue is expressed additively as parent compound, with calculation for differences in molecular weight when differences are > 20%}

b. Single metabolite or alteration product {(i) when the parent compound is converted to another chemical entity; (ii) or when analytical method measures the total residue as a single metabolite; (iii) or when analytical method measures the metabolites and parent compound separately and the total residue is expressed additively as metabolite, with calculation for differences in molecular weight when differences are > 20%} or

c. As parent compound and metabolite separately (JMPR, 1995)

On tea, applied pesticides are generally lost by evaporation, growth dilution, photodegradation and rainfall. Some are metabolized/degraded in plant system. Further, degradation takes place due to thermal decomposition during manufacturing process when tea is exposed to high temperature. Generally, the rate of loss of pesticides during manufacture is 30 – 70% (Muraleedharan., et. al., 2001). Pesticides with high vapour pressure exhibit a higher rate of loss.

In tea cultivation, the spectrum of pesticides used ranges from the organochlorine insecticides, to the modern substances, the pyrethroids and urea compounds, based on geographical location, incidence of pests and availability of compounds. For the examination laboratory, this means that analysis is to be aligned in such a way that substances not detected up to now are always detected.

2. GENERAL CONSIDERATION OF RESIDUE ANALYTICAL METHODS

(i) SAMPLES :

Tea is derived from the leaves of *Camellia sinensis* L. and is consumed as extracts of the dried or processed product. For residue analysis, samples are taken in a manner reflecting common practice of processing for the marketed end product (green leaves). Freshly harvested produce is not required to be analyzed for tea.

Special care need to be taken in collecting samples and the collected samples should be truly representative in order to obtain meaningful residue data. The sampling procedure must be fully accommodated both to the commodities involved and to the levels of pesticide residues to be determined.

(ii) VALIDITY OF THE METHOD EMPLOYED

The method selected, needs to be validated for parameters like specificity, linearity, accuracy, precision and limit of quantitation (LOQ), for each analyte, before adopting for regular sample analysis.

(iii) SAMPLE STORAGE

Normally, a time lag occurs between sample collection and sample analysis. Hence, stability of residues in the substrates during storage needs to be evaluated; or, along with the samples, control samples fortified with known concentrations of the pesticide may be stored under exactly the same storage conditions and finally analysed along with test samples in order to know if any degradation has taken place during storage.

(iv) ANALYTICAL METHOD

Typically, a pesticide residue analytical method includes residue isolation, concentration, separation, identification and quantification. Chromatographic methods continue to be the most used for residue analysis. Increasingly important concerns for chromatographic analysis of agrochemical residues are related to high throughput, rugged methods, minimum sample handling, growing number of analyses, improved accuracy and precision, most importantly submicrogram detection limits and increased documentation to meet certifications such as Good Laboratory Practices, etc.

In pesticide residue analysis, the first step is separation of the pesticide residues from the substrate material by solvent extraction. The solvent selected must extract the pesticide residues in a reproducible manner without extracting large amounts of extractives from the substrate. And also the solvent system should be applicable to a large variety of analytes. Several extraction techniques such as shake-filter, liquid-liquid extraction, Soxhlet extraction or blending are widely used. Also modern extraction and clean-up methods like solid-phase extraction (SPE), accelerated solvent extraction (at elevated temperatures or micro-wave assisted), centrifugal partition chromatographic extraction, etc., are in use.

After extraction the next important phase to be accomplished is the purification of the extract. In other words, after extraction of the analyte from its previous environment/matrix (eg., tea) by a suitable solvent a "clean-up" procedure is often devised to quantitatively separate the analyte from the sample. The extracted analyte must be free of most accompanying interfering extractants before precise and valid chemical analysis can be undertaken. Clean-up techniques are often as varied as the analytical methods. Most clean-up procedures described in the literature are based essentially on one or more of the following techniques: (i) Chromatographic separation with materials exhibiting a selective adsorption (or lack there of), (ii) Chemical removal of interference through oxidation, reduction, saponification or hydrolysis without detrimental effect on the compound itself, (iii) Physical separation by solvent partition, steam distillation or freezing.

Column chromatography is one of the most widely used and readily adopted clean-up technique. A large number of adsorbents having varying degrees of polarity are available. Choice of the proper adsorbent for a given compound will be dependent on the polarity of the compounds themselves. Compounds exhibiting low polarity can readily be separated from more polar extractive interferences through the use of a wide variety of adsorbents. Chemicals exhibiting a polarity equal to or greater than their

extractive interferences can be purified by the employment of an adsorbent having a high affinity for the compounds under study. In this particular instance, the interfering substances are eluted off the column leaving behind the analytes. The analytes can then be recovered by introducing a more polar solvent to the column. If chromatographic purification is not feasible, sometimes the extractive interferences can be altered chemically through reaction with acids, bases or oxidizing agents to form products having different solubility characteristics from the analytes being determined. Clean-up by physical separation through solvent partition can be advantageously employed where the analytes have a solubility preference for one solvent of the pair while the extractive biological interferences have a greater affinity towards the other solvent.

If the analytes cannot be estimated directly using the available instruments like GC, HPLC, Spectrophotometers, etc., derivatization of the analyte into a form, which can be analysed, is required. But the conversion needs to be reproducible and the derivative should be stable.

Even after purification/clean-up of the samples, most of the times the sample contains a few more interfering substances along with the analytes. The analytes require separation from each other and also from the interference before reaching detection point. To meet this requirement chromatographic analytical techniques are extensively used. Analysis of volatile analytes is usually performed by gas chromatography while high performance chromatography is used for semi volatile, nonvolatile, polar and thermolabile compounds. Other methods like thin layer chromatography, capillary electrophoresis, etc., are also in use. Biotechnology based methods involving immunoassays, bioassays and biosensors have been developed in recent years. In GLC and HPLC analyses column selection plays a major role in separation of analytes from each other and from interferences. Depending on the chemical nature of the analytes and required detection limits of the method, a suitable detector is selected.

3. MULTIRESIDUE METHODS

Multiresidue methods are the analytical methods capable of determining many pesticide residues in a single analysis. It is difficult to develop a single multiresidue method, which can analyse residues of all the pesticides. Hence, depending on the properties of analyte and the substrate, various multiresidue methods have been developed, which include several combinations and varieties of instrumental techniques. Choice of the multiresidue method depends on the properties of the analytes and the substrate and also on availability of laboratory equipment.

Multi-residue analysis in foods, soil, water, etc is a time consuming process and often entails several post-extraction clean-up steps before analysis. The use of organic solvents such as acetone, acetonitrile, or ethyl acetate for extraction provides high recoveries of pesticides over a wide range of polarity, but further clean-up steps which often include solid-phase extraction (SPE), liquid-liquid partitioning, adsorption column chromatography, and/or gel permeation chromatography (GPC), are required before analysis. GLC with Electron Capture detector for final estimation of Organochlorines, GLC with Nitrogen/Phosphorus detector for Organophosphates/Organonitrogens, HPLC using post-column derivatization for carbamates, etc., are examples of the techniques used in final estimations in Multi-residue methods. Either GC or LC with Mass Spectrometric Detector also widely used in multiresidue determinations as it helps in identification and confirmation of the analytes in addition to routine quantifications.

4. ANALYTICAL METHODS FOR ANALYSIS OF PESTICIDE RESIDUES IN/ON TEA

As in case of other substrates, for tea also, no single method is available for analysis of residues of pesticides. Some of the methods recommended in literature for analysis of pesticide residues in tea are briefed below.

In one of the method recommended by Nakamura Yumiko et. al., (1993), natural pyrethrins & pyrethroids were determined by Gas Chromatograph/ electron capture detection (GC/ECD) by using a methyl silicone-coated fused-silica capillary column, and recoveries were calculated by summing peak areas of the components. The green tea leaves were extracted with acetone, filtered after addition of coagulating solution, partitioned into n-hexane, and cleaned up on a Florisil column (as necessary).

Manikandan et. al.,(2001), proposed adsorption column chromatography for estimation of residues in ethion, dicofol, endosulfan & quinalphos wherein the residues were extracted from tea sample along with the additive, anhydrous sodium sulphate and using hexane by shaking for two hours on a mechanical shaker. The filtrate was then concentrated to near dryness in a rotary vacuum evaporator & co-extractives were removed using adsorption column chromatography. Analysis was carried out using a Hewlett Packard 5890 series II Gas Chromatograph equipped with nitrogen phosphorus detector (NPD) and Ni-63 electron capture detector (ECD).

Multiresidue analysis of pesticides by Supercritical Fluid Extraction (SFE) and HPLC method was recommended by Akiko Kaihara et.al. (2000) in which a screening method was established for determination of 27 pesticides in tea & other vegetables and fruits by SFE, cleaned up with cartridge columns and HPLC. In this method, wet samples were not suitable for the SFE instrument, so the water in the samples was removed with an absorptive polymer prior to SFE. The pesticides were extracted by SFE, the extracts trapped with Extrelut NT + Bond Elut C_{18} and eluted with acetonitrile. After washing with n-hexane, the pesticides were eluted from the cartridges with 15% ether in *n*-hexane, 15 & 50% acetone in *n*-hexane. These three fractions were individually determined by HPLC with a photodiode-array detector. The pesticides spiked in samples at 0.5 ppm showed satisfactory recoveries except for thiabendazole, imazalil & clofentezine. Detection limits were 0.005 – 0.01 ppm for the 27 pesticides.

SOFIA, Gmbh, an expert for the chemically analytical examination of tea for pesticides developed/published recommendation in co-ordination with TEA Association describing minimum requirements made for quality control[7&8] . In this method, analysis is aligned in such a way that substances that are not detected up to now, are detected. The method recommended by SOFIA is DFG S19. The designation DFG S19 describes a chemically analytical examination method, which has developed into a standard. This method permits the proof of more than 270 pesticides in vegetable foodstuffs. Due to the large number of compounds to be proven, it is also called a multi-method. Important substance groups such as the urea herbicides and the carbamates as well as the phenoxy-carbonic herbicides demand the use of other specialized methods.

Residue analytical methods for some of the pesticides recommended for use in Tea are listed in Table 1.

5. GUIDANCE RESIDUE METHODS FOR TEA

With our experience in the field of residue analysis, we are presenting a guidance method of multiresidue analysis in Tea. Tea, being in dry form, need to be soaked in water for a minimum of 30 minutes before subjecting it for residue analysis. Two multiresidue methods are proposed, which cover most of the pesticides used in Tea.

5.1 METHOD FOR ORGANOCHLORINES, ORGANOPHOSPHORUS AND PYRETHROIDS

PRINCIPLE

Tea samples are soaked in water for 30 minutes and extracted with acetone. The extract is evaporated, dissolved in water and re-extracted into n-hexane. Further clean-up is done on adsorption column with neutral alumina (activity grade V) as adsorbent and hexane-acetone mixtures as eluting solvents. Final estimation is by Gas Chromatography with either electron capture detector or nitrogen phosphorus detector.

APPARATUS

- High-speed blender fitted with leak-proof glass or stainless steel jar and explosion-proof motor or Homogenizer
- Buchner funnel
- Filtration flask
- Filter paper (Whatman No.1)
- Rotary vacuum evaporator, 30-40°C
- Separatory funnels with Teflon stopcock and ground joint, 1000 ml
- Round bottom flasks with ground joints, 250 ml 1and 500 ml
- Chromatographic glass tubes with Teflon stopcocks, 30 cm long and 15 mm i.d.
- Graduated cylinders, 250 ml, 100 ml
- Gas chromatograph equipped with Electron Capture Detector (ECD) and Nitrogen Phosphorus Detector (NPD).
- Micro syringe, 10µl
- Gas Chromatographic columns : DB-608, 30 m long, 0.53 mm i.d., 0.5 µm film thickness or equivalent (for Organochlorines); DB-1701, 30 m long, 0.53 mm i.d., 0.5 µm film thickness or equivalent (for Organophosphorus); DB-1, 30 m long, 0.53 mm i.d., 1.0 µm film thickness or equivalent (for pyrethroids).

REAGENTS

- Acetone, Analytical reagent grade or better
- Water, Double distilled
- n-Hexane, Analytical reagent grade or better
- Sodium sulphate, Anhydrous, granular
- Sodium chloride, Analytical reagent grade or better
- Alumina, Neutral, activity adjusted to grade V
- Reference standards of all the pesticides that are to be determined in tea

SAMPLE EXTRACTION

Soak accurately weighed 25 g tea sample in 60 ml water for at least 30 minutes, add 150 ml acetone and homogenize at 10000 rpm for 2 min. Filter the homogenate through Whatman No.1 filter paper placed on a Buchner funnel under suction. Rehomogenize the filter cake with 150 ml acetone and filter again. Rotary evaporate the combined filtrate to about 100 ml.

CLEAN-UP STEP 1

Transfer the contents from the above step into 1000 ml separatory funnel, add about 400 ml water, 10 g sodium chloride and 100 ml hexane. Shake vigorously for 5 min. and allow the layers to separate. Collect the hexane layer, re-extract the aqueous layer twice with 75 ml portions of hexane and collect the hexane layers. Dry the pooled hexane portion over sodium sulphate and concentrate to near dryness.

CLEAN-UP STEP 2

Prepare a chromatographic column with 2 g sodium sulphate, 10 g alumina and 2 g sodium sulphate in tandem in hexane. Transfer the concentrated hexane extract to the column with a minimum of rinsings using hexane, allowing the solvent layer to run into the sodium sulphate layer between additions. Elute the column with 150 ml of 3% acetone in hexane, collect the eluate and rotary evaporate to dryness.

DETERMINATION

Reconstitute the residues from the above step in suitable volumes of acetone + hexane (2 + 8, v/v) solvent mixture and inject a suitable volume (preferably 3 μl) to the Gas Chromatograph operated under optimal conditions for separation using the columns specified under 'Apparatus'.

Examples of GC conditions are given below which need to be optimized to get the required separations.

- Detector : ECD (for Organochlorines and Pyrethroids); NPD (for Organophosphorus pesticides)
- Column : As given under 'Apparatus'
- Carrier : Nitrogen @ 5 ml/min.
- Injector Temperature : 270°C
- Detector Temperature : 270 °C (NPD); 300 °C (ECD)
- Column Temperature : 60 °C (hold 2 min.), increase 30 °C/min. to 150 °C, increase 5 °C/min. to 270 °C (hold 15 min.)

CALCULATION

Residue (μg/g) = (As/Astd) x (V1/V2) x C x (D/W) x F

OR

$$= \left(\frac{As}{Astd}\right) \times \left(\frac{V1}{V2}\right) \times C \times \left(\frac{D}{W}\right) \times F$$

Where,

Astd = Standard response

As = Sample response

V1 = Volume of sample injected

V2 = Volume of standard injected

C = Standard concentration, μg/ml

D = Total dilution, ml

W = Weight of the sample, g

F = Recovery factor, (100/ % recovery)

5.2 METHOD FOR TRIAZOLES

For analysis of triazole pesticide residues in tea, same method described above may be adopted with the following variations. In 'Clean-up step 1', replace hexane with dichloromethane. In 'Clean-up step 2', replace the

eluting solvent i.e., '3% acetone in hexane' with 'dichloromethane + hexane (60 + 40, v/v) solvent mixture' and '10 g alumina' with '5 g alumina'. For determination on GC the Gas Chromatographic column need to be DB-5, 30 m long, 0.53 mm i.d., 0.5 μm film thickness with all other conditions remaining the same and the detector being either NPD or ECD.

The detection limits of the above methods should meet the set MRL requirements. These methods need to be optimized by the laboratory to suit the respective laboratory equipment and Method validation is a pre-requisite.

6. METHOD FOR ANALYISIS OF RESIDUES IN TEA BREW

Tea brew from tea may be prepared as per the standard practice. For various pesticide residue analyses in tea brew, methods given above for tea may be adopted starting from CLEAN-UP STEP 1, by taking 100 ml tea brew.

REFERENCES

ANNA SANNINO, MIRELLA BANDINI AND LUCIANA BOLZONI, 1999, Multiresidue Determination of 19 fungicides in Processed Fruits and Vegetables by Capillary Gas Chromatography after Gel Permeation Chromatography, J. AOAC International, 82(5) : 1229 – 1238

GUNTER ZWEIG & JOSEPH SHERMA, 1972, "Analytical Methods for Pesticides and Plant Growth Regulators", Vol. VI, Academic Press, London

GUNTER ZWEIG & JOSEPH SHERMA, 1980, " Analytical Methods for Pesticides and Plant Growth Regulators" , Vol. XI, Academic Press, London

HANS-PETER THIER & HANS ZEUMER, 1988, Manual of Pesticide Residue Analysis, Vol. 1, 383 – 400, Pesticide Commission, Germany

HANS-PETER THIER & HANS ZEUMER, 1988, Manual of Pesticide Residue Analysis, Vol. II, 317 – 322, Pesticide Commission, Germany

INDIAN STANDARDS, Bureau of Indian Standards, New Delhi

KAIHARA AKIKO, YOSHII KIMIHIKO, TSUMURA YUKARI, NAKAMURA YUMIKO & ISHIMITSU SUSUMU, 2000, Multiresidue Analysis of Pesticides in Fresh Fruits and Vegetables by Supercritical Fluid Extraction and HPLC, Journal of Health Science, 46(5) : 336 - 342

MURALEEDHARAN, N., SELVASUNDARAM, R. & MANIKANDAN K.N., 2001, Pesticide Residues in Tea : The present Scenario, The Planter's Chronicle : July 279 - 285

MANIKANDAN, K.N., MURALEEDHARAN, N. SELVASUNDARAM, R. & SUDHAKARAN, R., 2000, Studies the residues of certain pesticides and their persistence in tea, Recent Advances in Plantation Crops Research, 355 - 359

NAKAMURA YUMIKO, TONOGAL YASUHIDE, TSUMURA YUKARI. & YOSHIO ITO, 1993, Determination of Pyrethroid residues in Vegetables, Fruits, Grains, Beans and Green Tea Leaves: Applications to Pyrethroid Residue monitoring studies, J. AOAC International, 76(6) : 1348 - 1361

Pesticides Residues in Food, JMPR, 1995, 2.8.1, FAO, Rome

Sofia GmbH, Internet data (www.sofia-gmbh.de/en/lebensmittel)

TABLE 1: COMMON METHODS OF ESTIMATION OF PESTICIDE RESIDUES IN TEA

COMPOUNDS	EXTRACTION	CLEAN-UP	ANALYSIS	REFERENCE
ACARICIDES/INSECTICIDES				
Acephate	Waring Blender - using Ethyl acetate	Silica gel column with 10% methanol in ether as eluant	GLC with TID or FPD	IS : 13831-1993
Chlorpyriphos	Blender - with acetone	Silica gel column with 5% ethyl acetate in hexane as eluant	GLC with FPD	IS : 12385 – 1988
Cypermethrin	Homogenizer – using hexane - acetone mixture	Florisil column with hexane + toluene (4 : 6) mixture as eluant	GLC with ECD	Manual of Pesticide Residue Analysis Vol I & II, 1992, Pesticide Commission, Germany) Cypermethrin (S 23) Dicofol (S 12)
Dicofol	Homogenizer – using acetone	Sweep co-distillation	GLC with ECD	
Dimethoate	Blender - using CH_2Cl_2	Silica gel column with CH_2Cl_2 as eluant	GLC with TSD	IS : 11021 – 1984
Endosulfan	Blender using n-hexane	Water – hexane partitioning	GLC with ECD	IS : 12611 – 1989
Ethion	Blender - using n-hexane	Activated alumina column with 10% diethyl ether in hexane	GLC with TID or FPD	IS : 11773 – 1986
Fenitrothion	Waring blender – using acetone	Charcoal, celite, magnesium oxide (2:2:1) column with chloroform as eluant	GLC with TID or FPD	IS : 10168 – 1982
Fenvalerate	Homogenizer - using hexane-acetone mixture	Florisil column with hexane + toluene (4:6) mixture as eluant	GLC with ECD	Manual of Pesticide Residue Analysis Vol I & II, 1992, Pesticide Commission, Germany) Fenvalerate & Lambda-cyhalothrin - S 23 Quinalphos – S 8
Lambda-cyhalothrin	Homogenizer - using hexane-acetone mixture	Florisil column with hexane + toluene (4:6) mixture as eluant	GLC with ECD	
Quinalphos	Homogenizer using 200 ml acetone	Activated carbon silica gel column with dichloromethane + toluene + acetone mixture as eluant	GLC with FPD or NPD	
Phorate	Mechanical shaker - using acetone	Florisil column with chloroform and acetone.	GLC with FPD or NPD after oxidation	IS : 14914 – 2001
Malathion	Blender - using chloroform	Hexane-acetonitrile partition	GLC with NPD	IS : 5863 – 1985

COMPOUNDS	EXTRACTION	CLEAN-UP	ANALYSIS	REFERENCE
Monocrotophos	Waring blender – using acetone	Charcoal, celite, magnesium oxide (2: 2: 1) column with chloroform as eluant	GLC with TID	IS: 11374 – 1985
FUNGICIDES				
Hexaconazole	Blender using acetone	Gel permeation chromatography with ethyl acetate – cyclohexanone (50 + 50) as eluant	GLC with ECD or MS	J. AOAC Intl. 1999, Vol. 82, P. 1229
HERBICIDES				
2,4,D.	Mechanical shaker - using chloroform	Alumina column with 1% sodium carbonate as eluant	GLC with ECD after methylation	Analytical Methods for Pesticides and Plant Growth Regulators, Vol. VI, 1972 2,4,D – P. 630 Diuron – P. 664
Diuron	Blender – using acetone	-	GLC with FID after hydrolysis and bromination	
Glyphosate	Homogenize with 0.1 M HCl solution plus Dichloromethane	Ligand exchange resin column with 6 M HCl solution as eluant	HPLC with Fluorescence detector	Manual of Pesticide Residue Analysis, Vol I , 1992, Pesticide Commission, Germany Vol II, P. 229
Oxyflurofen	Blendor with 35% water in acetonitrile	Alumina column with diethyl ether + petroleum ether mixture as eluant	GLC with ECD	Analytical Methods for Pesticides and Plant Growth Regulators Vol. XI, 1980, P. 330
Paraquat	Reflux with strong sulphuric acid	Cation exchange resin column with saturated ammonium chloride solution as eluant	Spectro photometry	Manual of Pesticide Residue Analysis, Vol I , 1992, Pesticide Commission, Germany) p. 177