ANALYTICAL METHODS AND INSTRUMENTATION FOR THE DETERMINATION OF ORGANOPHOSPHORUS PESTICIDES IN FRUIT AND VEGETABLE SUBSTRATES

by

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IN THE NAME OF GOD

TO

JAMARAN

CONTENTS

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Abbreviations used	Glossary of Terms Used
EI-MS	Electron impact - Mass spectrometry
ADI	Acceptable Daily Intake
UV	Ultraviolet
TLC	Thin layer chromatography
GLC (GC)	Gas liquid chromatography (Gas chromatography)
GC-MS	Gas chromatography - Mass spectrometry
GC-FTIR	Gas chromatography - Fourier transform infrared spectrometry
GC-OES	Gas chromatography-Optical emission spectroscopy
LC-MS	Liquid chromatography - Mass spectrometry
LC-FTIR	Liquid chromatography - Fourier transform infrared spectroscopy
LC-NMR	Liquid chromatography-Nuclear magnetic resonance spectroscopy
GC-ECD	Gas liquid chromatography - Electron capture detector
op	organophosphorus
SIM	Selected Ion Monitoring
HPLC	High Performance Liquid Chromatography
PCI	Positive Ion Chemical Ionisation
NCI	Negative Ion Chemical Ionisation
NBS	National Bureau of Standard
CLENI	Chloride Enhanced Negative Ionisation
TID	Thermionic Detector
FPD	Flame Photometric Detector
ECD	Electron Capture Detector
FID	Flame Ionisation Detector
NPD	Nitrogen Phosphorus Detector
amu	atomic mass unit
FC-43 (PFTBA)	Perfluorotributylamine

DFTPP	Decafluorotriphenylphosphine
TCD	Thermal Conductivity Detector
KD	Kuderna-Danish
GC/EIMS/SIM	Gas Liquid Chromatography/Electron Impact Mass Spectrometry/Selected Ion Monitoring
GSC	Gas Solid Chromatography
CI	Chemical Ionisation
FI	Field Ionisation
FAB	Fast Atom Bombardment
RIC	Reconstructed Ion Chromatogram
TOF	Time-of-Flight Mass Spectrometer
m/z	Mass to charge ratio
RSD	Relative Standard Deviation
WHO	World Heath Organisation
FAO	Food and Agriculture Organisation of the United Nations
BAA	British Agrochemicals Association
EPA	Environmental Protection Agency
df	Film thickness
id	Internal diameter

ABSTRACT

The chromatographic behaviour of four organophosphorus pesticides has been investigated, using capillary gas liquid chromatography-mass spectrometry (GC-MS), and packed and wide bore capillary gas liquid chromatography with electron capture detection (GC-ECD).

The first part of this thesis (Chapter 3) embodies the application of high performance capillary gas liquid chromatography-mass spectrometry and the development of the methodology for analysis of pesticides in fruit and vegetable substrates. Manipulation of GC-MS data during the sample analysis is necessary in order to eliminate the problems arising from the complexity of the samples.

The application of selected ion monitoring (SIM) in GC-MS is to a large extent limited by selection of the ion for monitoring, selection of internal standard, optimisation of electrometer zero, the electron multiplier voltage, the selected mass interval, column selection for GC-MS analysis and tuning of the quadrupole GC-MS. A major concern was with the determination of the optimum conditions for the analysis of substrates containing organophosphorus pesticides by GC-MS following solvent extraction (without clean up steps). The analysis of organophosphorus samples using GC-MS-SIM provides detection limits lower than Pg pesticide injected and very good recovery data.

The second part (Chapter 4) deals with the application of GC-ECD. Here the role of packed column support, packed and wide bore capillary column temperature and detector temperature was investigated. Comparisons of the relative responses of the pesticides analysed (i) on packed columns and (ii) on wide bore capillary column under different column and detector cell temperatures were undertaken and the optimal condition for each of the organophosphorus pesticides was determined, under the optimal conditions the detection limits and recovery for fruit and vegetable samples were examined. The results from this method were correlated with the data obtained by GC-MS and found to be in very good agreement.

The data obtained from analysis using GC-ECD was found to have a higher precision than that obtained by GC-MS, however GC-MS exhibited a higher selectivity for the determination of these pesticides.

DECLARATIONS

We, the undersigned, declare that the work presented in this thesis is the candidate's own investigation.

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7.2.91

Date

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Dr F Vernon

Dr P J Baugh

I declare that the work has not already been in substance for any other degree or being concurrently submitted in candidature for any other degree.

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Ali Mostafavi Dehzoei

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Date

This work was carried out at the University of Salford from October 1988 to February 1991.

CHAPTER 1

General introduction to pesticide analysis and survey of analytical methods

1.1 General Introduction to Pesticide Analysis

1.1.1 Introduction

Organophosphorus pesticides are widely used to control household, agriculture and forestry pests. The application of pesticides for controlling pests has recently shown a tendency towards combination of two or more pesticides for diverse reasons, such as for increasing their effectiveness, for controlling more than one pest at a time, and for increasing the storage life of insecticides.

The interest in the development of good methods for the simultaneous determination of pesticides has increased greatly in recent years because of the heavy use of organophosphorus pesticides in agriculture and their pre ence in the environment and foodstuffs.

Gas chromatography with selective detectors such as EI-MS and electron capture provide sensitive methods for measuring organophosphorus pesticides.

The comprehensive review of the organophosphorus compounds may be found in a number of publications [1-7].

1.1.2 History

The original synthesis of an op is credited to de Clermont from his presentation (in 1854) of the synthesis of tetra ethyl pyrophosphate (TEPP), although de Clermont attributes the original synthesis to his colleague, Moechine [5]. It has been reported [5] that de Clermont actually tasted this highly toxic compound, yet he experienced no untoward effects. This is of note because it is the lack of knowledge concerning the pharmacological effects of the op's that is principally responsible for the slow development of these agents. As such, the highly toxic nature of these compounds remained largely unknown for 80 years.

As cited in Holmstedt [5,6], the important discoveries of op chemistry were:

1. The synthesis of compounds containing the carbon-phosphorus bond by Hoffmann in 1873, which is employed in the making of methyl phosphoryl dichloride, an important intermediate in insecticide and nerve gas synthesis.

2. The synthesis of compounds containing nitrogen-phosphorus and cyano-phosphorus bonds by Michaelis and his co-workers in the late 1800's, which led to other insecticides and nerve gases.

3. The discovery by Arbuson in the early 1900's of the isomerisation reaction which serves to stabilise the C-P bond.

4. The availability of TEPP in relatively pure form from Nylen Ca 1930, which permitted investigations into the biochemistry and pharmacology of the compound.

5. The phosphorus-fluoride linkage which was synthesized by Lange's group in 1932.

It was only with Lange's work that the pharmacological effects of the op's began to be appreciated. According to Holmstedt [5], Lange and Van Krueger published observations on the effects their compounds had on themselves. It was these findings which prompted Lange to entice I.G. Farbenindustrie into evaluating op's for use as insecticides. Within two years I.G. Fabenindustrie has successfully launched the first commercial effort into the development of synthetic insecticides. In 1937 Schrader, who had been assigned the insecticide project, began to develop organofluorine compounds. From the time of his first patent for a fluorinated contact insecticides in 1937 until the end of World War II, Schrader is reported to have synthesized over 200 op's, many of which were extremely toxic to man.

Because of the potential for use of the op's as chemical weapons, much of this work remained classified throughout the war era, a consequence of which was the independent discovery of many of these same agents by researchers in England. A larger portion of this work was carried out by McCombie and Saunders and included the synthesis of diisopropyl fluorophosphate [6,8].

1.1.3 Current use

Following World War II, the insecticidal properties of the op's began to be exploited as problems arose with the use of the polyhalogenated pesticides such as DDT, chlordane and kepone, the demand for organophosphorus agents increased markedly. According to the Pesticide Task Group report submitted to the Federal Working Group on Pest Management in 1974 [9], over 130 million pounds of op's were being produced annually for agricultural use with a potential human exposure calculated to be in excess of four and one half million individuals in the farming industry alone. Very few commercial farms were expected not to use the organophosphorus agents.

Since the task group report of 1974, the use of organophosphorus agents has steadily increased. Many more agents have become available not only to the farmer but to the general public, with the op's now being routinely employed as household pesticides. In addition, they are used by state and local government agencies for the eradication of mosquitos, fruit flies, gypsy moths and other insect pests [10]. The combination of environmental and agricultural uses makes it likely that virtually every human being in the United States has been exposed to op's at some point in his or her life. At the present time, it is not known what the typical exposure levels are, or what the long term effects of these exposures might be.

1.1.4 Mechanism of action

Organochlorine and organophosphorus insecticides act as general nerve poisons. Organochlorine insecticides pose a greater problem to our ecosystem because of their

greater stability compared to organophosphorus compounds. They are generally resistant to biodegradation[11].

1.1.4.1 Effects on enzymes of the esterase type

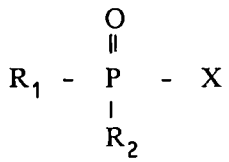
The op's are known principally for their ability to interact with the serine moieties of a number of enzymes [12-19], particularly those possessing esterase activities. Such interaction generally prevents normal enzymatic function. Most notable among these enzymes are the cholinesterase, the inhibition of which yields profound alterations in the normal physiological status of any life form possessing a nervous system. These vital enzymes are predominantly responsible for the rapid inactivation of acetylcholine, one of the most common neurotransmitters. Many essential neurological functions are regulated through cholinergic activity including motor activity (neuromuscular), much of basal central nervous system activity (brain stem), peripheral ganglionic function, and a large portion of end organ autonomic regulation [20]. Any disruption of these neurological processes effectively unbalances normal homeostasis. Extensive disruption is incompatible with life.

The properties of potency, selectivity and specificity were responsible for bringing commercial attention to the op's. However, so much attention was paid to the neuromuscular effects that the activities of these compounds on other systems, especially non-cholinergic systems, were virtually ignored. Only recently has it begun to be appreciated that the op's are highly efficient and potent inhibitors of a great variety of serine esterases[21], many of which have profound effects on homeostasis.

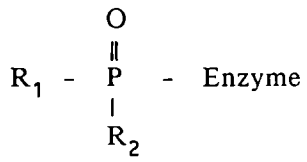
The esterases are an extremely diverse group of enzymes which are difficult to isolate, purify, and characterise. They have been tentatively divided into three major classifications: those which rapidly hydrolyse op's, carbamates and the like (A-esterase), those which are inhibited in a progressive, temperature-dependent fashion by the op's, carbamates, etc (B-esterase), and those which do not interact with op's at all (C-esterase)[1].

The B-esterase are of interest to this investigation, because they alone are prevented from functioning normally by binding to the op's. These hydrolytic enzymes possess both esteractic and proteolytic activities and have broad substrate specificities [1].

B-esterase are inhibited by op's of the general formula:



where R_1 and R_2 may be almost any group (alcohol, phenol, mercaptan, amide, alkyl or aryl) linked to the phosphorus moiety either directly or indirectly through O, N, or S and where x can be acyl radical such as fluoride, nitrophenyl, or phosphates, or a thiocyanate, carboxylate, phenoxy or thiophenoxy group [5]. These compounds phosphorylate the active site of the B-esterase such that the enzyme is inhibited, usually irreversibly [12,13,15,16,22,23].



The fact that op's react with specific residues within the active site of esterase is supported by the following findings:

a) The addition of more substrate competitively blocks the effects of op's,

b) the inactive precursors of enzymes will not bind the op's, and

c) when the reaction occurs between op's and esterase, one mole of phosphorus is bound per mole of enzyme, and one mole of acid is liberated [1,13,14,24].

Mammalian tissues have been shown to possess a broad spectrum of esterase both with regard to substrate specificity and to physical properties. Esterase polymorphism has been demonstrated in many organs such as kidney, liver, and nervous tissue.

The most studied esterase of all, acetylcholinesterase, appears to possess two sites of particular importance within the active centre. One, termed the anionic site, includes at least one carboxyl group bearing a negative charge which serves to orient substrate molecules by attracting the substrate positive charge such that the tailing end of the substrate may interact with the second, or esteratic site [25,26]. The esteratic site possesses both acidic and basic groups which are purported to be the residues primarily responsible for the catalytic cleavage of substrate. The basic group is thought to be the hydroxyl portion of a serine moiety (hence the name "serine esterase") [12,16,26,27,28], with the acidic group being found on a histidyl residue. These groups are essential for esteratic cleavage. A general discussion of the biochemical specificity is presented in reference 29.

The op's as a rule, do not possess positive charges which would interact with the anionic site, the exception is echothiophate [$(C_2H_5O)_2\overset{O}{\overset{\|}{P}} - SCH_2CH_2N^+(CH_3)_3$] [8,29]. Normally, the op's bind directly to the serine hydroxyl of the esteratic site [8]. In the formation of this bond, the acidic group of the op (labelled x in the diagram) is cleaved off in much the same manner that acetylcholine is hydrolysed. The two processes differ in that the op binds in a much more stable fashion than does acetylcholine [24,25,29-31]. The carbonyl carbon-acetate bond of acetylcholine lasts but a few microseconds, while the phosphorylated enzyme may remain as such for hours, days, or even weeks [26,27,32]. In many cases active enzyme can be regenerated only by the action of nucleophilic attacking substances such as the oxime (discussed below). Additionally, there are certain cases, usually involving the fluorinated op's where a second reaction occurs. This reaction termed aging, involves the dealkylation of the op through binding to an ionized hydroxyl group [29]. The result is a permanently inactivated enzyme. In situations like this a tissue may regain esterase function only through the synthesis of new enzymes [8,29].

1.1.4.2 Effect on other enzymes

There are several enzymes that are neither esterase nor protease which can be inhibited by op's. Examples include Na^+K^+ATPase, phosvitin kinase, and diglyceride kinase, all of which are inhibited by high concentrations of op's (greater than 10^{-3}M) [33-5]. In

reactions involving diisopropyl fluorophosphate, the inhibition appears to be due to fluoride ion rather than to phosphorylation of the active sites [36]. As extremely high concentrations are required to affect the enzymes, these findings may be considered of questionable toxicological relevance and therefore are not in need of further consideration.

1.1.5 Pesticide toxicity [38-46]

The toxicity of pesticides are given by their LD(50) values [37]. The LD(50) value is the dose required to kill 50% of population of test animals, and is expressed as milligram per kilogram of the body weight of the animal. The smaller the LD(50) value the more toxic the compound.

Both the concentration and the formulation effect of a pesticide are most important. The higher the concentration of active ingredient, the more hazardous it becomes. A pesticide formulated as a solution or as an emulsifiable concentrate is, as a rule, more hazardous than when formulated as a dust or as a wettable powder.

1.1.5.1 Pesticide used in this project [38-46]

Maximum permissible pesticides in food commodities at the time of harvest or after post-harvest treatment and LD(50) and ADI data are presented in Table 1.1.5.1.

pesticide	ADI mg/kg body mass	LD(50) mg/kg	commodity	maximum residue limit mg/kg
dimethoate	0.02 *	200	apples	2.0
			wheat	2.0
			beans	2.0
			grapes	2.0
			pears	2.0
			pineapples	0.5
			plums	2.0
			potatoes	0.1
			tomatoes	
			lettuces	
			carrots	
			fruits and vegetables	0.5-2.0
heptenophos		96 - 121	wheat	0.05
			apples	
			carrots	
			lettuce	
			tomatoes	
malathion	0.02 *	2800	apples	2.0
			apricots	4.0
			bananas	2.0
			beans	2.0
			fruit (dried)	8.0
			grapes	2.0
			nuts (dried)	8.0
			tomatoes	3.0
			vegetable (dried)	8.0
			lettuces	
			carrots	
			fruits and vegetables	0.5-8.0
pirimiphos-methyl	0.01 *	2050	sunflower seeds	3.0
			maize	8.0
			apples	
			tomatoes	
			lettuce	
			carrot	

Table 1.1.5.1

* ADI (acceptable daily intake) as set by World Health Organisation

1.2 Survey of analytical methods

1.2.1 Spectrometric methods

1.2.1.1 UV-visible spectrophotometry

The use of UV-visible spectroscopy as a primary method of determination in op pesticide residue work has declined to an insignificant level in recent years [47]. This method is based upon the measurement of the absorbency of the pesticides or derivatised pesticides. For example, Turner [48] has described a procedure using 4- (4-nitro-benzyl)-pyridine as a spectrophotometric reagent for most op pesticides, which is sensitive to low microgram amounts. The direct utility of a UV-visible spectroscopic method in pesticides residue analysis is limited because of its relatively low sensitivity and selectivity. However, it can be useful in conjunction with other techniques.

1.2.1.2 Infrared spectroscopy

Although recent advances have been made in infrared instrumentation and techniques that have resulted in better sensitivity than was possible in the past, the general utility of infrared spectroscopy in pesticide residue work is limited to conditions where microgram or greater amounts of pesticides are present in the sample matrix. Infrared spectroscopy can be useful for confirming the identity of a pesticide residue [49]. Gore et al [50] have compiled the infrared and UV spectra of 76 pesticides for this purpose. The use of infrared spectrometry as a selective detector in chromatographic methods appears to have good potential for future applications in the pesticide area [51].

1.2.1.3 Chemiluminescence spectroscopy

A highly sensitive method for the determination of some alkyl phosphates based upon chemiluminescence has been developed by Fritsch [52]. The alkylphosphate is reacted with sodium perborate ($NaBO_4$) or hydrogen peroxide (H_2O_2) to produce a

peroxophosphate, which then oxidises an amine such as luminol in alkaline medium to produce the chemiluminescence. This method can also be employed for spectrophotometric or fluorometric measurement when the appropriate amine is substituted for the luminol. This method has only been applied to phosphorus-containing nerve gases thus far, but application to op pesticides appears to be possible [53].

1.2.2 Electrochemical methods

1.2.2.1 Polarography

The polarographic determination of op pesticides in foodstuffs has been reviewed [54]. Electroanalytical techniques are applicable to those op pesticides which posses nitro- (e.g. parathion) or other electrochemically-active substituents. It appears that sulphur-containing op pesticides may be determinable after a hydrolysis step at a mercury electrode in an anodic mode [54]. However a chromatographic separation step would be desirable to reduce interferences from the sample matrix. A piezoelectric crystal detector which is sensitive to op pesticides has been reported [55]. This detector, although useful for gas-phase determinations, requires further development before it can be more widely applied.

1.2.2.2 Enzymatic methods

The inhibition of acetylcholinesterase activity can be useful for the sensitive detection of op pesticides [56], especially when used in conjunction with chromatographic separation [57,58], by using a substrate with favourable spectrophotometric or fluorometric measurement properties. However, when used by itself, this technique is non-selective in that no information as to the identity of the inhibitor is provided, and thus its use is possible only under very carefully controlled conditions. The high cost and the difficulty in maintaining consistent activities of the enzyme preparation are important limitations to be considered. The determination of blood acetylcholinesterase activity in farm workers is a good indicator of op pesticides [59], but, again, the identity of the pesticides

must be determined by other means.

1.2.3 Chromatographic methods

1.2.3.1 Thin layer chromatography

Considerable advances in the separating power of thin layer chromatography (TLC) have been made in recent years through improvement in sample application and plate technology. After separation, the plates are normally dried and scanned with a densitometer or fluorometer to quantitate the components. In many cases, including the trace-level determination of op compounds, treatment of the plate with a development reagent is necessary to obtain adequate sensitivity and selectivity. Because the utility of TLC in op pesticide residue analysis depends to a large extent upon the detection scheme, considerable effort has been made to devise sensitive and selective spray reagents for op pesticides [60-62]. For example 4-(4-nitrobenzyl)-pyridine reacts with most op compounds and results in a blue spot which can be quantified to low microgram amounts[63]. The formation of heteropolymolybdenum blue can be employed as a TLC detection technique after in-situ mineralisation of the op compound on the plate [64,65]. The detection limits are greater than 0.1 microgram using this technique [65]. Inhibition of acetylcholinesterase provides a more sensitive detection scheme [66]. While TLC is used to a limited extent as a primary method for pesticide residue analysis [67], it is especially valuable for screening [68] and confirmation of identity [76,77].

1.2.3.2 Gas chromatography

Numerous papers have been written about gas-liquid chromatography since the first description by James and Martin [69]. It has been applied most frequently in modern analytical chemistry and has been used to solve a wide range of problems in medical, biological, and environmental science areas, as well as having industrial applications. In spite of developments in spectroscopy and liquid chromatography, gas chromatography remains the most widely used separation tool in analytical chemistry and is likely to

remain so for the foreseeable future [70]. This technique has more resolving power and sensitivity for the separation and determination of volatile organic compounds than any other method in analytical chemistry. The limitations of the technique are established primarily by the thermal stability of the samples and chromatographic substrate. Generally, one is restricted to an upper temperature of around 400 °C and a molecular weight less than 1000, although higher temperatures have been used and larger molecular weight samples have been separated in a few instances [70]. In gas chromatography, the sample or some convenient derivative samples must be thermally stable at the temperature required for volatilisation.

Gas chromatography has become the method of choice for pesticide residue analysis because of the excellent sensitivity and selectivity afforded by the specialised detectors which are available [70-74]. The detectors which are the most useful in the determination of op pesticides are the flame photometric detector (FPD) [70,75,77,78], thermionic detector (TID) [70,76,79] and the electron capture detector (ECD) [70,73,74,76,80,81]. The FPD operates by combusting the op compounds in a hydrogen-rich medium, which results in the formation of electronically-excited HPO. The luminescence is monitored at 526 nm. The FPD is very reliable and achieves detection limits of the order of 100 pg or less for most op compounds [71]. The TID (also known as the alkali flame ionisation detector) is sensitive to both nitrogen and phosphorus. The mechanism of its operation is not known with certainty, although, it is believed to be due to the enhancement of the ionisation of the alkali halide salt which is placed in the flame plasma [82]. The TID is about ten-fold more sensitive than the FPD but is less reliable [72]. The ECD is the second most widely used ionisation detector [83,84] and it owes much of its popularity to its unsurpassed sensitivity to a wide range of toxic and biologically-active compounds. Consequently, it is widely used in trace analysis for the determination of pesticides, herbicides, industrial chemicals in the environment, drugs and other biologically active compounds in biological fluids, and for the determination of the fate of the volatile organic compounds in the upper atmosphere.

These highly selective and sensitive detectors have enabled the development of GC methods for the residue-level determination of op pesticides which have excellent signal-to-noise ratios at legally important limits. Typical examples of the applications of those methods to the determination of op pesticide residues in fruit and vegetables is taken from the same workers [76,85,86]. Their clean-up procedure consisted of extraction, evaporation and column adsorption prior to GC analysis. The selectivity of the TID and FPD permits the use of a less rigorous sample clean-up procedure than is the case for highly sensitive detectors such as the electron capture detector [71].

There are six selective detectors readily available which can cover the requirements for the GC analysis of the majority of op pesticides. No one detector can fulfill all residue requirements and often there is a choice between several detectors for a given application. However, the percentage of published paper on FPD, TID, ECD, MS, FID and NPD GC detectors are 30.89, 16.26, 14.63, 10.57, 9.76 and 4.88, respectively and the publishing percentage for LC-MS is 13.01.

1.2.3.2.1 Gas chromatography-mass spectrometry

A chromatogram gives information including number of components, quantity (peak height or area), and qualitative (retention parameter) of the components in a mixture. The identification is based solely on retention and is considered very suspect. Even for a simple system, a peak at a certain position in a chromatogram may be a substance other than the one anticipated (two or more components may elute exactly with the same retention). The identity can be firmly established, when the selectivity of information from the chromatogram is very good. Spectroscopic techniques provide a rich source of qualitative information with high degree of certainty. Spectroscopic instruments have, however two practical limitations: in that it is often difficult to extract quantitative information from the signals, and pure or single-component samples are required. Thus chromatographic and spectroscopic techniques provide complementary information about the complexity and the concentration of components in the sample. Their tandem operation provides more information about a sample than the sum of the information

gathered by either instrument independently [87], thus combinations of this type can be particularly fruitful.

The coupling of gas chromatography with mass spectrometry was at the forefront of the development of combined analytical instrumentation, or so-called "hyphenated" systems. The principal hyphenated techniques are gas chromatography interfaced with mass spectrometry (GC-MS), fourier transform infrared spectrometry (GC-FTIR), and optical emission spectroscopy (GC-OES); liquid chromatography combined with mass spectrometry (LC-MS), fourier transform infrared spectrometry (LC-FTIR), and nuclear magnetic resonance spectroscopy (LC-NMR).

GC-MS has had an impact on op pesticide residue analysis [88]. The technique of selected ion monitoring (SIM) can be operated at very high sensitivity for a mass selective chromatographic detector, and selectivity is obtained because the mass is a substance-specific parameter. Selected ion monitoring is now a widely used technique in the area of biomedical [89-91] and environmental analysis [92].

1.2.3.3 High performance liquid chromatography (HPLC)

The role of high performance liquid chromatography in pesticide residue analysis has been assessed by several authors [93-96] and its potential is beginning to be more fully explored. It is generally accepted that HPLC detectors must be improved for applications to pesticide residue analysis to be fruitful. Since the first reported use of HPLC in an op pesticide residue determination in 1971 [97], a number of publications have appeared in this area and will be discussed below to illustrate the approaches towards detection which have been taken. Most workers have empioyed UV detection of the intact pesticides. For example, Wilson and Bushway [98] determined azinphos-methyl and its exon analogue in fruit and vegetables using reversed-phase HPLC with UV detection set at 224 nm. The precision at the lower level of determination (0.16 ppm) was less than 4%. A further example of the use of a UV detector in the determination of op pesticide residues by HPLC is from the work of Cabras et al [99]. Several op pesticides were determined in

grapes by simple extraction with benzene, evaporating to dryness and reconstituting the extract in the mobile phase, followed by injection. Detection was accomplished at 221 nm and detection limits were in the range 0.04 to 0.2 ppm. UV detection is applicable only to those pesticides which possess suitable chromophoric substituent and which are in relatively uncomplicated sample matrices. The UV detection sensitivities for most op pesticides have been compiled [100,101].

Several attempts have been made to devise alternative detectors for op compounds which are more sensitive and selective. Acetyl cholinesterase inhibition has been used to detect op pesticides by coupling HPLC with an autoanalyser [57,58]. The combination of HPLC and the autoanalyser has also been exploited by Att [102] in connection with the determination of parathion and its exon in dust samples. A diazotization reaction was used, but low detection limits were not achieved. The specificity of the autoanalyser detection allowed confirmation of the UV response.

Some workers have attempted to adapt flame spectroscopy techniques for detectors in liquid chromatography. McGuffin and Novotny [103] have demonstrated the potential of an FPD used in conjunction with microcolumn HPLC. A detection limit of 2 ng was achieved. Chester [104] examined the problem of quenching of HPO emission by organic solvents and found that alcohols and acetone did not cause quenching at moderate concentrations, but acetonitrile caused nearly complete quenching at concentrations above 10%. Szalonti [105] has used flame ionisation detector for his study of the normal-phase HPLC of op pesticides. Graphite furnace atomic absorption has been employed for the detection of op compounds [106], but further work is needed on this technique to increase sensitivity and to decrease processing time. Cope [107] investigated a rotating molecular emission cavity detector for possible applications to op compounds, but found it to be relatively insensitive and noisy.

The use of electrochemical detectors in the determination of pesticide residues by HPLC has been reviewed. Electrochemical detector is expected to be applicable only to the few op pesticides whose structural features permit it, i.e. samples component is electroactive

(e.g. parathion).

The applicability of on-line LC-MS was demonstrated by Parker and co-workers [108,109] for op pesticides. The use of liquid chromatography-mass spectrometry (LC-MS) has been successfully applied by several workers in recent years [110-113]. The work of Barcelo was aimed at expanding the general insight into the usefulness of PCI and NCI and chloride-enhanced negative ionisation (ClENI) for the determination of organophosphorus compounds in on-line LC-MS. Several workers [114,115] show some op pesticides are thermally-labile, such as trichlorfon, or polar such as parathion [116]. Because of these problems, the analysis of organophosphorus pesticides and their corresponding metabolites has been carried out by LC using UV [117] and selective MS detectors [118-120].

The on-line combination of liquid chromatography-mass spectrometry (LC-MS) plays an important role in environmental organic analysis and compared with gas chromatography-mass spectrometry (GC-MS) offers major advantages for analysing polar pesticides and herbicides such as op pesticides for determination [119-122] and confirmation [123].

1.2.3.3.1 Combined HPLC-MS

The introduction of HPLC has made it possible to separate and also identify highly polar compounds which are not gas chromatographable. If compared with capillary GC, HPLC has two major disadvantages. 1) There are only a few detection methods: ultraviolet (UV), fluorescence and electrochemical detection. 2) The separation efficiency of HPLC is considerably poorer than that of capillary GC. This lower performance increases the probability that several components coelute and cannot be differentiated by non-specific detectors. A mass spectrometer represents a specific and at the same time sensitive detector. Unfortunately HPLC-MS coupling is far more difficult than GC-MS coupling as the vacuum system of the mass spectrometer has to handle up to 2 ml min^{-1} of solvent (corresponding to ~ 2000 ml min^{-1} gas), and soft ionisation methods are required for polar compounds.

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CHAPTER 2

Introduction to analytical methods

Instrumental Introduction

2.1 Introduction to Chromatography [1]

Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one stationary phase and one mobile. The sample is carried in the mobile phase through the stationary phase in a glass or metal column. The components of the sample interact with stationary phase and separate into bands. These emerge from the end of the column in order of their increasing interaction with the stationary phase. Those compounds that interact least emerge first.

The mobile phase may be either gas or liquid, and the stationary phase either liquid or solid. Gas chromatography has a gaseous mobile phase and a solid or liquid stationary phase. It is used extensively for thermally stable and volatile organic and inorganic compounds. High performance liquid chromatography (HPLC) has a liquid mobile phase and a solid stationary phase, either normal phase or reversed phase. As a result, it complements GC by being able to analyse compounds that are insufficiently volatile to pass down the GC column or compounds that are thermally unstable and tend to decompose.

2.2 Principles of Gas Chromatography (GC)

Gas Chromatography is essentially a separation technique in which the sample mixture in the vapour phase is subjected to a competitive distribution between two phases, one of which is a moving gas stream and the other is a stationary solid or liquid.

There are two separation mechanisms used:

a) Adsorption

b) Partition

In adsorption chromatography the sample constituents after volatilization are separated by passing them in a stream of carrier gas through a bed (the column) of powdered

adsorbent. This technique was first suggested by Ramsey [2] in 1905 and it is called gas solid chromatography (GSC). James and Martin [3,4] introduced partition chromatography in 1952. In partition chromatography a competitive partition of the sample between the moving carrier gas and a stationary liquid is used to achieve the separation. This technique is called gas liquid chromatography (GLC).

The apparatus required to produce a gas chromatogram is shown schematically in Figure 2.1. The heart of every chromatograph consists of a length of tubing filled with a powdered material with or without a liquid phase coating through which an inert carrier gas (N_2, He, etc) is flowing. This tube normally coiled for convenience, is responsible for separating the individual components of the mixture and is more properly known as the column. The type of column packing selected depends on the nature of sample to be analysed.

The sample is usually introduced into the carrier gas stream by means of microlitre syringe via a rubber septum in an injection port at the front of the column. The mixture is then separated according to GSC or GLC mechanisms as the case may be. The speed and efficiency of separation are temperature dependent and the column is therefore mounted in an oven provided with facilities for precise control of temperature to ensure good reproducibility of results.

Separation is achieved by virtue of the fact that individual components in the mixture tend to reside in the column for varying periods of the time depending on their affinities for the column packing, and, therefore they reach the column outlet sequentially. A detector, placed at the column outlet, responds to the emergence of these compounds and produces an electrical signal which may be amplified and displayed on a recorder. The response of the detector as displayed on a recorder in the form of a trace or chromatogram is the ultimate objective of the analytical chemist since it is the source of both qualitative and quantitative information on the composition of the sample. Figure 2.2 gives an example of a chromatogram of a mixture containing 3 components (A, B and C).

2.3 Gas liquid chromatography

In gas liquid chromatography (GLC) the separation is achieved by passing the sample in the gas phase, through a tube packed with an inert powder (called support) on the surface of which is coated a film of an involatile liquid (the stationary phase). The separation is achieved by a partition process involving the sample, the moving carrier gas and the stationary liquid phase.

In GLC analysis of any mixture the accuracy and precision depends on a vast number of variables. Some of the most important variables are the temperature of the column and detector, the support and liquid phase in the column, the surface area and composition of the column tubing, the flow rate of H_2 carrier gas and air in the case of the FID detector, the carrier gas flow rate (especially if peak height instead of peak area is used for quantitative analysis), impurities present in the carrier gas (e.g. oxygen), and electrode corrosion in the case of the FID.

2.4 Theory of Gas Liquid Chromatography [1, 5-9]

Theoretical considerations are a useful guide for the practical design and operation of the chromatographic experiment.

2.4.1 Partition Coefficient

When a small quantity of solute A is injected onto the column, it immediately distributes between the stationary phase (s) and mobile phase (m) as it passes through the column. At any stage of chromatography there is an equilibrium established with Cs and Cm, the concentrations in the stationary and mobile phases, respectively:

$$K_A = Cs/Cm \qquad (1)$$

where K_A is the partition coefficient (distribution constant) for component A. When K=1, the solute was equally distributed between the two phases.

Partition coefficient varies for different molecules, because the affinity will vary and so in a mixture, component will proceed through the system at differing speeds. Components with stronger affinity for the stationary phase, thus large K, being retained longer than those have weak affinity (small K) for the stationary phase. If a solute has no affinity for the stationary phase (K = 0, e.g. air) and the column is operated at a temperature in excess of the solute boiling point, then that solute will pass through the column at the same rate at which the carrier gas is flowing. This behaviour enables the measurement of dead volume to be carried out.

2.4.2 Partition ratio

Partition ratio (or capacity ratio) k' is a more fundamentally important parameter in chromatography. For a given set of operating parameters, k' is the ratio of the time spent by the solute in the stationary phase relative to the time spent in the mobile phase. It is defined as the ratio of the moles of a solute in the stationary phase to the moles in the mobile phase.

$$k' = Cs\ Vs/Cm\ Vm = k\ Vs/Vm \qquad (2)$$

where the ratio, Vm/Vs is often denoted by the symbol ß. Thus

$$k' = K/ß \qquad (3)$$

in gas chromatography, retention time and adjusted retention time are generally used rather than retention volume and adjusted retention volume respectively.

$$k' = (t_R - t_M)/t_M = t'_R/t_M \qquad (4)$$

$$t_R = t_M (1 + k') \qquad (5)$$

$$t'_R = k' t_M \qquad (6)$$

t_R = the retention time of a given sample component

t'_R = the adjust retention time ($t'_R = t_R - t_M$)

t_M = the time required to displace the mobile phase between the injection port and the detector (the hold up time)

k' = partition ratio (capacity factor)

2.4.3 Efficiency of column

Column efficiency (CE) describes the rate of band broadening as the solute travels through the column. CE is usually measured by column theoretical plate number (N) and HETP (CE increasing with N and decreasing H). It relates to peak sharpness or column performance. The various factors contributing to the efficiency of separation may be described by the concept of the height equivalent to a theoretical plate (HETP), H.

The number of theoretical plates in the column is given by $16(t_R/W_b)^2$ where t_R is the retention time and W_b is the tangential base of the peak measured as "distance" on a recorder chart.

$$N = L/H = 16(t_R/W_b)^2 \text{ or } \qquad H = L/16(t_R/W_b)^2 \qquad (7)$$

where L is the column length. The effective number of plates (Neff) in the column is:

$$Neff = 1/Heff = 16(t'_R/W_b)^2 \qquad (8)$$

It is usually difficult to measure W_b thus the width at half the peak height, $W_{½}$ was used:

$$Neff = 5.54(t'_R/W_{½})^2 \text{ or } \qquad N = 5.54(t_R/W_b)^2 \qquad (9)$$

2.4.4 Resolution [10]

Another measurement of column performance is given by the degree of separation between two adjacent peaks

$$R = \frac{t_{R(A)} - t_{R(B)}}{0.5(W_{(A)}+W_{(B)})} \qquad (10)$$

where the $t_{R(A)}$ and $t_{R(B)}$ are the retention time of two adjacent solute
$W_{(A)}$ and $W_{(B)}$ are the tangential base width of the two adjacent peak.

In a pair of equal peaks, resolution is generally considered to be complete for R = 1.5 (where the overlap of adjacent peaks is 0.3 %).

2.5 Apparatus

Several instruments varying in sophistication and ranging in price are available for gas chromatography. The basic components of these instruments are shown in Figure 2.1.

2.5.1 Carrier Gas [11]

The mobile phase must be chemically inert and not react with the sample solute and stationary phase. Helium, hydrogen and nitrogen are the most generally used mobile phases. The carrier gas chosen is dependent on the detector to be employed; Nitrogen is used with the most popular detector, the flame ionisation detector, and the electron capture detector. For regulation of the gas supply are pressure regulators, gauges and flowmeters. In addition, the carrier gas system often contains a molecular sieve to remove water or other impurities.

2.5.2 Flow controller

The flow controller is a needle valve or other device used to control the gas flow rate. Flow rates can be established by a rotameter at the column head. This device, however, is not as accurate as a simple soap-bubble meter, which is located at the end of the column. A flow rate of around 75 cm^3/min is most often for 1/4" (6.4 mm) o.d. column; a flow rate of approximately 25 cm^3/min is used for 1/8" (3.2 mm) o.d. column; and a flow rate of around 4 cm^3/min is used for wide bore capillary column; pressures of around 4 psi and 10 psi are used for medium and narrow bore capillary column, respectively.

2.5.3 Injection system

It is important to deliver a representative sample onto the column in the shortest time and the smallest volume possible. The sample is injected from a hypodermic syringe through a self-sealing silicone rubber septum into the injection port, a small heated chamber capped with a septum. Liquid samples typically 1 - 5 ul in volume are injected. The injection port temperature should be high enough to vaporise the sample instantly but overheating of the rubber septum must be avoided.

Gaseous samples can also be injected using a gas-tight syringe or a gas-sampling valve (valve method) as an injection device attached to the chromatograph.

2.5.4 The column

There are two types of GC columns, packed columns and capillary columns. Packed columns are constructed of stainless steel, nickel or glass. They have an inner diameter of approximately 4 mm and are generally 1 - 3 m in length. They are packed with an inert phase coated with a liquid film. The support phase is usually a diatomite earth, called chromosorb W, chromosorb P, etc. The stationary phases range from non-polar to very polar. Therefore, different sets of compounds can be separated using different stationary phases.

Capillary columns have an internal diameter lower than 1 mm and have a length between 12 - 30 m or greater. They may be constructed from fused silica or borosilicate glass. Fused silica has a high degree of cross-linking within the silicon-oxygen matrix, this gives it a high tensile strength. Capillary columns have a high efficiency but are expensive to prepare. The column is coiled and is mounted inside an oven which must be able to be rapidly heated and cooled whilst the temperature program takes place.

2.5.4.1 The support

The support material is used to provide a surface onto which is coated the stationary phase film. The support should have a large surface area relative to its volume and it should be inert towards both the stationary phase and the sample, and the particles should be of uniform size.

Many materials have been investigated as potential supports but the only ones that are used to any extent are diatomites and teflon. The diatomite supports can be divided into three main types whose designation depends on the supplier. Chromatographic supports manufactured by Johns Manville were named as follows:

a: Chromosorb P

b: Chromosorb W

and

c: Chromosorb G

Ottenstein [12,13] and Supia [14] have published good reviews on solid supports. According to these reviews, the Chromosorbs are prepared by heating to high temperatures, sometimes after mixing the diatomite with a small amount of sodium carbonate flux. When the diatomite alone is calcined (to reduce to a calx by the action of heat) at temperatures of up to 1600 deg F, a pink support is produced. The pink colour of the support is caused by the mineral impurities (iron, aluminium, etc) present. The mineral impurities form complex oxides which impart the pink colour to the support. This support is called Chromosorb P, where P stands for pink.

When diatomite is mixed with a small amount of flux, sodium carbonate, and calcined at temperatures of 1600 deg F, a fused material is produced. The original grey diatomite becomes white in colour because of the conversion of iron oxide to colourless complex, sodium iron silicate. This support is named Chromosorb W. The W stands for the white colour of the support.

2.5.4.1.1 Chromosorb W [15]

These are derived from flux-calcined diatomite. They are more friable, less dense, have a smaller surface area [9] but are relatively inert and useful for the analysis of polar samples.

They are commercially available in several forms:

i) Chromosorb W-NAW (non acid washed)

ii) Chromosorb W-AW (acid washed)

iii) Chromosorb W-AW-DMCS

2.6 Detector

The ideal requirements for a detector are that it should have a high sensitivity, provide a linear response over a useful range, should be independent of operating parameters and be stable. The main types are: 1) the flame ionization detector (FID); 2) the nitrogen phosphorus detector (NPD); 3) the flame photometric detector (FPD); 4) the thermal conductivity detector (TCD); 5) the electron capture detector (ECD); and 6) the mass spectrometer (MS).

2.6.1 Detector performance

The requirements for a detector for GC are:

i) Adequate sensitivity to monitor the eluted samples components present in very low concentrations compared to the major eluting component

ii) the carrier gas (nature of)

and

iii) a rapid response to the changing concentration of the minor component.

2.6.2 Minimum detection level (MDL)

The minimal detectable level is the level of sample being measured by the detector, when the detector signal (S) is at least twice the mean noise signal level (N) (Fig. 2.3)

$$S/N \geq 2$$

MDL concentration is usually expressed in $ugml^{-1}$, gml^{-1} or g injected. The MDL of the common detectors is given below:

Type of detector	MDL (g)
FID	10^{-12}
NPD	10^{-14}
FPD	10^{-11}
TCD	10^{-9}
ECD	10^{-14}
MS (coupled with GC)	10^{-10} - 10^{-15}
MS (coupled with HPLC)	10^{-12}

2.6.3 Electron capture detector (ECD)

The functional mechanism of the ECD is based on the reduction of an electron current flow due to the removal of free electrons from the system by sample components eluted from a gas chromatographic column [16].

The schematic diagram in Figure 2.4 illustrates the electron capture detector. The current is produced by electrons present in the cell, which are derived from two sources.

(a) Primary electron or beta-particles (high energy electrons) are emitted by nickel 63 (or ^{3}H) at a rate of about 10^{9} beta-particles per second, which is equivalent to about 10^{-9} amps [16].

(b) Secondary electrons are formed by the collision between primary electrons and molecules of the carrier gas. In the latter case, an additional electron and a positive ion is produced.

$$N_2 + ß^- \text{ <--> } N^+_2 + e^- \qquad (11)$$

or

$$Ar + ß^- \text{ <--> } Ar^+ + Ar^* + e^- \qquad (12)$$

When an electron capturing component is introduced into the cell, it captures the thermal electrons to form negative molecular ions or neutral radicals and negative ions

$$AB + e^- \text{ --> } AB^- \quad \text{non-dissociative mechanism} \qquad (13)$$

$$AB + e^- \text{ --> } A. + B^- \quad \text{dissociative mechanism} \qquad (14)$$

The net result is the removal of electrons from the system and the production of negative ions having a far greater mass. These ions will combine with positive ions (e.g. positive nitrogen molecule in the case of nitrogen carrier gas) and will be purged from the cell as neutral complexes.

When a potential is applied to the cell, essentially all the free electrons are collected at the anode. However, at least one electron is captured for every molecule of electron capturing substance that passes through the cell. This loss of electrons results in a corresponding decrease in cell current which after amplification is proportional to the quantity of electron capturing sample introduced.

The detector response follows a Beer's law type relationship

$$I = I.e^{(-ack)} \qquad (15)$$

I = current when electron capturing materials are present

I. = current when electron capturing materials are not present

a = electron affinity of material

c = concentration

k = proportionally constant related to the geometry of the cell and the operating conditions

The sensitivity of ECD is reduced when there is sample overloading of the cell. The introduction of too much sample can happen very easily when working with the ECD due to its extreme sensitivity to certain types of compounds. Since response varies widely, depending on the nature of the compound, careful judgement is required of the operator as to the appropriate amount of sample that should be used for any particular analysis.

ECD has high sensitivity to electrophilic molecules, but has a small linear range. Thus careful preparation of sample is required to ensure that the sample component concentrations fall within the operating range. Relative response factors have been determined for a range of compound types [17].

The choice of carrier gas for use with the ECD is limited to nitrogen and the noble gases[18]. Pure argon and helium are unsuitable because they can easily transfer excitation energy by collision with solute vapours [6] producing undesirable ionisation effects (Penning reaction). The addition of 5 - 10% v/v of an alkane, usually methane overcomes these problems. Methane molecule collides with the slow ß-electron and with the argon in excited state, thus the meta stable ions, argon and electron are formed by deactivating collisions (equation 18). Proposed mechanisms for this process are [16]:

$$Ar \xrightarrow{\beta} Ar^{+} + e^{-} \qquad (16)$$

$$Ar \xrightarrow{\beta} Ar^{*} \qquad (17)$$

$$CH_4 + Ar^{*} \xrightarrow{\beta} Ar + CH_4^{+} + e^{-} \qquad (18)$$

The maximum response of the ECD to different organic compounds is temperature dependent. For a single compound the change in response of 100 to 1000 fold should occur for a 100 °C change in detector temperature [20,21]. This high temperature dependence can be derived directly from the kinetic model of the ECD process [22,23].

The temperature dependence can be used to diminish in order to minimise unwanted interferences during measurement of a particular solute [23].

2.7 GLC of organophosphorus pesticides

2.7.1 Packed column analysis

Bevenue [24] found that the Chromosorb W supports, the high purity (HP) grade, gave the best results both in terms of peak symmetry and lower detectable limits for organophosphorus pesticides. The Chromosorb P supports proved to be unsuitable with pesticides used (malathion, paraoxon, malaoxon and methyl parathion). The poor response of chromosorb P was attributed to the greater adsorption properties of the support. Chromosorb P has a greater surface area per unit volume than chromosorb W. Kawahara et al [25] were unable to chromatograph methyl and ethyl parathion at a column temperature of 195 °C on Chromosorb P-NAW. Using the silicone liquid phase QF-1 an Aeropak 30 (a type of diatomaceous earth support), it was found that once a temperature of 200 °C was reached in the column, the thermal stability of the column changed [25]. Analysis of malathion at 200 °C on this column gave erratic results. It was suggested that malathion can begin to decompose at temperatures of about 150 °C depending on the reactivity of the support surface.

It was concluded that the variation in the response of the organophosphorus pesticides with the supports gave evidence that some form of interaction of the compound with the support was taking place. Apparently the more reactive the support the lower temperature of decomposition of organophosphorus pesticides.

Gunther and Jaglan [26] found that Teflon is a superior support for gas chromatographic analysis of organophosphorous pesticides with low (2 - 5%) liquid phase loaded columns. Egan[27], Beckman and Bevenue [28] reported that stainless steel columns give a higher recovery of organophosphorus pesticides than most other metal columns (copper, aluminium, nickel).

Beroza and Bowman [29] found that with lightly-loaded columns (less than 4% liquid phase), the adsorption of compounds on the supports become especially critical relative retention times of organophosphorus pesticides have been observed to be higher on low-loaded columns than on high-loaded columns (e.g. 3% Apiezon L on Gas-chrom Q compared to 12%). This was explained as being probably due to adsorption effects.

Bevenue et al [30] concluded from their work that Chromosorb G-NAW, Gas-chrom Q, and chromosorb W-HP were among the best supports for malathion, parathion and malaoxon. Lives and Giuffrida [31] improved non-polar columns for analyses of organophosphorus pesticides by adding a polar phase to the non-polar liquid phase.

Lindgren et al [32] coated Gas-chrom Q with 1% OV-101 and found that organophosphorus pesticides tailed badly on this column. Chromosorb G showed similar results to gas-chrom Q and chromosorb W-HP was the least adsorptive diatomaceous earth. Lindgren et al found that columns coated with 1% OV-1 as the stationary phase on diatomaceous supports were inferior to those coated with 1% OV-101. The explanation given for this inferiority was that there is a higher molecular diffusion of the solute molecule into the more viscous OV-1 and hence there is a greater possibility for interaction with the support material. Such differences in molecular diffusion have been observed [33] for the two silicon phase SE-30 and SF-96, which are similar to OV-1 and OV-101, respectively.

The stationary liquid phases most commonly used for multi-residue analysis of organophosphorus are listed in Table 2.1. Silicone DC-200 (10%) on Chromosorb W is one of the best and most widely used phase support packing available for organophosphate and it is specific in the official AOAC method [34-37]. Separation of compounds cannot always be achieved on this packing [38].

2.7.2 Wide-bore capillary column

An open tubular capillary column in gas chromatography is capable of better separation efficiencies and sample detectability for a given analysis time than any packed

column[39]. The principal disadvantages of the general use of open tubular columns are that, they are more demanding of instrument performance and have a lower sample capacity than packed columns.

In 1983, developments of open tubular capillary column permitted users to readily bridge the gap between open tubular capillary column and packed column GC, and made use of the new generation of capillary columns more favourable.

First, wide-bore 0.53 mm i.d. fused silica capillary tubing became available. Second, as a result of the work of the Grobs [40] with thick films (5 to 8 um) of crosslinked methyl polysiloxane, coated on wide bore conventional glass capillary columns, Duffy and Manke [41] were able to advance the technology required to prepare a series of crosslinkable stationary phases with different polarities and film thickness extending to 5.0 um on 0.32 and 0.53 mm i.d. fused silica glass capillary columns. Third, Ryder and his colleagues [42] described the different performance characteristics of a 0.53 mm i.d. fused silica capillary column, coated with a 2.5 um film of methyl silicone.

The major advantage of wide-bore capillary column is good separation and speed of analysis. Using packed column flow rates, the wide-bore column produces separations with packed column efficiencies but with a total run time about three times faster. However, when the flowrate is optimised for the tubular diameter, the wide-bore column produces far superior efficiencies and therefore superior separations, with analysis time approximately equal to those for packed columns [1]. Thus, there are six extremely good reasons for changing from packed columns to wide-bore capillaries: lower retention times, greater inertness, longer life, lower bleed, higher efficiencies, and greater reproducibility[1].

Zielinski [43] established the capability of high-temperature thermotropic nematic liquid crystal phases for providing enhanced separations of important solute isomers that have been difficult to resolve by other chromatographic methods. Such liquid crystals can be successfully used as wall coatings in wide-bore open-tubular columns. Gas

chromatographic analysis of cholesteryl esters was investigated in fused silica columns coated with a non polar and polar-bonded liquid phase. Wide-bore (0.3 mm i.d.) capillary column completely separated the cholesteryl esters according to degree of unsaturation [44]. Wide-bore capillary columns were used in the determination of organic (methyl-, ethyl-, propyl-, and phenyl-) Hg species as their chloride derivatives by electron-capture gas liquid chromatography (GLC) [45].

Specific environmentally significant arsenic compounds are determined by wide-bore capillary gas liquid chromatography [46]. Kelly evaluated the feasibility of using wide-bore capillary columns for GLC arsenic specification. Wide-bore borosilicate glass and fused-silica columns under conditions of temperature programming can separate inorganic (arsenite, arsenate) and organic (monomethyl arsonate, dimethyl arsinate) arsenicals [46]. Wide-bore capillary gas liquid chromatography method was used for quantification of acetoin and diacetyl from bacterial cultures [47]. GLC proved to be a sensitive method for diacetyl when a wide-bore capillary column was used to analyse culture head spaces by ether extraction.

Aldoses and alduronic acids can be identified and determined by wide-bore capillary gas liquid chromatography. The aldoses are converted to alditol acetates and the alduronic acids are converted to their corresponding N-propyl aldonamide acetate [48]. A rapid and specific method is described for the determination of orphenadrine concentration in human plasma. It involves a one-step sample preparation, using n-hexane/isopropyl alcohol (98:2) extraction, and analyse by gas chromatography on a wide-bore capillary column using N/P detection [49]. Thus, packed columns are still very much in use, because the majority of retention data and conditions are obtained on packed column and thus can be used by all chromatographers. To date there are no reports on the application of wide-bore capillary column gas liquid chromatography for the determination and identification of OP pesticides.

2.8 Gas chromatography-mass spectrometry

Gas chromatography is now increasing coupled to mass spectrometry, the so-called GC-MS combined technique, which is a powerful aid for the identification of separated compounds. This technique was introduced by Holmes and Morrell [50] in 1957 and Gohlke [51] in 1959. The gas chromatography-mass spectrometer technique was later improved by Ryhage [52] and Watson and Biemann [53] who devised methods for overcoming the difficulty encountered in coupling the two instruments.

There are a number of possible arrangements in interfacing the gas chromatograph to a mass spectrometer depending on the type of the column used and the mass spectrometer, but in general the column exit gas stream is passed to the spectrometer ion source, via an intermediate stage, which reduces the carrier gas pressure to the high vacuum conditions required by the mass spectrometer.

At the present, most GC-MS involves the use of fast scan high resolution instruments coupled to capillary or porous layer open tubular columns. Mass spectra are run continuously as the chromatogram proceeds and each spectrum takes only a few seconds.

Today, with the aid of computers GC-MS performance is enhanced and chemists are better equipped to solve more complicated problems from complex mixtures. The computer can control the machine from the beginning to the end of the operation, i.e. from injection to identifying and quantifying the compounds separated.

2.9 Gas chromatogram-mass spectrometer system

The major components (apparatus) required to produce as GC-MS are shown in Figure 2.5

The major components are

1) Gas chromatograph

2) Interface/transfer line

3) Mass analyser

4) Vacuum system

5) Data system

2.9.1 Inlet system (injection port)

The column inlet system must be capable of delivering a sample onto the column without any discrimination and with no compromise to chromatographic performance. Today the technology of inlet systems is more important for capillary columns.

Capillary column inlet systems are

1) Direct injection

2) Split injection

3) Splitless injection

4) Cold on-column injection

2.9.2 Interface

The successful operation of mass spectrometer as the detector for GC-MS is dependent on the interface. The interface capability is most important for the transfer of sample without compromise of chromatographic or mass spectrometer performance; various interface have been designed [54-56]. Direct coupling is employed for high performance capillary columns and are most commonly used.

2.9.2.1 Direct coupling

The development of narrow bore capillary and highly flexible vitreous capillary columns has enabled the column to be connected directly to the ion source of MS [57,58]. This system eliminates dead volumes and allows transfer of 100 % sample from the outlet of GC to the inlet of MS (ion source). The advantages of this technique are, thermal

stability, mechanical stability and chemical inertness. The major disadvantages of direct coupling are:

1) For columns of internal diameter 0.2 - 0.3 mm the flow of gaseous sample into the ion source must be at a level capable and acceptable for vacuum.

2) The pressure of the outlet of the column is less than atmospheric and there is a decrease in the chromatographic performance of up to 20 % [59,60].

3) Column switching also proves time consuming and for mass spectrometer optimisation replacement of columns requires accurate alignment.

Another kind of direct coupling, using a capillary restrictor between the column and ion source can be employed [61,62]. This form provides a low dead volume connection with pressure drop between the end of column and the ion source.

2.9.3 Ion source

The mass spectrometer is an instrument that only separates ion fragments introduced from neutral molecules, and thus analysis of a sample component depends on the successful ionisation of the substance. Several types of ionisation methods can be used [63] for GC-MS such as (i) electron impact (EI), (ii) chemical ionisation (CI), (iii) field ionisation (FI) and (iv) fast atom bombardment (FAB) and the use of these techniques has been reviewed[64-66]. The most common ionisation techniques applied to GC-MS are electron impact and chemical ionisation. Figure 2.6 shows the cross section of an EI ion source. In this technique, neutral molecules are bombarded with electrons after entering the ionisation chamber. The electrons are produced by thermionic emission from a heated wire (tungsten or rhenium filament). Collision of beam electrons with neutral molecules produces positively charged ions which are extracted by a field applied between a positive repeller electrode and negative accelerator plate [67,68]. Than ions are focused and accelerated towards the mass analyser.

For optimal operation the electron beam is given an energy of 50 to 70 eV, which yields the most reproducible spectra. The ionisation potentials of most organic molecules are

about 10 eV and with this minimum energy the primary process is the production of singly charged molecule ions [9]. ($M + e^- \rightarrow M^+ + 2e^-$). The excess energy may result in low abundance of molecular ion species and a corresponding excessive fragmentation.

2.9.4 Mass analyser

The mass analyser separates the ions that are produced in the ion source according to their different mass to charge ratios. After leaving the ion source, the ions are accelerated by a system of electrostatic slits and then enter a mass analyser. There are two main types of mass analyser, quadrupole and magnetic sector.

2.9.5 Quadrupole mass analyser (Figure 2.7)

The quadrupole mass analyser is the most successful commercially. It can separate ions according to the m/z ratio without the need for heavy magnets as in sector instruments. The quadrupole mass analyser is formed from four short and circular metal rods (stainless steel), with 6 mm diameter arranged symmetrically around the beam. To one of the two pairs parallel rods (x direction), a positive voltage, U, from a DC source and a radio frequency potential v cos wt are applied, whereas to the other pair of rods (y direction) an opposing dc voltage is applied.

The first pair of rods is held at +udc volts and the other pair at -udc volts. An rf oscillator supplies a signal to the first pair of rods that is + v cos wt, and an rf signal retarded by 180° (-v cos wt) to the second pair. Thus a region is produced between the four rods such that when the positive particles are ejected from the ion source, the combined fields cause the particles to oscillate about their central axis of travel. At a specified dc/rf ratio only those ions with a certain mass to charge ratio can pass through the array and reach the collector without being removed by collision with one of the rods. All other ions undergo unstable oscillation and are removed by collision with the rods. The mass spectrum can be obtained by varying the voltage (dc) and the radio frequency (rf) while keeping the ratio between these variables constant.

The quadrupole mass analyser has some advantages;

1) It is easily used for selected ion monitoring. The voltage applied to the rods can be switched very rapidly from one value to any other to detect several ions of different mass. It can be scanned over the entire mass range very rapidly (in a few milliseconds).

2) It is desirable for sensitive quantitative mass spectrometry.

3) It is more easily automated.

2.9.6 Detector [9,69]

The commonest detector of mass spectrometer is the electron multiplier which comprises a series of about ten electrodes (dynodes). When the positively charged particle (ion) impinges onto the first electrode, dynode it produces the release of a shower of electrons to the second electrode, thus a larger shower of electrons hit the third dynode and so on. This effect continues through the whole series of electrodes and provides gains of the order of 10^6. The current resulting is converted to a voltage which is digitised and transferred to the data system and recorded as an electronic signal.

2.9.7 Vacuum system

For operation of the mass spectrometer, the ion source, the mass analyser and the detector must be kept under high vacuum conditions (about 10^{-6} - 10^{-7} torr). The efficiency of maintaining high vacuum is related to the capacity and speed of the vacuum system. Most systems use a combination of oil diffusion pumps to maintain high vacuum together with backing rotary pumps to reduce the initial pressure to approximately 10^{-3} torr. Turbomolecular pumps contain no working fluid, and the pumping effect is purely mechanical. Therefore, background spectra are practically non-existent and even accidental venting does not create any problems [1].

A large amount of hardware visible on GC-MS instruments concerns the pumping and vacuum system [64]. The very high vacuum is essential in the mass spectrometer for the following reasons.

1) High voltage breakdown may occur in the multiplier, source and analyser if the pressure becomes too great.

2) Oxygen from residual air and leaks will cause burn out in the ion source (filament at 70 volt heats up to about 150 °C).

3) Ion-molecular collision will occur in the mass analyser leading to unwanted fragmentation of primary ions.

4) Regulation of the electron current at the ion source becomes difficult if the pressure increases.

5) High background pressure produces unwanted ion fragments and these lead to interfering mass spectra, making interpretation difficult.

6) The pressure rise lead to contamination ion source, slit, rods.

2.9.10 Data system

The GC-MS system is interfaced to a data system for control of both the gas chromatograph and the mass spectrometer, acquisition of raw data from the interface and calibration, conversion, and reduction of raw data acquired.

The data system comprises many software programs, which are stored on the systems disk. A variety of data systems [70-76] is available for on-line acquisition and processing, operating on the same principle. A data system is usually capable of three basic operations (1) control of the scanning of the mass spectrometer, (2) data acquisition from the mass spectrometer and (3) data processing. It should be capable at least of the following data processing requirements:

2.9.10.1 Reconstructed ion chromatogram (RIC)

At the time of acquisition the data system displays peaks of separated compounds as they are scanned by the mass spectrometer after elution from the column. The reconstructed ion chromatogram (RIC) is similar to a chromatogram produced by GC/FID or GC/ECD. During the acquisition and after the completed run, the data system can be commanded

to display and hardcopy the mass spectrum of a certain GLC peak in a bar chart or list form, mass chromatogram, forward library search and quantification. The data system is able to subtract the background noise to produce the enhanced spectrum.

2.9.10.2 Mass chromatogram

The data system can be instructed to display the ion abundances of a few selected m/z values extracted from RIC. The Finnigan 1020 is able to scan up to 12 mass ions at one time in the SIM mode. In the full scan mode, the scan range generally employed is from 40 - 450 amu. The mass chromatograms can be used as diagnostic data for characterising and locating particular components. This information is also used for rapid survey of GC-MS data obtained from complex of samples, particularly if more than one component is emerging in one GC peak.

Application of the mass chromatogram processing in quantitative analysis is described in a later chapter (experimental chapter).

2.9.10.3 Library search and identification of mass spectra

Any mass spectra can be matched against NBS library which contains spectra of known compounds. The result can be displayed or hardcopied in a list or spectra form. There is an accurate algorithm for computing a numerical estimate of the similarity between two mass spectra. The algorithm calculates three match factor parameters, i.e. PURITY, FIT and RFIT which have values between 0 - 1000. The similarity between the unknown spectrum and that the library spectrum is measured as purity. A purity of 1000 indicates that the two compounds have identical mass lists and peak intensities are exactly the same. FIT measures the degree to which the library spectrum is included in the unknown spectrum. A FIT of 1000 indicates that all library peaks occur as peaks in the unknown and that all intensities are the same for common peaks. In the same way, RFIT measures the degree to which the unknown is contained in the library spectrum.

2.9.11 Selected ion monitoring (SIM)

With full scan analysis the dwell time on a given mass ion is very small. Today for increasing the sensitivity and detection limits for a particular analysis selected ion monitoring (SIM) can be used, in which the mass spectrometer acts as a highly sensitive and selective detector for GC.

Henneberg [77] described the first application of SIM, and in 1966, Sweeley et al [78] used this technique for the analysis of biological samples. Since these two efforts and early impetus given by Hammar and his co-workers [79-81], SIM has found widespread application in biological, medical and environmental research. In 1972, Gordon and Frigerio [82] described the application of SIM using a magnetic sector instrument. In 1973, Jenden and Cho examined its use in quadrupoles and in 1970, Merritt [83] investigated the Time-of-Flight instrument.

Initially this technique was called mass fragmentography [79]. In 1973, Jenden and Silverman [84] named it multiple "specific detection". A variety of descriptive phrases have been used namely: ion specific detection (Gaffney et al, 1971), multiple ion monitoring (Stillwell et al, 1973), multiple ion analysis (Hamberg, 1973), tuned ion analysis (Kelly, 1972), selected ion peak recording (Merritt Jr., 1970), and in 1974, Hoffenberg et al [85] introduced selected ion monitoring, which has predominated since. Selected ion monitoring is preferred since the word selected implies both choice and specificity with no restriction to the number of ions to be monitored and the word ion describes the species being monitored which are not restricted to fragment ions but may include molecular ions or adduct ions, e.g. $(M + H)^+$ as in chemical ionisation mass spectrometry.

The ions selected for SIM should be the most abundant and characteristic of the compounds of interest and should not be characteristic ions of bleed from the GC column. High mass ions should be selected if possible because low mass ions are more likely to be present as background.

In SIM mode technique, the mass spectrometer is used as an extremely sensitive and specific detector for GLC. By monitoring one or a few selected m/z instead of full scanning (usually 40 - 450 amu), an increase in sensitivity by a factor of 10 - 100 is possible. The quadrupole mass spectrometer is more suited than the magnetic sector type since it shows more flexibility in the choice and number of m/z to be monitored which can be accomplished by rapidly switching the voltages used to produce mass dispersion. The voltage changes can focus on selected masses of interest throughout the entire mass range of the spectrometer, thus improving sensitivity and selectivity. However, the sector instrument unit be employed for high resolution SIM application.

2.10 Quantitative aspects of environmental analysis

Analysis of environmental samples employing GC or GC/MS usually involves the transfer of components of interest from the bulk original matrix to another matrix and reduction of the volume of solvent extract containing the components sample in order to match the sensitivity of the detector. This procedure is usually followed by the probable or positive identification of the components in the sample. These analyses are incomplete unless some quantitative analysis accompanies the qualitative work. If a compound of interest is identified in a sample, then it is necessary to estimate the amount/concentration of the particular compound. It is also necessary to have a reproducible chromatographic procedure and a linear response to the detector for that particular compound for which either a peak height or peak area can be used as a basis for the method involving quantification. There are several different mathematical manipulations required (internal standardisation, normalisation, external standardisation and standard addition) to convert the chromatographic peak area or height data to an absolute concentration in the samples.

2.10.1 Internal standardisation

This technique is the most commonly employed in quantitative environmental and biomedical research analysis. There is the possibility during transfer of the component from the original matrix to the matrix which is suitable for analysis by GC or GC-MS,

i.e. organic solvent that losses of the component will be uncontrolled because of an incomplete transfer from one vessel to another, and poor extraction.

In an internal standardisation approach a suitable reference compound(s), an internal standard(s) is selected and introduced into the sample or extract at a certain stage of analysis, and is used to obtain reference peak areas/heights for each analyte. Five important criteria have been suggested for selecting an internal standard suitable for environmental analysis.

The internal standard should be miscible with the sample to be analysed, should not react with any components in the sample, should give a single peak and this should not overlap with any other component peaks. The retention time should be close to the retention times for components of interest and should not be indigenous to the sample.

However, GC-MS with the addition of a data system and mass specificity is able to resolve two components which overlap in time of elution but possess different molecular and/or fragment ions and furthermore, quantification can be effected on the basis of these different ions. Studies carried out on both retention times and quantitative response using capillary column GC-MS indicate that good reproducibility of retention time can be achieved when computed based on the most closely eluting references compounds. Higher precision can be obtained when quantification is calculated based on relative response to an internal standard that is chemically similar to the analyte. Isotopically-labelled internal standards which are chemically identical to the compounds of interest, provide the most accurate results for methods using reference compounds.

However, in environmental analysis where complex mixtures are inevitably found in extracted samples, it is not possible to use internal standards that correspond to all of the compounds to be quantified. A limited number of internal standards can be employed to analyse and compensate a multicomponent system having a wide chromatographic range with precise and accurate results [86,87] with the provisor that the internal standards elute within 10 minutes of the components of interest. Deuterated compounds such as

d_8 naphthalene, d_{10} anthracene, d_{10} phenanthrene and d_{12} chrysene are most commonly used as internal standards because of the low probability of their occurrence in real samples and the normal and deuterated compounds can be distinguished by mass spectrometry.

To use these methods of quantification, the standards are prepared at least at four different concentrations with the concentration of the internal standard held constant. A calibration graph of the ratio of peak area of the component of interest to the internal standard(s) versus the concentration or concentration ratio is constructed and a response factor calculated from the slope of the linear graph.

The concentration of components are calculated based on the formula:

$$\text{Concentration} = A_S \times C_{IS} \times RF \times DF / A_{IS}$$

where A_S = peak area of component of interest

A_{IS} = peak area of internal standard

C_{IS} = concentration of internal standard

DF = dilution factor

RF = response factor, i.e. slope^{-1}

Liquid Phase	Solid support	Dimensions	Column temperature (°C)	Flow rate ml /min
10% DC-200	Chromosorb WHP	185 cm x 4 mm	200°	120
5% DC-710	Gas Chrom Q	240 cm x 4 mm	140° - 240°	160
5% OV-17	Gas Chrom Q	240 cm x 4 mm	150° - 300°[1]	160
10% OV-101	Chromosorb W-HP	185 cm x 4 mm	205°	60
5% OV-210	Gas Chrom Q	240 cm x 4 mm	150° - 300°[1]	160
5% OV -225	Gas Chrom Q	240 cm x 4 mm	150° - 300°[1]	160
5% QF-1	Gas Ghrom Q	240 cm x 4 mm	140° - 240°	160
5% Dexsil 300	a.w. Chromosorb w	240 cm x 4 mm	150°[1] - 300°[1]	160
2% DEGS	Gas Chrom Q	183 cm x 4 mm	165°, 210°, 220°	60
QF-1/DC-200[2]	Gas Chrom Q	183 cm x 4 mm	200°, 220°	120
4% SE-30 + 6% QF-1	Chromosorb W	183 cm x 3.5 mm	180°	80

1 also isothermal between 150° and 300°C
2 equal parts of 15% QF-1 and 10% DC-200

Table 2.1 Columns for the analysis of multicomponent residue

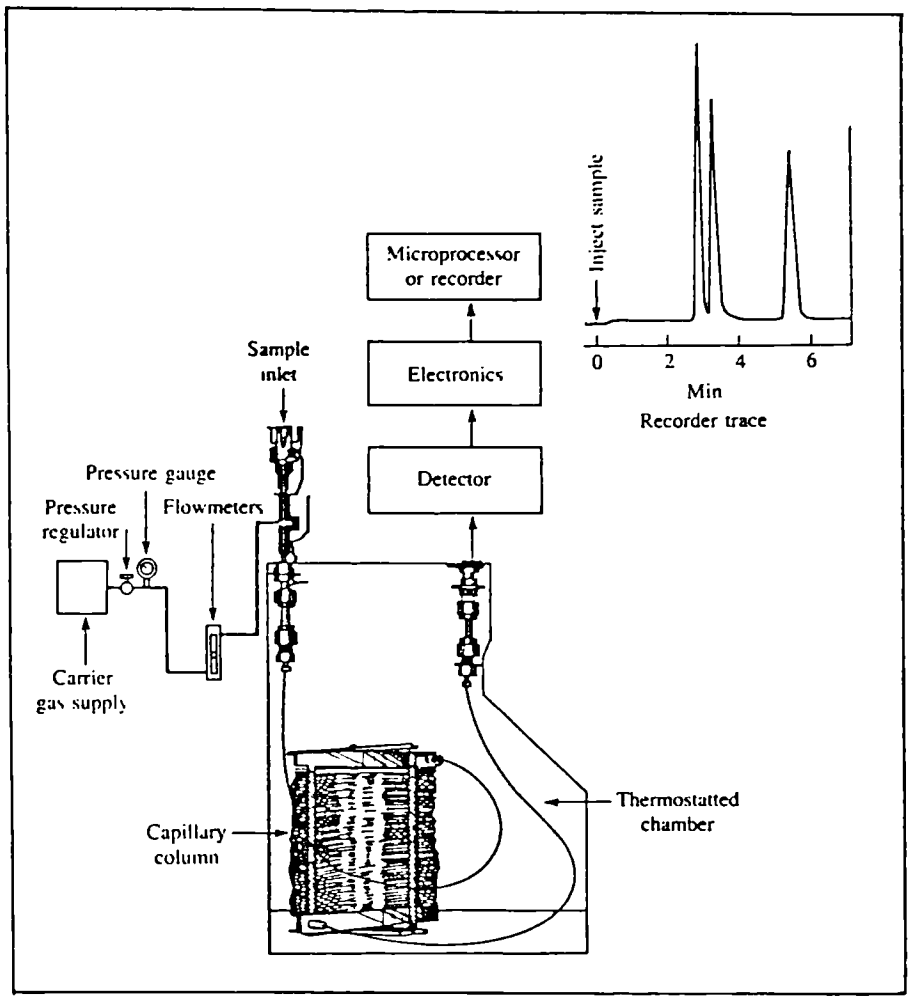


Figure 2.1 Schematic diagram of a gas chromatogram

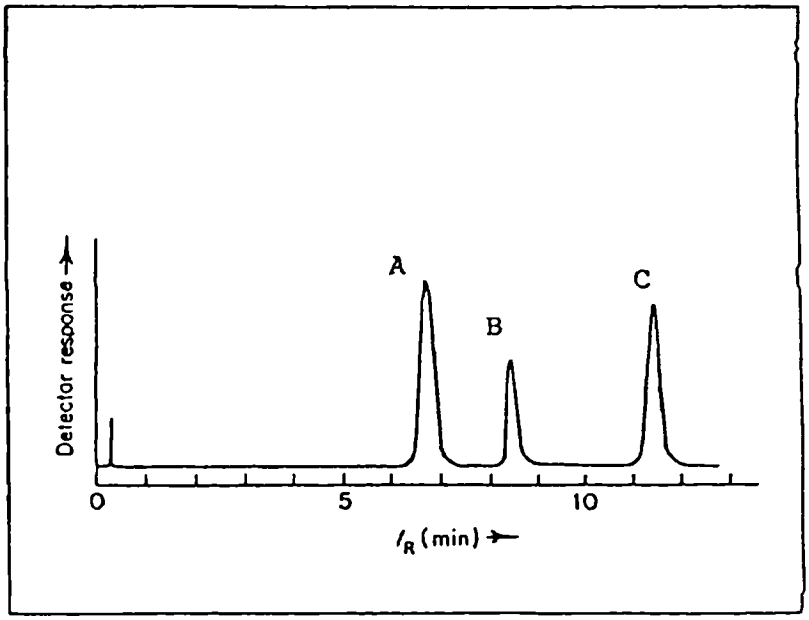


Figure 2.2 Typical chromatogram of a three component (A, B and C) mixture

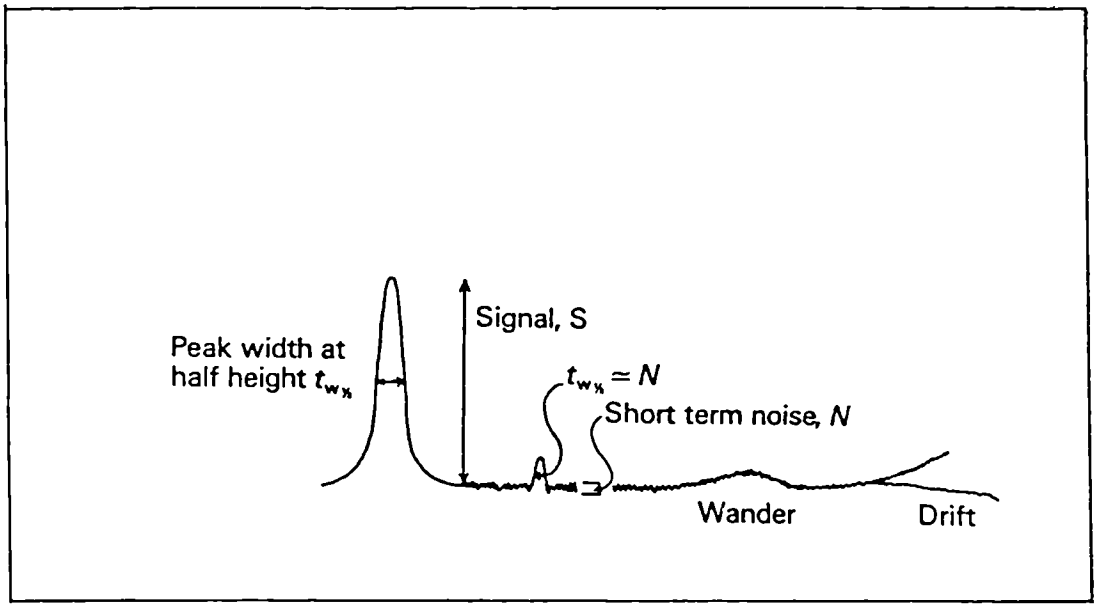


Figure 2.3 Signal-to-noise ratio and determinable levels

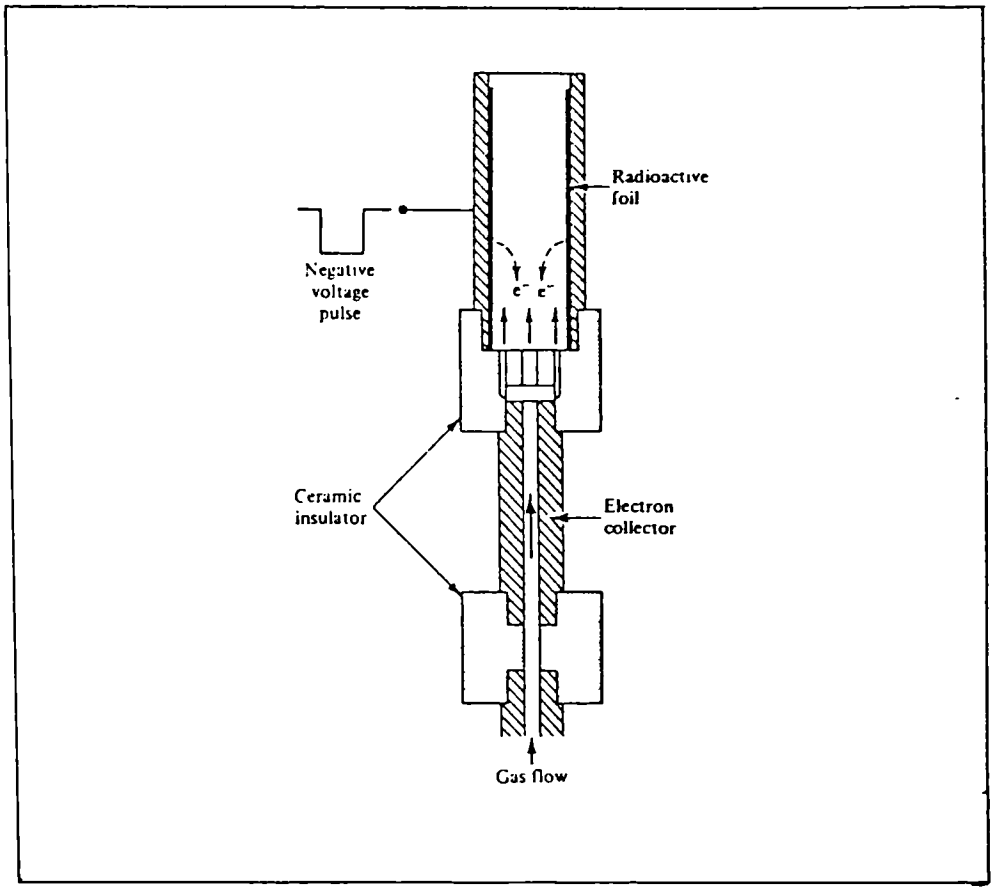


Figure 2.4 Electron capture detector

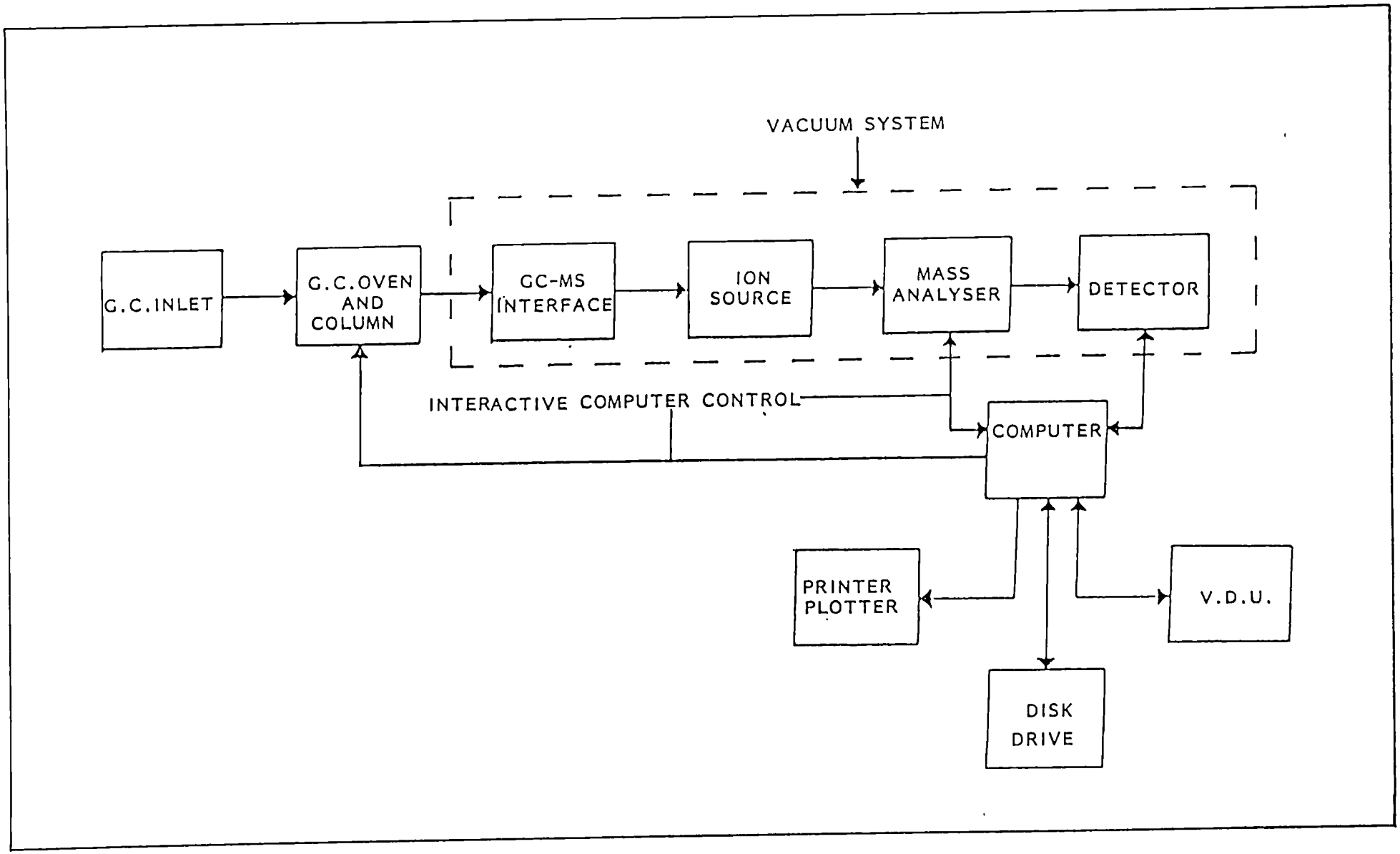
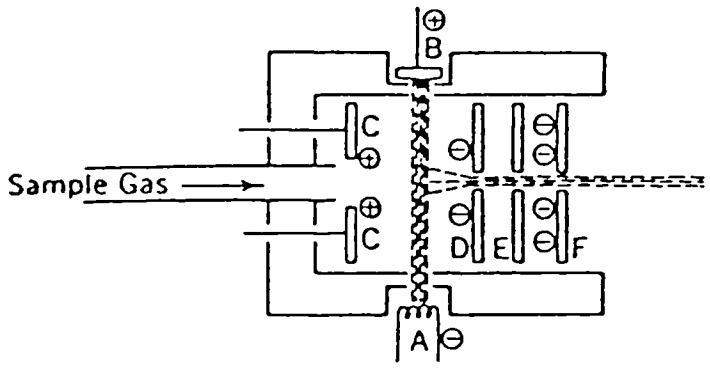


Figure 2.5 The major components of a Gas Chromatograph-Mass Spectrometry System



A: Filament

B: Anode

C: Repeller

D: First accelerating slit

E: Intermediate plate

F: Second accelerating slit

Figure 2.6 Electron impact ion source

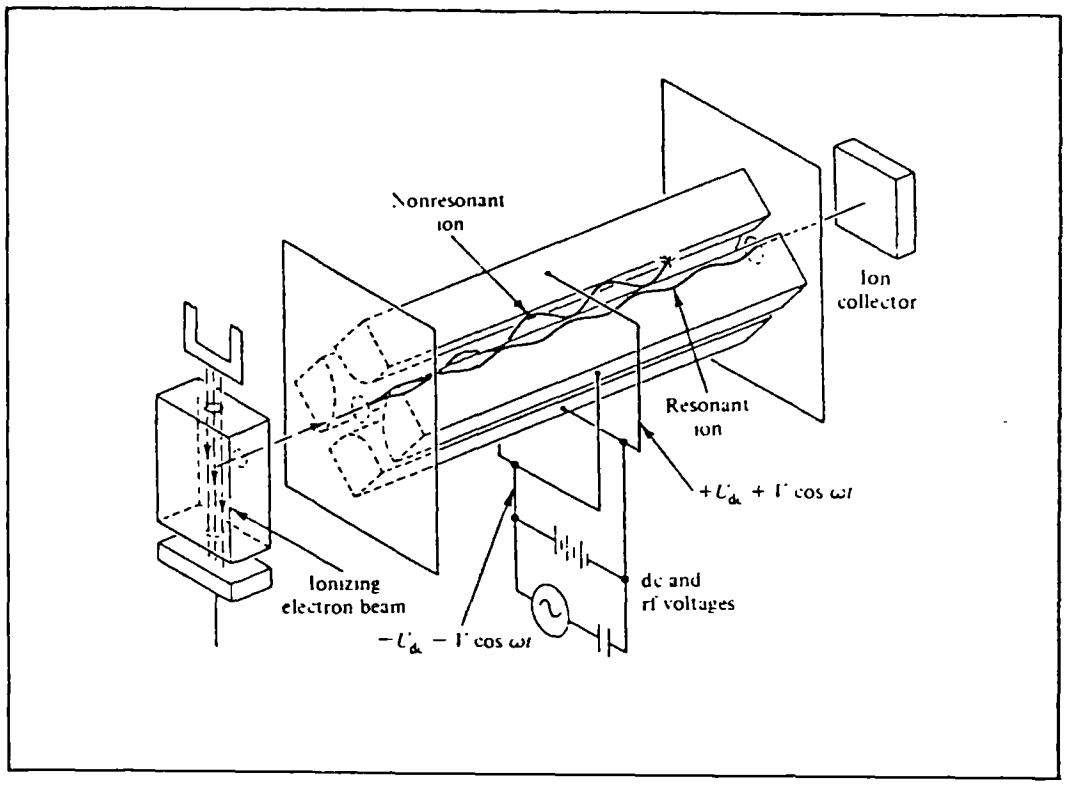


Figure 2.7 Quadruple mass analyzer

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CHAPTER 3

Determination of organophosphorus pesticides in fruit and vegetable materials by gas liquid chromatography-mass spectrometry

3.1 EXPERIMENTAL

3.1.1 Introduction

Recent advances in detector systems of gas chromatography have enabled analytical chemists to determine microgram, nanogram and even picogram quantities of pesticide residues[1,2,3]. The importance of the detection of such small quantities of pesticides cannot be overemphasized because of the potential danger of these compounds to humans and to the environment. The increased use of various types of pesticides in the modern world has led to much greater emphasis on the possibility of serious contamination of the environment.

The scientific attack on pests dates from about the middle of The Twentieth Century. In 1963, Rachael Carson's book "Silent Spring"[4], made people aware of the potential dangers of pollution from pesticides. Whenever certain chemical pesticides are applied to the foliage or seeds of crops or to the soil there is the possibility that some of the material will be persistent and may lead to serious contamination of the ecosystem.

The application of gas chromatography-mass spectrometry is to a large content limited by the volatility and thermal stability of the sample, the thermal stability of the stationary phase and also optimisation of GC-MS. Recent advances in gas chromatography using high performance inlet systems combined with high temperature bonded-phase capillary columns has extended applications of GC-MS. Further advantages of the enhanced performance resulting in improved peak profiles, can be obtained using the SIM technique, with the result that the MS can be employed as an highly sensitive and specific detector for GLC (both qualitative and quantitative applications).

This project was based an attempt to analyse four organo-phosphorus pesticides, dimethoate, heptenophos, malathion and pirimiphos methyl, in fruit samples by solvent extraction with subsequent analysis by GC-MS as the detection system.

3.1.2 Instrumentation

3.1.2.1 Gas liquid chromatography-mass spectrometry

GC-MS analysis was carried out on a Finnigan 1020, using a quadrupole analyser as the mass spectrometer. It was operated in the electron impact mode as ion source and interfaced to a Perkin-Elmer Sigma 3 gas chromatograph by direct coupling of the capillary column. The chromatograph was fitted with split/splitless and J & W telescopic on-column injector inlets. The injection port was operated entirely in the splitless mode, and ultra high purity helium was employed as the carrier gas.

The mass spectrometer was operated in the full scan mode over a mass range selected between 4 to 800 amu (instrumental limits), and with multiple interval scanning to monitor selected ions.

The manual tuning programme was used to provide interactive mass spectrometer optimisation and chromatographic conditions were applied through the GC programme. The data system comprised a Data General Nova 4 Minicomputer with 32 K memory, and a 20 megabyte dual disk drive, with 10 megabyte disc storage dedicated to software control of operational conditions, processing of acquired data and containing reference libraries. The remaining 10 megabyte removable disc was used for storage of acquired data files.

The GC-MS conditions used for running the samples are shown below. The ramp rates used were 10 and 15 deg/min, higher and lower ramp rates were tried but did not have much effect on the separation or chromatography.

3.1.2.1.1 The optimum operating conditions (all of the samples were run both full scan and under selected ion monitoring conditions) were found to be:

(i) Full scan mode (EI)

Parameter	Conditions
Zone temperature	= 250°C
Initial oven temperature	= 55°C
Final temperature	= 300°C
Initial time (column hold at 55°C)	= 2 min
Ramp rate	= 15°C for Dimethoate and heptenophos
Ramp rate	= 10°C for malathion and pirimiphos-methyl
Manifold set point	= 80°C
Final time (column hold at 300°C)	= 10 min
Interface temperature	= 240°C
Scan time	= 1 sec
Split/sweep valve time	= 30 sec
Injection mode	splitless
Helium pressure	= 8 psi (UHP-99.9995%)
Mass spectral range	= 45-450 amu
Electron multiplier volts	= -2350 volt
Volume of sample injected	= 1µl
Filament/multiplier time	= 75 sec

(ii) Selected ion monitoring (MI)

Almost the same conditions were used for the MI as for the EI but the basic differences were mass spectral range, scan time and electron multiplier voltage.

The mass spectrometer scans from 40 to 450 amu and EI mode at a rate of one second per scan or over selected mass intervals (up to 12 ions) in a total of one second or less using the selected ion monitoring (SIM) mode.

The SIM mode, optimum operating condition (mass spectra range) were found to be:

Sample		Selected Mass Interval
Malathion	Two ions	from 126.75 amu to 127.25 amu
		from 172.75 amu to 173.25 amu
Pirimiphos methyl	Two ions	from 275.5 amu to 276.5 amu
		from 289.75 amu to 290.25 amu
Dimethoate		from 86.5 amu to 87.5 amu
Heptenophos		from 123.5 amu to 124.5 amu
Parathion as internal standard		from 108.5 amu to 109.5 amu
D_{10} anthracene as internal standard		from 187.5 amu to 188.5 amu
D_8 naphthalene as internal standard		from 135.5 amu to 136.5 amu

3.1.2.1.2 Adjustment of the electrometer zero

The electrometer zero parameter (for optimum background electronic noise levels) is variable from +6.0 to -6.0 and can be changed by entering:

MT: 13,xx or MT: Ez xx

where xx is the new electrometer value.

For optimisation of the electrometer zero, most of the sample were run, and the optimum operating conditions were found to be:

Parameter	Condition
Electrometer zero	-1.01

3.1.2.1.3 Electron multiplier voltage

This high negative potential is dependent upon the age of the electron multiplier and the sensitivity needed for a particular application. Usually this voltage was set at -2350 volts in EI mode at a rate of one second per scan or over selected mass intervals in a total time one second using the SIM mode, and at a higher value also when using the SIM mode.

To optimise the electron multiplier voltage, all of the samples were run and the optimum operating conditions were found to be:

Parameter	Condition for highest sensitivity
Electron multiplier voltage	-2600 volts

3.1.2.2 Capillary columns

Several bonded-phase open tubular capillary columns of various lengths, internal diameters, liquid phases and phase thicknesses were used. The characteristics of the columns were as follows:

Stationary phase	internal diameter(mn)	polarity	phase thickness (um)	length (m)
Dimethyl siloxane BP1	0.32	N.P. *	0.1	9
14% Cyanopropylphenyl dimethyl siloxane BP10	0.22	S.P. **	0.1	10
Polydimethyl siloxane DB1	0.32	N.P.	0.1	10
Diphenyl dimethyl siloxane DB5	0.3	N.P.	0.25	30

* N.P. = non polar ** S.P. = slightly polar.

Table (3.1.2.2.1) The characteristics of the capillary columns

3.1.3 Reagents

Reagents required for standard solutions must be of high purity. Analytical grade chemical reagents are suitable for GC-MS purposes and other preparation standard.

All reagent and standard solutions should be stored in glass bottles unless otherwise stated.

3.1.2.1 Acetone

Acetone * (propanone), Analar grade purchased from MAY & BAKER LTD DAGENHAM (ENGLAND) was used for primary selection of samples (four pesticides) and washing of the apparatus.

* Highly flammable. Keep container in a well ventilated place. Keep away from source of ignition - No Smoking. Do not breathe vapour. Take precautionary measures against static discharge.

3.1.3.2 Hexane

Hexane* (HPLC grade) purchased from RATHBURN CHEMICALS LTD, was used for the preparation of standard solution from stock solution.

* Highly flammable, Helsekadelig.

3.1.3.3 Ether

Ether* (Diethyl Ether), analar grade purchased from MAY & BAKER LTD DAGENHAM (ENGLAND), was used for column cleaning.

* Highly flammable. Keep container in a well ventilated place. Keep away from source of ignition - No Smoking. Do not empty into drains. Take precautionary measures against static discharges.

3.1.3.4 Sodium Sulphate

Sodium sulphate, granular, anhydrous analar grade (purity = 99.5%) purchased from VICKERS LABORATORIES LTD was used for water absorption.

3.1.3.5 Extractant

(A) Chloroform

Chloroform* HPLC grade purchased from RATHBURN CHEMICALS LTD, was used for the extraction of samples from Acetonitrile contained in a large volume of aqueous sodium sulphate solution and pesticides.

* Harmful by inhalation, toxic.

(B) Acetonitrile

Acetonitrile* HPLC grade purchased from RATHBURN CHEMICALS LTD were used for extraction of sample (pesticide) from fruit sample.

* Highly flammable. Toxic, in contact with skin and if swallowed.

3.1.3.6 Pesticides

Standard pesticides of 91.5 - 99.5% purity (malathion, parathion, dimethoate, pirimiphos methyl and heptenophos) were purchased from RIEDEL-DE HAEN AND SHERMAN CHEMICALS and were used as received. The structural formulae common and chemical names for each of these pesticides and internal standards are given in Table 3.1.3.6.1.

Common name	Chemical name and formula	purity
Heptenophos	7-chlorobicyclo[3,2,0]-hepta-2,6-yl dimethyl phosphate $(C_7H_6(Cl))\ OP(OME)_2$	98%
Malathion	0,0-dimethyl S-(1,2-dicarboethoxy) ethyl phosphorodithioate $(CH_3O)_2P(=S)-S-CH(CH_2COOC_2H_5)COOC_2H_5$	91.5%
Pirimiphos-methyl	O,0-dimethyl 0-(2diethylamino-6-methyl-4-pyrimidinyl) phosphorothionate $(CH_3O)_2P(=S)-O-((CH_3)C_4N_2H)-N(C_2H_5)_2$	99.3%
Dimethoate	0,0-dimethyl S-(N-methylcarbamoylmethyl)-phosphorodithioate $(CH_3O)_2-P(=S)-S-CH_2CONHCH_3$	99.5%
Parathion	0.0-diethyl 0-4-nitrophenyl phosphorothioate (1) $(Eto)_2P(=S)O-(C_6H_4)-NO_2$	
D_{10}-anthracene	Anthracene-d_{10} $C_{14}D_{10}$	99%
D_8-naphthalene	Naphthalene-d_8 $C_{10}D_8$	98%

Table 3.1.3.6.1 The pesticides and internal standards employed in this project.

3.1.4 Apparatus

3.1.4.1 Blender

The blender* used in the present work is the OSTER DESIGNER to obtain fine and desirable, chopped and mixed fruit samples.

* Glass container (glass blender cup).

3.1.5 Stock standard solutions*

The pesticide purity is stated in the specification provided by RIEDEL-DE HAEN AND SHERMAN CHEMICALS. These sources of pesticides were used to make up calibration standards and also for recovery studies. Three internal standards have been employed during this study i.e. parathion ethyl, d_8-naphthalene and d_{10}-anthracene.

A stock solution containing 1000 ppm of particular pesticides (each sample pesticide separately) was prepared by accurately weighing directly into a 50 ml volumetric flask and made up to volume with HPLC grade solvent. The procedure for dimethoate, 50 mg of dimethoate was weighed directly into a 50 ml volumetric flask. Then about 5 ml ethanol HPLC grade was added and the volume was made up with HPLC grade hexane to give a 1000 ppm dimethoate stock solution (see figure 3.1.5.1).

* Warning: organophosphorus pesticides in the undiluted state are very toxic. Extreme caution must be exercised when preparing the stock solutions. Skin contact, ingestion and inhalation must be avoided.

A standard solution of 100 ppm pesticide in hexane was prepared by dilution of 1000 ppm stock and a standard solution of 10 ppm was prepared by dilution of the 100 ppm standard solution.

The calibration standards for full scan GLC-MS were prepared using stock solution and consisted of six different concentrations, i.e. 1, 2, 4, 6, 8, 10 ppm with concentration of internal standards held constant (e.g. 2ppm d_8 naphthalene for dimethoate pesticides). The calibration standards employing GC/MS/SIM were prepared from the calibration standard for full scan, i.e. 0.1, 0.2, 0.4, 0.6, 0.8, 1 ppm and 0.01, 0.02, 0.04, 0.06, 0.08, 0.1 ppm (concentration of internal standard held constant).

The working ranges of calibration standards for this project are given in Table 3.1.5.1 - 3.1.5.4 below:

Name of sample	Scan mode	working concentration range for sample (ppm) / internal standard	internal standard used
malathion	full scan	(1, 2, 4, 6, 8, 10)/5	parathion
malathion	SIM	(0.1, 0.2, 0.4, 0.6, 0.8, 1)/0.5	"
malathion	SIM	(0.01, 0.02, 0.04, 0.06, 0.08, 0.1)/0.05	"
malathion	full scan	(1, 2, 4, 6, 8, 10)/2	d_8-naphthalene
malathion	SIM	(0.1, 0.2, 0.4, 0.6, 0.8, 1)/0.2	"
malathion	SIM	(0.01, 0.02, 0.04, 0.06, 0.08, 0.1)/0.02	"
malathion	full scan	(1, 2, 4, 6, 8, 10)/2	d_{10} Anthracene
malathion	SIM	(0.1, 0.2, 0.4, 0.6, 0.8, 1)/0.2	"
malathion	SIM	(0.01, 0.02, 0.04, 0.06, 0.08, 0.1)/0.02	"
malathion	full scan	(1, 2, 4, 6, 8, 10)/5	pirimiphos-methyl
malathion	SIM	(0.1, 0.2, 0.4, 0.6, 0.8, 1)/0.5	"
malathion	SIM	(0.01, 0.02, 0.04, 0.06, 0.08, 0.1)/0.05	"

Table 3.1.5.1 MS operation mode and working concentration range for malathion

Name of sample	Scan mode	working concentration range for sample / internal standard ppm	internal standard used
pirimiphos-methyl	full scan	(1, 2, 4, 6, 8, 10)/5	parathion
"	SIM	(0.1, 0.2, 0.4, 0.6, 0.8, 1)/0.5	"
"	SIM	(0.01, 0.02, 0.04, 0.06, 0.08, 0.1)/0.05	"
pirimiphos-methyl	full scan	(1, 2, 4, 6, 8, 10)/2	d_8 naphthalene
"	SIM	(0.1, 0.2, 0.4, 0.6, 0.8. 1)/0.2	"
"	SIM	(0.01, 0.02, 0.04, 0.06, 0.08. 0.1)/0.02	"
pirimiphos-methyl	full scan	(1, 2, 4, 6, 8, 10)/2	d_{10}-anthracene
"	SIM	(0.1, 0.2, 0.4, 0.6, 0.8, 1)/0.2	"
"	SIM	(0.01, 0.02, 0.04, 0.06, 0.08, 0.1)/0.02	"
pirimiphos-methyl	full scan	(1, 2, 4, 6, 8, 10)/5	malathion
"	SIM	(0.1, 0.2. 0.4, 0.6, 0.8, 1)/0.5	"
"	SIM	(0.01, 0.02, 0.04, 0.06, 0.08, 0.1)/0.05	"

Table 3.1.5.2 MS operation mode and working concentration range for pirimiphos-methyl.

Name of sample	Scan mode	working concentration range for sample / internal standard ppm	internal standard used
Dimethoate	full scan	(1, 2, 4, 6, 8, 10)/2	d_8-naphthalene
"	SIM	(0.1, 0.2, 0.4, 0.6, 0.8, 1)/0.2	"
"	SIM	(0.01, 0.02, 0.04, 0.06, 0.08, 0.1)/0.02	"
Dimethoate	full scan	(1, 2, 4, 6, 8, 10)/2	d_{10}-anthracene
"	SIM	(0.1, 0.2, 0.4, 0.6, 0.8, 1)/0.2	"
"	SIM	(0.01, 0.02, 0.04, 0.06, 0.08, 0.1)/0.02	"

Table 3.1.5.3 MS operation mode and working concentration range for dimethoate.

Name of sample	Scan mode	working concentration range for sample / internal standard ppm	internal standard used
Heptenophos	full scan	(1, 2, 4, 6, 8, 10)/2	d_8-naphthalene
"	SIM	(0.1, 0.2, 0.4, 0.6, 0.8, 1)/0.2	"
"	SIM	(0.01, 0.02, 0.04, 0.06, 0.08, 0.1)/0.02	"
Heptenophos	full scan	(1, 2, 4, 6, 8, 10)/2	d_{10}-anthracene
"	SIM	(0.1, 0.2, 0.4, 0.6, 0.8, 1)/0.2	"
"	SIM	(0.01, 0.02, 0.04, 0.06, 0.08, 0.1)/0.02	"

Table 3.1.5.4 MS operation mode and working concentration range for heptenophos.

3.1.6 Preparation of spiked samples

3.1.6.1 Spiked fruit sample (2mg/kg)

20 gram of pesticide-free finely chopped fruit was placed in a mixer (very clean). Using a 10 ml pipette, 4 ml of 10 ppm fresh standard solution added the sample swirled to mix well, prior to extraction (see figure 3.1.7.1).

3.1.6.2 Spiked fruit sample (0.2mg/kg)

20 gram of pesticide-free finely-chopped fruit was placed in a mixer. Using a 1 ml pipette, 1 ml of 10 ppm fresh standard solution added then the sample swirled to mix well prior to extraction.

3.1.6.3 Pesticide-free fruit sample

20 gram of pesticide-free fruit sample, finely-chopped was placed in a mixer prior to extraction. Fruit samples were purchased from a local fruit market in Manchester, England.

3.1.7 Procedures

3.1.7.1 Extraction

3.1.7.1.1 Principle of the method[5]

The finely chopped representative sample was mixed with anhydrous sodium sulphate and extracted with acetonitrile. The extract was diluted with a large volume of aqueous sodium sulphate solution. The pesticides, and other materials, were extracted into chloroform. The chloroform solution was dried and concentrated for gas liquid chromatography and gas liquid chromatography-mass spectrometry.

3.1.7.1.2 Materials section

Solvents should be checked for interference in GC-MS before use.

Acetone. Analytical-reagent grade

Acetonitrile. HPLC grade

Chloroform. HPLC grade

Sodium sulphate, granular, anhydrous. Analytical-reagent grade

Sodium sulphate, 2.9% aqueous solution. Analytical-reagent grade

Propylene glycol, 50% solution in acetone

Pure pesticides. For standard solution

Nitrogen. For concentration

Helium. Carrier gas for GC-MS

3.1.7.1.3 Apparatus

Blender. Glass blender cup

Separating funnel. Capacity 1 l

Cotton-wool

Anti-bumping granules

Filter funnel. About 15 cm diameter

Calibrated flasks

Micro-snyder condenser

Chromatography column. About 300 x 15 mm

Kuderna-Danish evaporator. Fitted with a 10-ml graduated tube, but no fractionating columns

Steam-bath

Water-bath

GC-MS

Microlitre syringes

3.1.7.1.4 Extraction method

The following extraction method was followed. A representative sample (20 g) was finely chopped into a blender cup and mixed in 30 g of anhydrous sodium sulphate, or sufficient to give a homogenous mixture, with the sample. Add 50 ml of acetonitrile and blend at high speed for 3 min. Decant the extract through a filter-funnel containing a small cotton-wool plug into a separating funnel containing 500 ml of 2.5% sodium sulphate solution.

Repeat the extraction and decanting procedures several times each with 50 ml of acetonitrile and the same sodium sulphate solution. Mix the contents of the separating funnel and extract the aqueous solution with 50 ml of chloroform. Ensure the complete separation of the two layers. Run the chloroform layer down a 10 cm chromatography column of contusing granular anhydrous sodium sulphate and into a Kuderna-Danish evaporator fitted with a 10 ml graduated tube. Repeat the chloroform extraction and drying procedure twice more, each time with 50 ml of chloroform. Add 0.1 ml of propylene glycol solution as a keeper, add a few anti-bumping granules and reduce the volume of the chloroform to about 2 ml on a steam-bath. The level of the liquid in the flask should always be above the level of water in the bath.

Remove the apparatus and allow it to cool to room temperature before removing the still head. Rinse the evaporator with 3 - 5 ml of acetone and allow 5 min for drainage. Remove any moisture collected around the outside of the joint of the graduated tube and evaporator with a cloth. Separate the tube from the evaporator and rinse the male joint with 1 ml of acetone. Fit a micro-Snyder condenser and carefully remove the solvent by boiling over a steam-bath until only 0.5 ml remains in the bottom of the tube.

Remove the apparatus from the steam-bath, wait about 1 min, then carefully run 0.5 ml of acetone into the top of the micro-snyder tube and allow the acetone to drain down into the graduated tube. Repeat the latter acetone washing twice and remove the micro-snyder tube. Place the graduated tube into a water-bath at 30°C and blow a stream of dry air

onto the surface of the extract until a small constant volume remains. Remove the graduated tube and make up to a suitable volume with acetone and/or hexane for gas liquid chromatography mass-spectrometry (see figure 3.1.7.1).

3.1.7.2 GC/MS analysis

The GC/MS instrument used was an automated GLC/MS Finnigan 1020 as described in previous sections.

The use of 9 metre BP1, 10 metre BP10 (SGE Ltd) and 10 metre DB1, 30 metre DB5 (J&W Company) capillary columns was investigated to determine the optimal performances in terms of separation, peak shape and analysis time for the pesticides studied.

The full scan mode (40 - 450 amu) of mass spectrometer was employed to analyse higher concentrations of samples (1 - 10 ppm). The full EI mass spectra of the four pesticides and five internal standards were acquired using this mode. The base peaks for each component, i.e. m/z 173 for malathion, m/z 290 and m/z 276 for pirimiphos-methyl, m/z 87 for dimethoate, m/z 124 for heptenophos, m/z 109 for parathion, m/z 188 for d_{10} anthracene and m/z 136 for d_8 naphthalene were selected for quantitative analysis using SIM.

The EI mode was also operated using the SIM. Splitless injection with 1 ul of analyte[6] was employed and the column temperature was programmed as described in section 2.2.1.1.

3.1.7.2.1 Optimisation of tuning

The tuning was adjusted with the manual tune program using the calibration gas FC-43 (pftba) by optimising the spectrum to obtain good amplitude, peak shape, resolution and minimal discrimination from low to high mass. The print out in the Appendices (A3.1) shows a spectrum of FC-43 with optimised tuning. For further optimisation the sensitivity

and isotope ratios were determined for m/z 299 and 298 using methyl stearate 10 ppm or by carrying out the EPA performance evaluation/abundance calibration procedure using DFTPP 60 mg/l. The mass spectra of methyl stearate and DFTPP are also included in the Appendices (A3.2-3).

3.1.7.2.2 Optimisation of electrometer zero

To obtain minimal background electronic noise levels and total sector usage (for SIM mode at high electron multiplier voltage), the MT file was used to vary the electrometer zero parameter (from +6.0 to -6.0).

3.1.7.2.3 Electron multiplier voltage

For maximum sensitivity, i.e. lower amounts detection limits for organophosporous compounds using selected ion monitoring the negative potential supplied to the electron multiplier was varied and increase to -2600 volts to determine the optimum voltage for each compound.

3.1.7.2.4 Chromatographic condition

To find the optimal efficiency of the chromatographic column for the separation of mixtures of samples and internal standard, the GC conditions should enable base line resolution of the pesticide and reference compound so that quantification data processing can be carried out. The reference compound should elute close in time to the pesticide to allow for any changes in mass spectrometer tuning and sensitivity (retention time window 60 seconds of less if possible). For the above reasons ramp rates (in the range of 7 - 20 degrees/minute) were investigated.

The effects of the capillary column length, internal diameter on different column coating on resolution were considered. In this work, the performance of four possible columns was investigated and the characteristics of the capillary columns are listed in Table (3.2.2.1).

3.1.7.2.6 Silanization

Silanization of the column using a Chrompak reagent was an attempt to improve the chromatography of pesticides, particularly dimethoate, but with little effect.

3.1.7.2.7 Selected mass internal

Optimisation the selected mass interval range in SIM mode, was accomplished by utilising the multiple mass interval scan program MI. The MI program provides multiple ion detection scanning (MID), also known as selective ion monitoring (SIM), and up to 25 separate mass intervals for each file. These files are designated by the letters A through Z.

Optimum operating conditions are listed in the previous section.

3.1.7.3 Procedure for calibration

Serial dilution of standards were made as described in Tables 3.5.1, 3.5.2, 3.5.3 and 3.5.4.

1 ul of each sample (as indicated before) was injected under the optimum GC/MS conditions as listed in previous sections.

The areas of the pesticide peak (or ion area) and internal standard peak were measured for each injection, and the ratios were plotted against concentration of pesticide.

3.1.7.4 Pesticide residue recovery procedure

Cut up sample of vegetable material and add a known amount of the organo phosphorous pesticides using the extraction procedure as described in section 3.7. The calculation of recovery of pesticide was performed by comparing the area ratio of extracted standards with calibration standards.

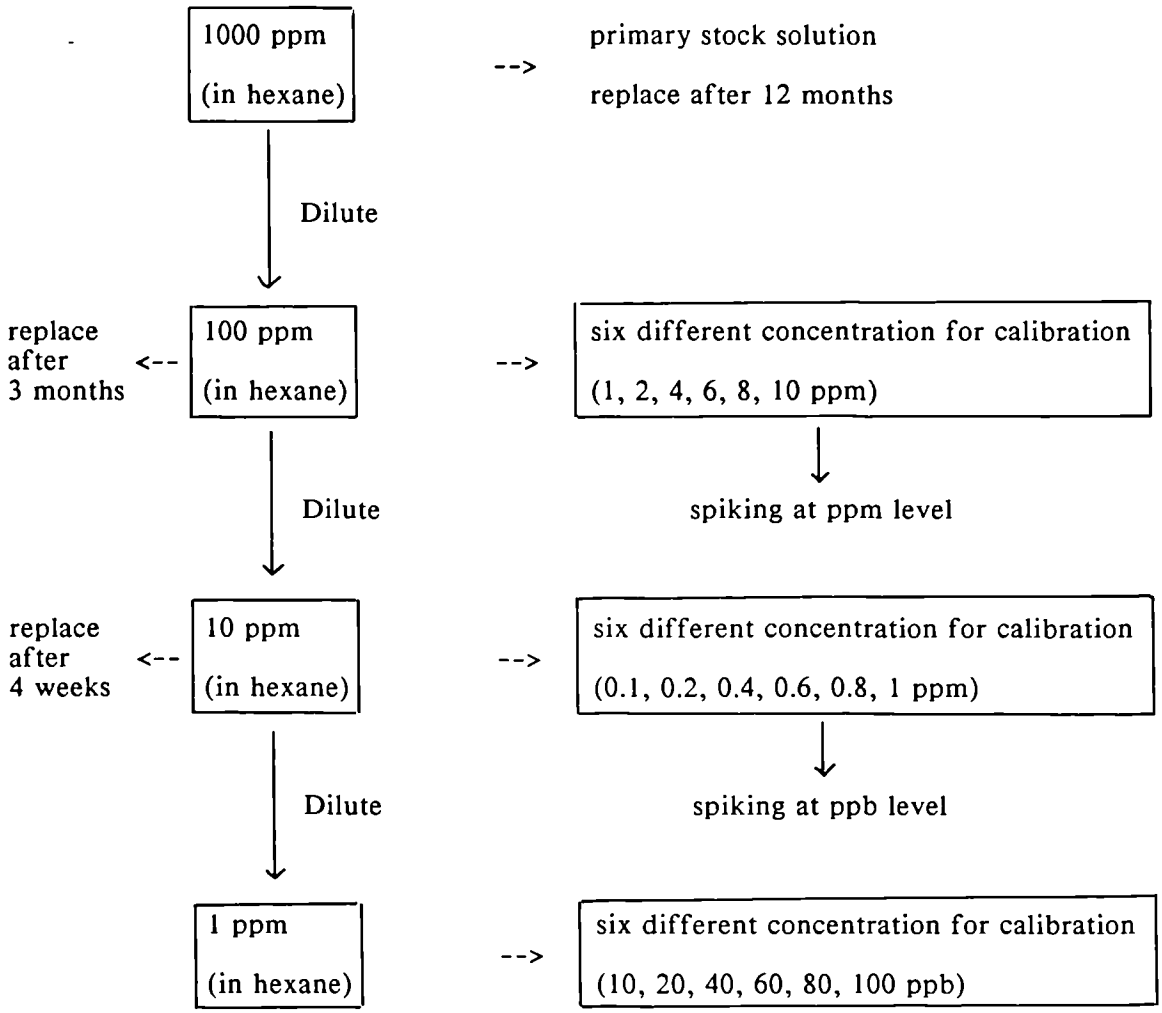


Figure 3.1.5.1 Scheme of preparation/dilution of standard solution from stock solution (stored at 0°C) of technical pesticides

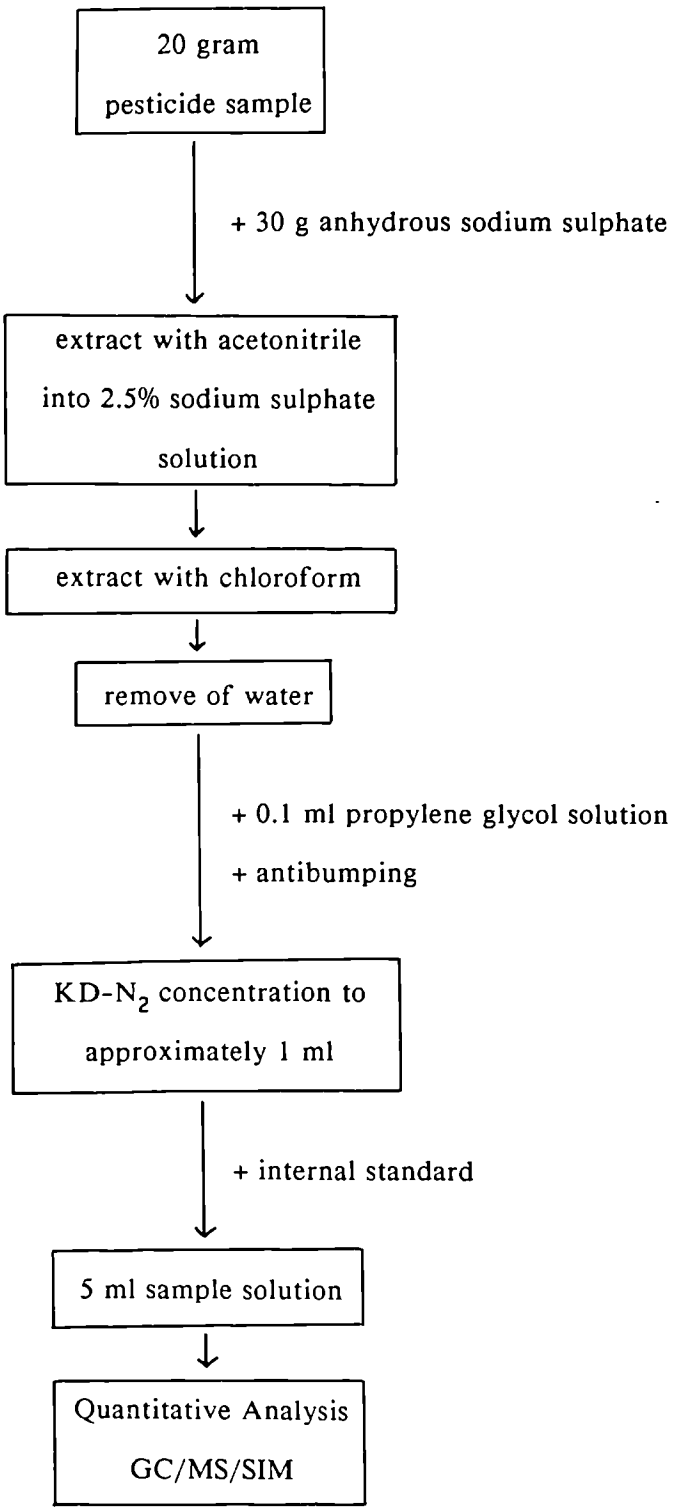


Figure 3.1.7.1 The scheme for the analysis of fruit and vegetable sample using extraction method

3.2 Result and Results Analysis

3.2.1 General considerations - spiking and sampling methodology

Consideration has been given to the method of spiking the sample (fruit or vegetable) in order to obtain reliable date for recovery studies. The analyte should be distributed uniformly within the sample and analyte mixing in the matrix should not be altered. Thus, a solvent (for pesticide solution) suitable for spiking should be easily dispersiable in the fruit or vegetable and mixed well to provide a homogeneous matrix. In this work, acetone was used as a solvent for spiking because of its high miscibility with water which will facilitate the mixing with a moist solid such as blended fruit or vegetable.

Another important problem to be considered in ultra-trace analysis is cross-contamination via the apparatus e.g. blender, calibrated flask, etc. This is particularly critical when employing sensitive methods such as GC-MS. This problem can be avoided or at least reduced by thoroughly washing the apparatus well.

3.2.2 Mass spectra of pesticides and internal standards

The mass spectra of pesticides have been reported by several workers (7 - 16). Table 3.1 shows the major ions observed in mass spectra (only eight ions for each component) of pesticides obtained under electron impact mode with ionisation voltage of 70 eV. The electron impact mass spectra exhibit base peaks (100% RA) at m/z 173, 124, 290, 87, 188, 136, 97 for malathion, heptenophos, pirimiphos-methyl, dimethoate, d_{10} anthracene, d_8 naphthalene and parathion, respectively.

3.2.3 Selection of ions for monitoring

The ions which give the highest abundance (100% RA) in the EI mass spectra, i.e. base ion peak, were selected for monitoring in GC-MS analysis, although other ions such as m/z 93 for dimethoate and m/z 89 for heptenophos could also be included for monitoring

because of their abundance in EI mass spectra. From the results (Table 3.2), increasing the number of ions monitored will only reduce (56.3%) the overall sensitivity of analysis, thus increasing the detection limit. Further more, this will contribute to more background when employed for real samples. Monitoring only m/z 87 provides a good selectivity and sensitivity because no other chromatographic peaks corresponding to m/z 87 interfere with this monitoring. Thus dimethoate can be specifically quantified and identified employing the base ion peak m/z 87.

Monitoring of m/z 124, an ion characteristic of heptenophos was also included in GC/EIMS/SIM to determine this pesticide residue in fruit and vegetable samples. Although, EIMS of heptenophos (Table 3.1) indicates that the base peak is at m/z 124 and m/z 89 is the second most abundance mass. Monitoring two ions (m/z 124, m/z 89) reduced the overall sensitivity of analysis by 70.10 % and increased the detection limit (see Table 3.3). Furthermore, this condition of scanning will contribute to more background when employed for real fruit and vegetable samples. Monitoring only m/z 124 provided a good selectivity and sensitivity because no other chromatographic peak with ions corresponding to m/z 124 interfere with the monitoring of this ion; thus heptenophos can also be specifically identified and quantified.

The monitoring of both m/z 290 and m/z 276 characteristic of pirimiphos-methyl was included in GC/EIMS/SIM to determine this pesticide in samples.Selection of these two ions increases (40.30 %) the overall sensitivity of analysis (see Table 3.4) thus decreasing the detection limit. Furthermore, these ions will not contribute to a higher background when employed for real fruit and vegetable samples. Monitoring m/z 290 and m/z 276 provides a good selectivity and sensitivity because again no other chromatographic peaks containing ions corresponding to m/z 290 and m/z 276 interfere with the monitoring. Thus by scanning these ions this pesticide can also be specifically identified and quantified without interference and referring to a standard.

Monitoring m/z 173 characteristic of malathion was also included in GC/EIMS/SIM to determine the malathion residue in samples. Because also m/z 173 provides a good

selectivity and sensitivity for the same reasons as stated for other pesticides, malathion can also be monitored with confidence at low levels. Although EIMS of malathion (Table 3.1) indicates that the base peak is at m/z 173 (sometimes m/z 125 and 127), m/z 173 was selected since monitoring of lower mass ions will accumulate a higher noise level.

Table 3.5 shows that monitoring m/z 173 reduced the sensitivity (compared with the monitoring of two ions) but because of interference with m/z 125 and 127 in the real sample quantification based on the scanning of these two m/z is not valid.

3.2.4 Qualitative analysis of pesticides

(A) Malathion and pirimiphos-methyl

Figure 3.1 shows the RIC of a 10 ppm mixture of malathion and pirimiphos-methyl with 6 ppm parathion as an internal standard using full scan mode (40-450 amu) with the pirimiphos-methyl eluting before malathion and malathion eluting before parathion. Good separation is indicated with retention time windows between pirimiphos-methyl and parathion of 21 seconds and less than 14 seconds between malathion and parathion, and malathion and primiphos-methyl, achieved at a temperature ramp rate, 10°C/min.

Figures 3.2-5 show that for RICs of samples (without pesticides) analysed in full scan mode (40-450 amu) the pesticide peaks (comparing retention time in figure 3.1 with figures 3.2-5) are completely masked by many co-extractants. The existence of these two pesticides and parathion as internal standard can only be confirmed by using mass chromatogram analysis at m/z 276, 290, m/z 173 and m/z 97, 109 respectively as shown in figure 3.6-9. These figures confirm that unknown peaks (co-extractant compounds) elute at the same retention times as these two pesticides but exhibit m/z 97 and m/z 109 and thus these ions are not characteristic of the internal standards. Many workers (16-25) commonly practice a clean-up step in residue analysis of pesticides resulting from environmental samples. However, it was considered that these tedious and time-consuming procedures are unnecessary since further loss of the pesticides will ensue via the adsorbent and the effect on recovery is more severe at the lower concentrations

determined here.

Figures 3.10-13 and figures 3.2-5 show the GC/EIMS/SIM method can be used with good separation without any interference. Using the conditions as described in section 3.2 (SIM), complete analysis is achieved in 17 minutes per sample. The characteristic ions used to determine spiked fruit and vegetable samples (malathion and pirimiphos-methyl) are listed in table 3.6. The relative retention time (RRT) of malathion and pirimiphos-methyl calculated based on d_8 naphthalene are summarised in table 3.7. RRT based on d_{10} anthracene using similar conditions are listed in table 3.8.

(B) Dimethoate

Figure 3.14 shows the RIC and mass chromatogram of 10 ppm dimethoate with 2 ppm of d_8 naphthalene and d_{10} anthracene as internal standards using the full scan mode (40-450 amu) with the d_{10} anthracene eluting after dimethoate and d_8 naphthalene prior to it. This figure also shows the mass chromatograms of dimethoate (m/z 87) and d_8 naphthalene (m/z 136) and d_{10} anthracene (m/z 188) with good separation. The retention time window between dimethoate and d_{10} anthracene is 29.4 seconds and 5.09 minutes between dimethoate and d_8 naphthalene, achieved at a ramp rate of 15°C/min.

Figures 3.15-18 show the RIC of samples (free of pesticides) analysed in full scan mode (40-450 amu). The dimethoate peak (comparing retention time in figure 3.14 with figures 3.15-18) is completely masked by many co-extractants (except for carrot samples). The existence of dimethoate can only be confirmed using mass chromatogram analysis for m/z 87 in full scan mode. Many workers have employed a clean-up step as described in part A but here again the GC/EIMS/SIM method can be used to obtain good separation, without any interference, with a reduced analysis time using the conditions as described in section 3.7.2, complete analysis being achieved in 12 minutes per sample. The characteristic ions used to monitor pesticides in spiked fruit and vegetable samples (dimethoate) are listed in table 3.6. The relative retention time (RRT) for dimethoate calculated based on d_{10} anthracene using conditions described previously (see 3.2.1.1) is 0.96 (RSD (n=5) 0.12%).

(C) Heptenophos

Figure 3.19 shows the RIC obtained for a standard solution containing of 10 ppm heptenophos with 2 ppm of d_8 naphthalene and d_{10} anthracene as internal standards using the full scan mode (40-450 amu) with the d_8 naphthalene eluting prior to heptenophos and d_{10} anthracene eluting after heptenophos. Figure 3.20 shows the mass chromatograms for heptenophos (m/z 124) and d_{10} anthracene (m/z 188) and d_8 naphthalene (m/z 136). Good separation of hepthenophos, d_8 naphthalene and d_{10} anthracene was achieved at a ramp rate i.e. 15°C/min.

Figure 3.15-18 show the RICs of a sample (free of heptenophos) analysed in full scan mode (40-450 amu) and the heptenophos peak (comparing the retention data here with that in figure 3.19) is completely masked by many co-extractants. The presence of heptenophos can only be confirmed using mass chromatogram analysis for m/z 124 in full scan mode and thus the GC/EIMS/SIM method must be used. Good separation, without any interference, with a reduced analysis time and using the conditions as in section 2, complete analysis was achieved in 12 minutes per sample. The characteristic ions employed to analyse spiked fruit and vegetable samples are listed in table 3.6. The relative retention time (RRT) of heptenophos calculated based on d_8 naphthalene using conditions as described previously (see 3.2.1.1) is 1.602 (RSD (n=5) 0.14%). The RRT of heptenophos calculated based on d_{10} anthracene is 0.859 (RSD (n=5) 0.11%).

3.2.5 Quantitative analysis

Quantitative GC/MS analysis of individual pesticides in extracts was performed by a technique of internal standardisation and this method has been described in section 3.7.4. A comparative study of internal standardisation methods with external standardisation has been carried out using full scan mode of GC/MS over the concentration range 1-10 ppm, and GC/MS/SIM mode for the concentration range 0.1-1.0 ppm and 0.01-0.1 ppm. Figures 3.21 and 3.22 show the internal and external standardisation calibration curves of malathion as typical, using full scan mode. The linearity of plots indicates the best fit by linear regression. The quantity of individual component was calculated from the

equation $y = mx + c$, where y was area ratio (internal standardisation) or area of mass chromatogram (external standardisation), m is the slope and c is y intercept at x=0. A simple program run on the University of Salford Prime Computer has been used to calculate several statistical parameters including correlation-coefficient, slope, and intercept at x=0. This program was also used to calculate the concentration of analyte in the samples.

The reproducibility of external standard method (Table 3.17-18) was poor and quantification can be greatly improved in GC/MS by the use of deuterated compounds as internal standards (Table 3.19-20). Two factors appear to contribute to the problem of lack of reproducibility (linearity) of the external standardisation method. The first was related to the lack of consistency in injection volume. The second can arise from the lack of consistency in detector sensitivity. It was difficult to maintain the sensitivity of mass spectrometer throughout the period of extended analysis of 2-3 days, particularly when the instrument was used routinely by other workers involved in many different types of sample analysis. The internal standardisation method which was based on the ratio of analyte to reference standard (internal standard) gives more satisfactory reproducibility as it was not affected by sensitivity but rather influenced by the consistency of the mass spectrometer to monitor mass ions in the range required. Optimisation can be carried out by daily manual tuning of the mass spectrometer as recommended by US EPA. Thus, the quantitative analysis of pesticides with external standardisation was difficult and less reliable, particularly in a long period of time where there is a dependency on the capability of detector , to maintain optimum and linear sensitivity.

This state of operation appears to be impossible when the instrument was used routinely for analysis involving a variety of types of sample and different workers. On the other hand, the internal standardisation method provides far better reproducibility and linearity. All the curves, including internal standardisation using d_8 naphthalene, d_{10} anthracene and others as described previously in table 3.5.1-4 exhibit good linearity with correlation coefficient R higher than 0.9946.

Quantitative analysis employing GC or GC/MS, quantitation of an unknown sample is as follows. The peak area (or peak hight) corresponding to the concentration of the m/z x (x is a characteristic ion) in the sample is converted to a concentration of pesticide from the calibration graph. The procedure is summarised in equation (1)

$$\text{Ratio of } \frac{\text{pesticide ion}}{\text{internal standard ion}} \xrightarrow{\text{calibration curve}} \text{concentration.pesticides} \quad (1)$$

3.2.5.1 Selection of internal standard

The use of the internal standardisation methods, particularly a multi-internal standard approach was considered essential to provide an accurate method for quantitative analysis which has been emphasised by several authors. Before the internal standard used for quantification were selected, their suitability as references for pesticides was first investigated. Several internal standards were used in investigations for quantitative analysis. The results are presented in table 3.6. References which chromatograph fairly close to pesticides give better reproducibility than other standards which elute outside a narrow retention time windows.

3.2.5.2 Quantitation analysis of malathion

Quantitation was conducted by peak area ratio correlation with a calibration curve. For GC/MS the calibration curve was obtained by plotting peak area (mean of two injection, 1 ul volume. 10 microlitre syringe) against the weight of malathion. It should be noted however, that the ion monitored was m/z 173 corresponding to M^+-$(CH_3O)_2\overset{S}{\overset{\|}{P}}$-S.
The regression line for this curve was y = 0.601x - 0.572 with a correlation coefficient of 0.9982.

3.2.5.3 Quantitation analysis of pirimiphos-methyl

Quantitation was performed by peak area ratio correlation with a calibration curve. For GC/MS the calibration curve was obtained by plotting peak area ratio (mean of three

injections, 1 ul volume) against the weight of pirimiphos-methyl. It should be noted that the ions monitored were m/z 290, 276, corresponding to M^+-CH_3 and CH_3-CH_2 respectively. The use of these two ions was recommended because of the greater sensitivity and specificity of these ions. The regression line for this curve was $y = 0.579x - 0.531$ with a correlation coefficient of 0.9990.

3.2.5.4 Quantitation analysis of dimethoate

Quantitation was performed by peak area ratio correlation with a calibration curve. For GC/MS the calibration curve for dimethoate was obtained as for pirimiphos-methyl above. It should be noted that the ions monitored was m/z 87 corresponding to M^+-$(CH_3\text{-}O)_2$-$\overset{S}{\overset{\|}{P}}$-S. The use of these ion was again recommended because of sensitivity and specificity. The regression line for this curve was $y = 0.963x + 0.325$ with a correlation coefficient of 0.9946.

3.2.5.5 Quantitation analysis of heptenophos

Quantitation was by peak area ratio correlation with a calibration curve. For GC/MS the calibration curve was obtained as for pirimiphos-methyl repetitive against the weight of heptenophos. It should be noted that the ion monitored was m/z 124 corresponding to M^+-C_7H_6Cl. The use of this ion was recommended because of sensitivity of response for this characteristic ion. The regression line for this curve was $y = 0.146x - 0.208$ with a correlation coefficient of 0.9973.

3.2.6 Maximum response optimisation

The tuning of mass spectrometer prior to analysis was of prime importance for optimising the sensitivity and ensuring that the mass spectrum is of good quality. Furthermore, a system which is incorrectly tuned or calibrated may give inaccurate isotope ratios, omit small but important peaks, misalign m/z values and also may use disc space very rapidly resulting in poor quality data. In this work for the application of GC/MS the operational

variables with the following parameters were investigated:

1. Optimisation of electrometer zero
2. Electron multiplier voltage
3. Selected mass interval (sensitivity and specificity)

3.2.6.1 Optimisation of electrometer zero

For the SIM mode at high voltage, optimisation is very important in order to minimise background electronic noise level and total sector usage. The MT file was used to vary the electrometer zero parameter (from +6.0 to -6.0).

The electrometer zero parameter was varied between +6.0 to -6.0 and optimisation was determined by peak area ratio (other parameters held constant) correlated with optimal background and reduction in sector usage. The results were obtained by comparing peak area ratio (mean of four injection, 1 ul volume) against the background and total sectors are presented in table 3.9.

In this case the results show that electrometer zero setting of -1.01 was the optimal because of good sensitivity and reasonable sector usage.

3.2.6.2 Electron multiplier voltage optimisation

Using the SIM technique, the mass spectrometer can be used as an extremely sensitive and specific detector for GC by monitoring one or a few selected m/z instead of full scanning (usually 40-450 amu). Thus optimisation to obtain maximum sensitivity for organophosphorus compounds was necessary. Using selected ion monitoring, the negative potential applied to the electron multiplier was varied and increased to -2600 volts to obtain the optimum voltage for each compound. Table 3.10 shows an increase in sensitivity by a factor of 33, 50, 173 and 200 for pirimiphos-methyl, malathion, heptenophos and dimethoate, respectively.

3.2.6.3 Optimisation of selected mass interval

With the Finnigan 1020 used, below the level of a few nanograms, quantification required a selected ion monitoring method (by GC/MS). The compound is identified on the basis of retention time and the presence of one or more m/z values known to be in the mass spectrum of the compound, although the most sensitive quantitative technique is to monitor characteristic ions as selected masses. Because of interference from impurity peaks which have masses at the monitored m/z values and also elute at the same retention time the identification can be erroneous in such circumstances. The specificity of the analysis should be as high as possible. A gas chromatographic capillary column should be used because retention time is more finely diagnostic than with a packed column. For the mass spectrometric analysis, recourse may be made to high resolution because of monitoring ions of selected elemental composition rather than selected integral mass.

This work was based on optimisation of the selected mass interval range (near to elemental composition rather than selected integral mass) or optimisation (increase) of the dwell time on the lower mass interval range as much as possible for each pesticide. To obtain maximum sensitivity 1 ul of each pesticides (standard solution) was injected under the same conditions (except for the mass interval range) and the results of peak area ratios are presented in table 3.11. Results show that under the optimum conditions an increase in sensitivity of pirimiphos-methyl 27%, heptenophos 28%, dimethoate 33% and malathion 30% was obtained.

3.2.7 Column performance and selection for GC/MS analysis

Capillary columns with a high degree of inertness and coated with stable chemically-bounded phase are commercially available at the present time. However, because of the wide range of options in either standard or customer-made columns, the selection of the best column for a given application is not easy. A certain stationary phase column may be purchased (or prepared) in different lengths, in non-bonded or bonded form, with a 0.1mm i.d. to 0.5mm 1.d diameter range and with film thicknesses ranging from 0.2 to

5um, until a few years ago chromatographers who did not make their worn columns had to rely on a very limited range of diameters and most standard columns were around 0.25mm i.d. with film thickness of 0.1-0.25um. Now the user can select a range of columns with the particular requirements for different applications. The GC/MS environmental analysis should be with the performance of the columns chosen for separation. The ideal column for GC/MS should have the following properties:

(a) good separation for complex samples

(b) good sample capacity to allow the analysis of minor components (closely eluting major compounds, and relative concentration around 100/1

(c) minimal stationary phase bleed at high temperature (with a mass spectrometer for the identification of trace samples at high temperature not overlapping with the bleed mass)

(d) the maximum degree of chemical inertness

(e) the column can be used with different methods of sample injection (direct liquid injection and head space methods).

It is important to point out that the ideal column described by properties a to e does not exist. Therefore, in this work the performances of four columns, all bonded phase, but having different phase thickness, internal diameter and lengths were investigated.

1 ul of each standard samples was applied to each column under the conditions described in section 3.2. The results for HETP (mm) and theoretical plates per metre (N/l) are presented in table 3.12.

The number 'N' of theoretical plates is calculated by equation 4.1

$$N = 16 \left(\frac{t_R}{w_b}\right)^2 \qquad (4.1)$$

t_R is the retention time of peak

w_b is the width at the peak base

The height equivalent to a theoretical plate (HETP), is usually calculated from the equation 4.2

$$HETP = \frac{\text{length of column}}{N} \qquad \text{or} \qquad H = l/N \qquad (4.2)$$

The efficiency of the column is improved by decreasing both the internal diameter and the thickness (df) of the coating.

The results show that columns about 30 m in length, medium-wide bores (0.30 - 0.32 mm i.d.) and coated with bonded phases of 0.25μm film thickness are the most satisfactory (good separation, good sample capacity) for general purpose use in the GC/MS analysis of pesticides.

To determine the optimal conditions for the separation of mixtures of sample and internal standard, the reference compound should elute close in time to the pesticide to allow for any changes in mass spectrometer tuning and sensitivity during acquisition. Thus for the above reasons different columns were investigated. Malathion and pirimiphos-methyl exhibited the best chromatography (retention time window less than 60 seconds); however, for heptenophos and dimethoate the performance was poorer (where the elution is outside a narrow retention time window).

3.2.8 Optimisation of the quadrupole GC/MS

The tuning of the mass spectrometer prior to analysis was one of prime importance for optimising the sensitivity and for good quality of mass spectra (correct mass spectra and abundance of ions). These optimised conditions allow valid interlaboratory comparisons of mass spectral data, mass spectral matching with mass spectral data in libraries, user created or NIST (compare mass with EPA mass). Furthermore a system which is incorrectly tuned or calibrated may give inaccurate isotope ratios, omit small but important peaks, or m/z value and use disc space very rapidly resulting in poor quality data. To find the best conditions, standard compounds (Reference compounds) are required. Several compounds and recommended for EI operation. They are methyl

stearate (26), FC-43 (Perfluorotributyl amine PFTBA) [27] and decafluorotriphenylphosphine (DFTPP).

The most frequently used are FC 43 and DFTPP. These compounds are recommended by US EPA in calibration and tuning of quadrupole GC/MS for priority pollutant legislation. FC-43 is used to tune and in mass calibration the GC/MS for general mass data product (ion acquisition). However the mass spectra of FC-43 has only low abundance ions in the range of higher 300 amu (and also contaminates the ion source rapidly).

DFTPP can be introduced into the mass spectrometer by injecting a standard solution via the gas chromatograph under normal operating conditions. The mass spectrum of DFTPP was originally used for optimisation criteria in the tuning of quadrupole mass spectrometers as described by Eichelberger et al [28] (see Table 3.13 DFTPP ion abundance criteria).

Methyl stearate can be introduced to the mass spectrometer by injecting a standard solution via GC under normal operating conditions. Methyl stearate has the disadvantage of having lower abundance of characteristic ions in the higher mass range. This feature can be used as an indicator in a sensitivity test for GC/MS by monitoring the intensity and ratio of m/z 298 and 299 ions. See Table 3.14 Relative abundance for major ions for methyl-stearate.

3.2.9 Recovery analysis

The GC/MS condition optimised for organophosphorus pesticide analysis as described in Section 2 was used for application of sample pesticides here.

Several workers have used acetonitrile for extraction of some organophosphorous insecticides from fruit and vegetable e.g. [29-32] because of high polarity of this solvent.

Chloroform is a popular organic solvent for all polar components (very good solvent for extraction of organophosphorous compound from water) [30]. Some samples containing few extractives can be analysed without clean up by GC using selective detectors such as the FPD or AFID [33] and GC/MS. However the latter method was used for this work and is one of the best choices.

Tables 3.15-16 summarises the recovery of malathion, pirimiphose, dimethoate and heptenophos at a spiking level amount of 2 and 0.2 mg/kg in four fruit and vegetable, the total pesticides at a spiking level amount of 40 ug and 4 ug in 20 g sample. As the table 3.15-16 shows that the percentage of recovery for malathion in tomato is less than in apple, carrot and lettuce determined by the same procedure. The reason for this difference could be described as follows.

The hydrolysis rate of organophosphorus pesticides and their metabolites is more important. The reaction between a phosphorus ester (A) and water, base, or acid (B) obeys second order kinetics.

$$A + B \longrightarrow \begin{matrix} C + D \\ \text{products} \end{matrix}$$

The rate equation may be represented as follows:

$$\frac{dx}{dt} = k_2 (a - x)(b - x)$$

where a and b are the initial concentrations of A and B, and x is the decrease in concentration after time t.

The hydrolysis rate is dependent upon the chemical structure and reaction conditions such as pH, temperature, the kind of solvent used, catalytic reagents [34]. In general organophosphorus pesticides have bonds connecting phosphorus with hetero atoms such as oxygen, nitrogen, sulphur and halogens, all of which have lone pairs of electrons. Thus a lone pair of electrons can be donated into the vacant 3d orbitals of the phosphorus atom. See Figure 3.2.7.1

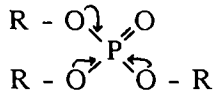


Figure 3.2.7.1

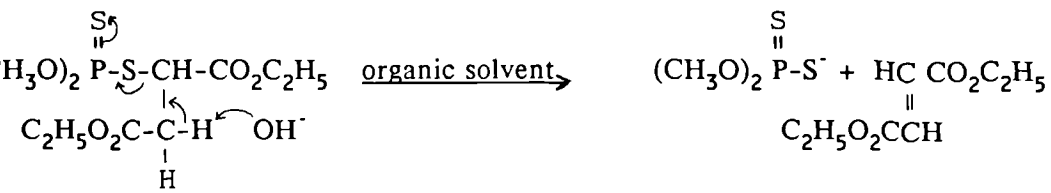
With this $p\pi$-$d\pi$ contribution (much evidence for such bonding has been reported [35]) the bondings is fortified, the electron density of phosphorus increased, and the phosphorus compounds become less susceptible to the attack of nucleophiles.

Electron-width drawing groups make the bond deficient in electrons and the compounds more reactive. However, the electron density of phosphorous decrease, consequently compounds are more reactive. Electron density of dimethoate is higher than malathion[36] and malathion exhibits 58.3% more hydrolysis than dimethoate under the same conditions [36].

The electron density of these project pesticides is as follows:

malathion < dimethoate < heptenophos < pirimiphos-methyl

other evidence indicates that malathion is decomposed in alkali solvent [37].



Because of the above evidence, in tomato sample (acidic sample), lower recovery is reasonable (see Table 3.15).

Table 3.16 shows (in some cases) recovery of lower (4 ug/20 g) residue level is higher than 40 ug/20 g.

3.2.10 Detection limit

The detection limit was determined using a sample without spiking chromatographed on the DB5 capillary column (under the optimum SIM condition of instrument operation). The detection limit for malathion, pirimiphos-methyl, heptenophos and dimethoate are 1×10^{-13} gram, 0.75×10^{-13} gram, 1.5×10^{-13} gram and 3×10^{-13} gram per injection respectively. This low level of detection has not been previously indicated in the literature, generally, although with clean-up such a level of detection may be difficult to achieve.

The detection limit for dimethoate is higher than the other, because of peak tailing and low mass ion (m/z 87) in SIM mode. The detection limit for pirimiphose-methyl is the highest because of higher mass ion (m/z 276,290) in SIM mode compared with the other this results show the higher mass ion selected for SIM gives lower detection limit.

MW	Compound	mass spectra obtained and % RA								mass spectra reported and % RA							
330	malathion	173	127	125	158	93	99	43	55	173	127	125	93	158	99	55	43
		100	83	84	41	66	31	19	35	100	81	80	73	44	30	28	20
250	heptenophos	124	89	109	126	77	127	79	51	124	89	109	126	77	127	79	51
		100	68	37	30	24	22	18	16	100	69	37	32	23	23	19	18
305	pirimiphos-methyl	290	276	305	125	233	180	93	262	290	276	305	125	233	180	93	262
		100	90	82	71	43	40	37	29	100	93	85	73	42	45	43	31
229	dimethoate	87	93	125	58	47	63	79	56	87	93	125	58	47	63	79	56
		100	61	45	21	19	20	15	9	100	53	35	20	25	23	15	12
188	d_{10}anthracene	188	84	94	189	80	186	160	158	188	184	94	189	80	186	160	158
		100	12	10	13	9	9	6	6	100	11	10	9	10	8	7	7
136	$d_8$naphthalene	136	134	108	54	68	137	132	66	136	134	108	54	68	137	132	66
		100	14	8	10	10	10	4	5	100	12	10	10	9	8	6	5
291	parathion	97	109	132	137	125	155	123	81	97	109	132	137	125	155	123	81
		100	95	52	44	42	32	28	16	72	100	-	59	44	33	22	22

Table 3.1 Major ions observed for organophosphorus pesticides and deuturated standards

Run	area of m/z 87/ m/z 188	area of (m/z 87 + m/z 93)/ m/z 188
1	2.10	1.07
2	1.84	1.05
3	1.97	1.21
mean	1.97	1.11

Table 3.2 **Comparison of the monitoring of one ion for dimethoate with two ions under the same conditions.**

Run	area of m/z 124/ m/z 136	area of (m/z 124 + m/z 89)/ m/z 136
1	1.59	1.09
2	1.76	1.21
3	1.88	1.35
mean	1.74	1.22

Table 3.3 **Comparison of the monitoring of one ion relative for heptenophos with two ions under the same conditions.**

Run	area of m/z 173/ m/z 109	area of (m/z 173 + m/z 127)/ m/z 109
1	4.22	10.08
2	5.37	12.11
3	4.75	11.01
mean	4.78	11.07

Table 3.5 **Comparison of the relative response for monitoring single ion with two ions under the same conditions for malathion.**

Run	area of m/z 290/ m/z 109	area of (m/z 290 + m/z 276)/ m/z 109
1	0.56	1.23
2	0.42	1.33
3	0.63	1.45
average	0.54	1.34

Table 3.4 **Comparison of the relative response for monitoring single ion with two ions under the same conditions for pirimiphos-methyl.**

Type of spiked vegetable sample	selected ion for quantification m/z	selected ions for / internal standard / m/z
lettuce	173, 276, 87,124	136, 188, 276, 173, 97, 109
carrot	173, 276, 290, 87, 124	173, 276, 290, 136, 188
apple	87, 173, 276, 290, 124	173, 276, 290, 136, 188, 97 109
tomato	124, 173, 276, 290, 87	173, 276, 290, 136, 188, 109

Table 3.6 Characteristic ions used for pesticides and internal standards in quantitation analysis.

N.B. Characteristic ions (m/z)	Pesticide
173	malathion
276, 290	pirimiphos -methyl
87	dimethoate
124	heptenophos
136	d_8 naphthalene
188	d_{10} anthracene
97, 109	parathion

Table 3.7 Retention times * (relative to d_8 naphthalene)

Pesticides	mean value (n=5)	standard deviation	relative standard deviation (%)
malathion	2.37	45.03×10^{-4}	0.19 %
pirimiphos - methyl	2.35	42.30×10^{-4}	0.18 %

GC column (DB5) was held at 55 °C for 2 min, then programmed at 10 °C/min up 200 °C

* RRT

Table 3.8 Retention times * (relative to d_{10} anthracene)

Pesticides	mean value (n=5)	standard deviation	relative standard deviation (%)
malathion	1.14	17.10×10^{-4}	0.15 %
pirimiphos - methyl	1.12	17.99×10^{-4}	0.16 %

GC column (DB5) was held at 55 °C for 2 min, then programmed at 10 °C/min up to 200 °C.

* RRT

Electrometer zero	area ratio	sector used
-5.01	$\frac{633}{9} = 70.3$	113
-2	$\frac{11871}{430} = 27.6$	108
-1.01	$\frac{49171}{6459} = 7.6$	152
-0.49	$\frac{28174}{4590} = 6.2$	193
0.02	$\frac{28789}{4627} = 6.2$	345

Table 3.9 **Results for electrometer zero analysis of 800 ppb malathion and 500 ppb parathion as internal standard**

Name of pesticide	peak area for pesticides [mean of four injection (1μl)]				
malathion	163	1028	6352	7873	8176
pirimiphos-methyl	287	1521	8289	9449	9593
dimethoate	124	1488	11647	20382	25885
heptenophos	99	1102	7965	13440	17093
Electron multiplier voltage (volt)	-2352	-2447	-2552	-2588	-2600

Table 3.10 **Result for Electron multiplier voltage analysis of 100 ppb pesticides**

Name of Pesticides	peak area ratios mean of four injection (1 ul)	mass interval range
malathion	8.54	172.75-173.25
malathion	5.98	172.5 - 173.5
dimethoate	0.42	86.75 - 87.25
dimethoate	0.63	86.5 - 87.5
heptenophos	1.05	123.75-124.25
heptenophos	1.46	123.5 - 124.5
pirimiphos-methyl	8.34	289.75-290.25
pirimiphos-methyl	6.09	289.5 - 290.5

Table 3.11 **Optimisation of SIM effect of variation of selected mass interval using 800 ppb pesticides, 500 ppb parathion and 200 ppb d_{10}anthracene as internal standards.**

Type of column	Internal diameter mm	Phase m thickness	length of column m	malathion		pirimiphos-methyl		dimethoate		heptenopos	
				Hmm	n	Hmm	n	Hmm	n	Hmm	n
BP1	0.32	0.1	9	0.1992	5020	0.1962	5080	0.2000	5000	0.1984	5040
BP10	0.22	0.1	10	0.1069	9348	0.1070	9346	0.10704	9342	0.1071	9338
DB1	0.32	0.1	10	0.2577	3880	0.2565	3898	0.2581	3875	0.2569	3892
DB5	0.3	0.25	30	0.1654	6045	0.1653	6050	0.1980	5050	0.1656	6040

Table 3.12 Comparison of performance of bonded column

m/z	ion abundance criteria
51	30 - 60 % of mass 198
68	less than 2 % of mass 69
70	less than 2 % of mass 69
127	40 - 60 % of mass 198
197	less than 1 % of mass 198
198	base peak, 100 % relative abundance
199	5 - 9 % of mass 198
365	1% of mass 198
441	less than mass 443
442	greater than 40 % of mass 198
443	17 - 23 % of mass 442

Table 3.13 Ion abundance criteria of DFTPP

m/z	ion abundance
55	28 - 30 % of mass 74
56	5 - 12 % of mass 74
69	13 % of mass 74
74	base peak, 100 % abundance
75	2 - 2 % of mass 74
87	57 - 60 % of mass 74
97	4 - 7 % of mass 74
129	4 - 6 % of mass 74
143	14 - 16 % of mass 74
185	2 - 3 % of mass 74
199	4 - 6 % of mass 74
255	4 - 6 % of mass 74
298	4 - 6 % of mass 74
299	0.5 - 1 % of mass 74
299	15 - 20 % of mass 298

Table 3.14 Relative abundance for major ions for methyl stearate.

sample	Recovery (%)			
	apple	carrot	tomato	lettuce
pesticides	mean ± RSD	mean ± RSD	mean ± RSD	mean ± RSD
malathion	97.75 ± 4.38	98.40 ± 5.25	92.83 ± 3.62	97.33 ± 6.01
pirimiphos-methyl	98.23 ± 4.41	95.48 ± 6.12	98.34 ± 4.98	96.67 ± 2.89
dimethoate	96.38 ± 5.64	93.60 ± 4.50	94.24 ± 3.33	96.00 ± 7.01
heptenophos	97.38 ± 3.12	96.73 ± 2.49	95.05 ± 4.81	95.25 ± 5.05

Table 3.15 Recovery of pesticides from spiked sample, 2 mg/kg.

sample	Recovery (%)			
	apple	carrot	tomato	lettuce
pesticides	mean ± RDS	mean ± RDS	mean ± RDS	mean ± RDS
malathion	100.26 ± 4.28	100 ± 4.99	91 ± 3.82	99.32 ± 5.88
pirimiphos-methyl	98.78 ± 4.14	98.24 ± 5.89	100 ± 5.06	98.75 ± 3.03
dimethoate	99.24 ± 5.46	92.31 ± 4.75	95.45 ± 3.62	98.14 ± 6.55
heptenophos	98.14 ± 3.21	101.56 ± 3.03	101.02 ± 5.15	100.46 ± 4.95

Table 3.16 Recovery of pesticides from spiked sample, 0.2 mg kg^{-1}

name of compound	Regression equation	Correlation coefficient
malathion	y = 0.601x - 0.572	0.9982
pirimiphos-methyl	y = 0.579x - 0.531	0.9990
dimethoate	y = 0.963x - 0.325	0.9946
heptenophos	y = 0.146x - 0.208	0.9973

Table 3.19 **Result of regression analysis of pesticides employing internal standard calibration using full scan GC-MS.**

Name of Compound	Regression equation	Correlation coefficient
malathion	y = 7584.5x - 13689	0.9894
pirimiphos-methyl	y = 3213.03x - 4466.17	0.9858
dimethoate	y = 490.28x +158.44	0.9836
heptenophos	y = 1294.38x + 1.25	0.9885

Table 3.17 **Result of regression analysis of pesticides employing external standard calibration using full scan GC-MS.**

Name of Pesticides	Regression equation	Correlation coefficient
malathion	y = 6.88x - 1.39	0.9982
pirimiphos-methyl	y = 3.34x - 0.33	0.9976
dimethoate	y = 0.96x + 0.33	0.9948
heptenophos	y = 0.25x - 0.26	0.9980

Table 3.20 **Results of regression analysis of pesticides employing internal standard calibration using GC/MS/SIM.**

Name of pesticides	Regression equation	Correlation coefficient
malathion	y = 28364x - 5116	0.9901
pirimiphos-methyl	y = 1295x - 92.49	0.9920
dimethoate	y = 13914x + 0.173	0.9985
heptenophos	y = 15991x - 0.17	0.9884

Table 3.18 **Results of regression analysis of pesticides employing external standard calibration using GC/MS/SIM.**

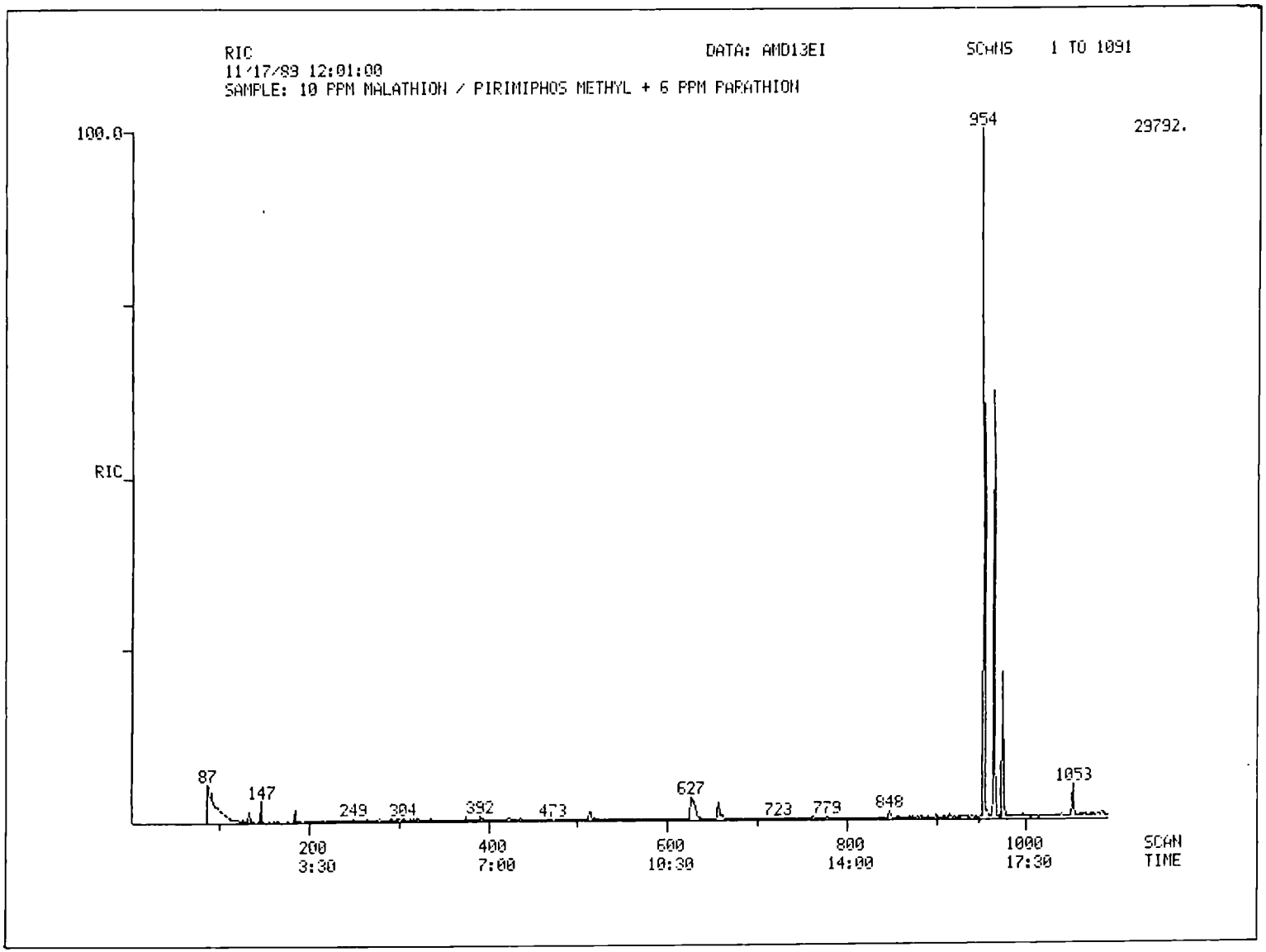


Figure 3.1 RIC of malathion and pirimiphos-methyl (10 ppm) with internal standard parathion (6 ppm), using full scan (40-450 amu)

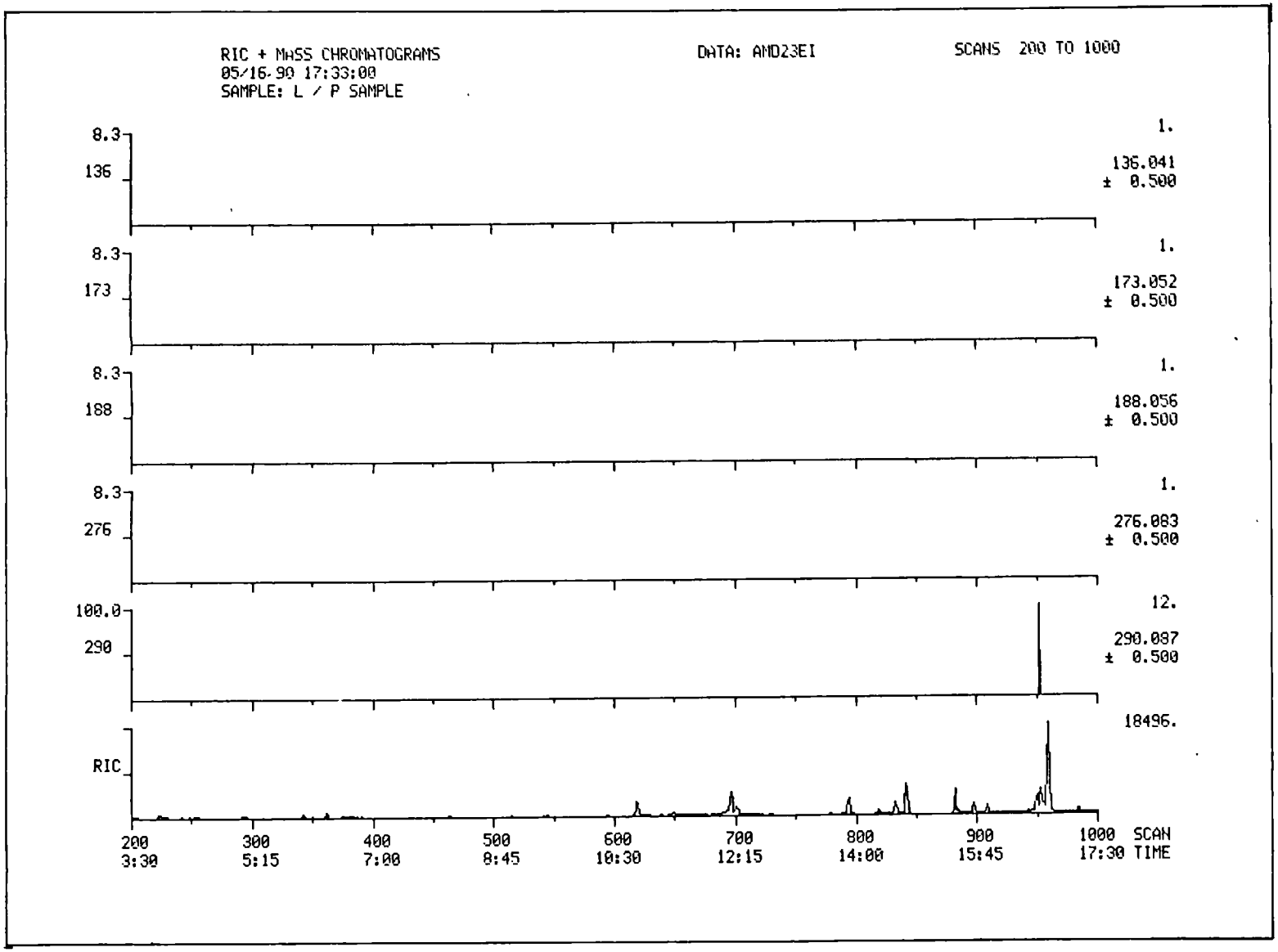


Figure 3.2 RIC of lettuce sample (without pesticides) analysed using full scan mode (40-450 amu); Comparing with the mass chromatogram for m/z 136, 173, 188, 276, and 290.

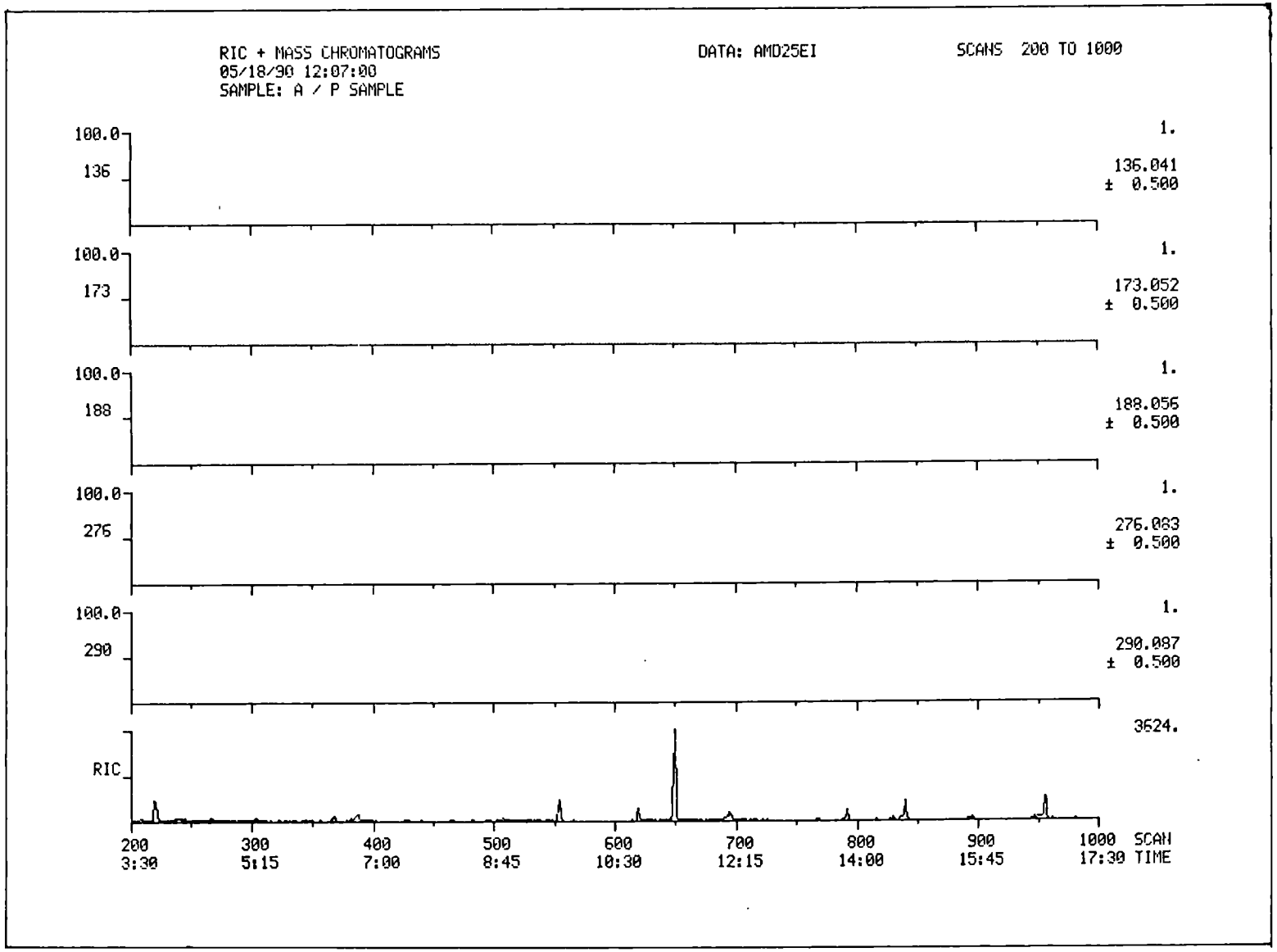


Figure 3.3 RIC of apple sample (without pesticides) analysed using full scan mode (40-450 amu); Comparing with the mass chromatogram for m/z 136, 173, 188, 276, and 290.

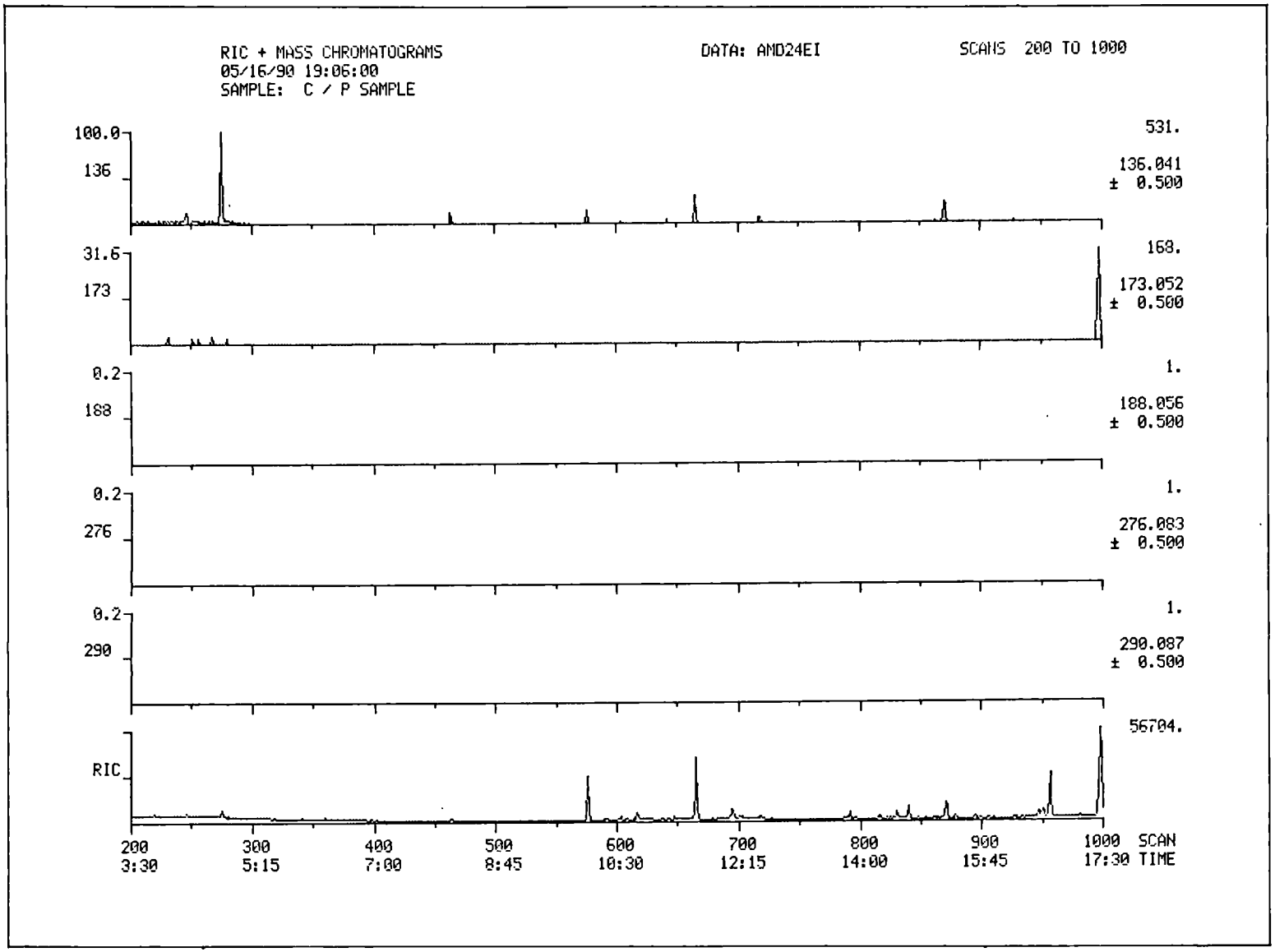


Figure 3.4 RIC of carrot sample (without pesticides) analysed using full scan mode (40-450 amu); Comparing with the mass chromatogram for m/z 136, 173, 188, 276, and 290.

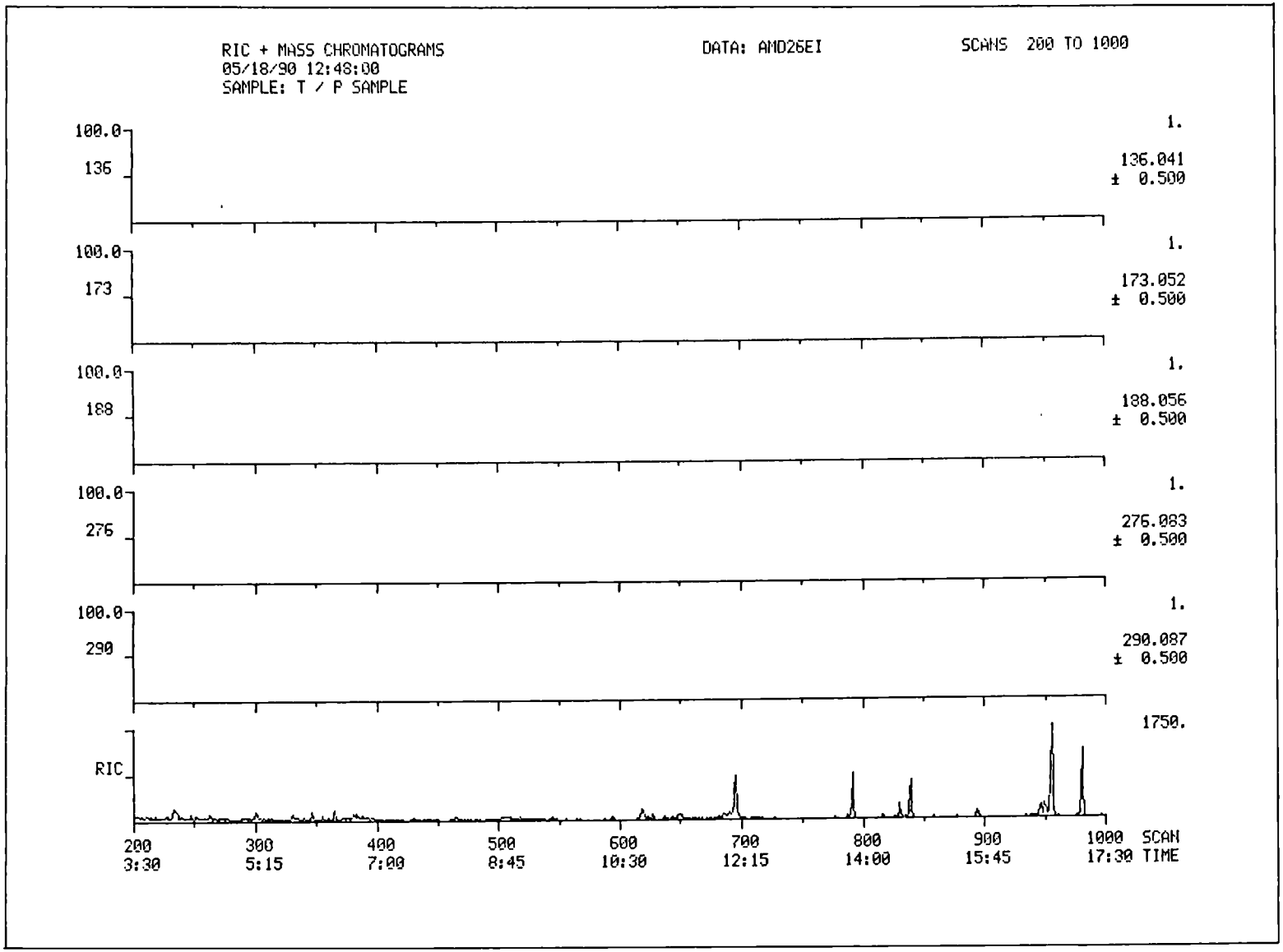


Figure 3.5 RIC of tomato sample (without pesticides) analysed using full scan mode (40-450 amu); Comparing with the mass chromatogram m/z 136, 173, 188, 276, and 290.

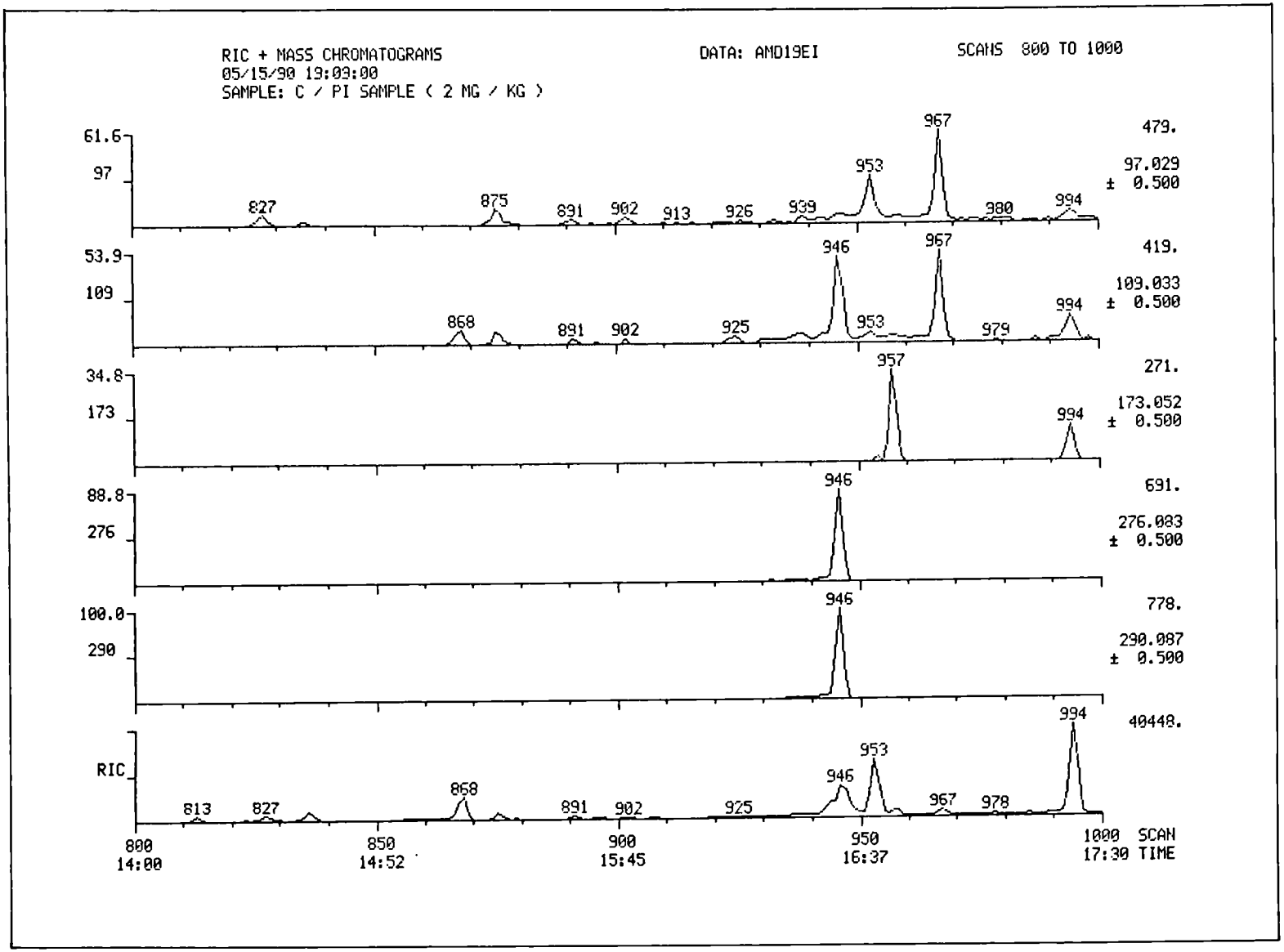


Figure 3.6 RIC of carrot sample (2mg/kg mixture of malathion and pirimiphos-methyl) analysed using full scan mode (40-450 amu); Comparing with the mass chromatogram for m/z 97, 109, 173, 276, and 290.

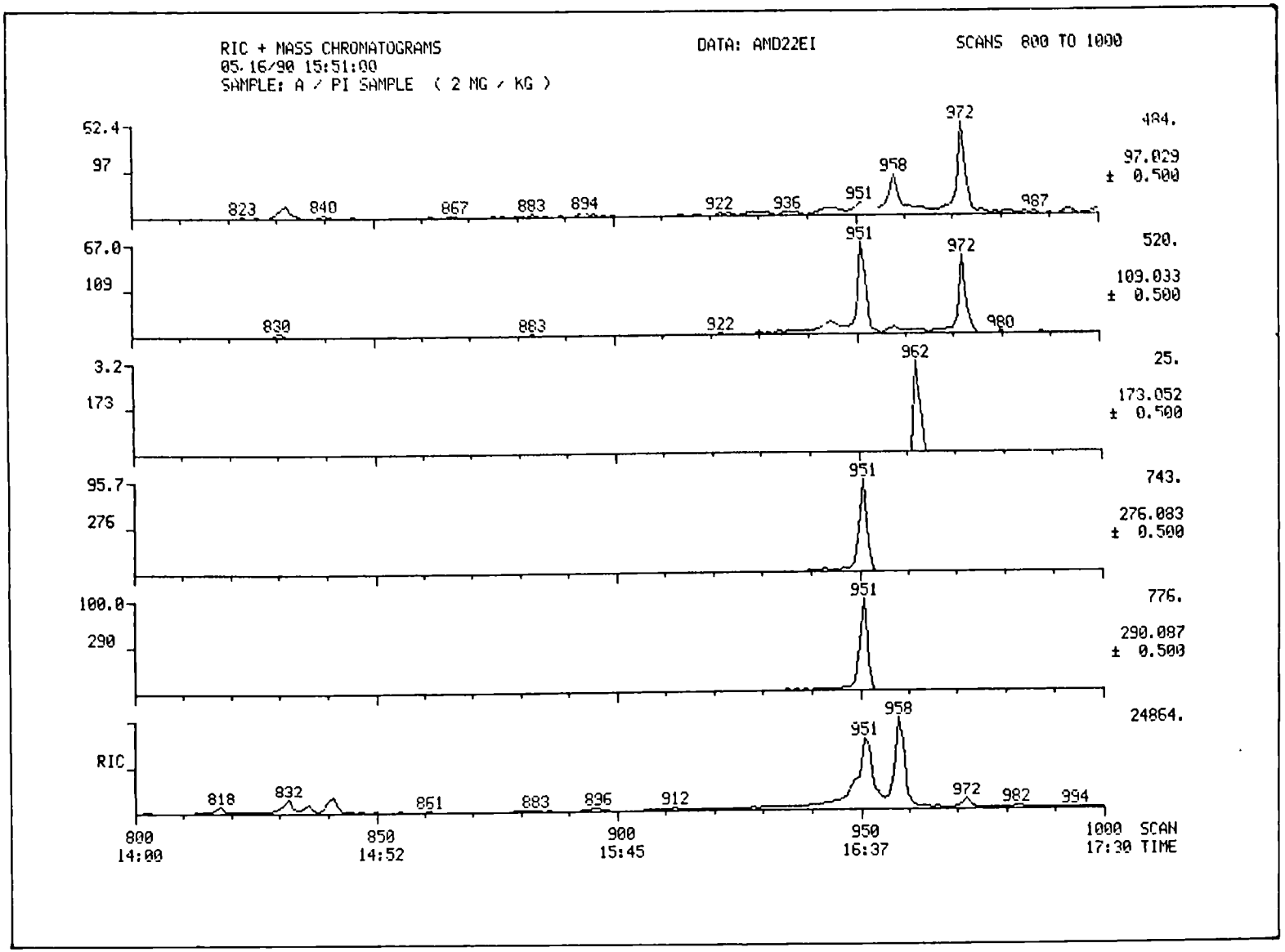


Figure 3.7 RIC of apple sample (2mg/kg mixture of malathion and pirimiphos-methyl) analysed using full scan mode (40-450 amu); Comparing with the mass chromatogram for m/z 97, 109, 173, 276, and 290.

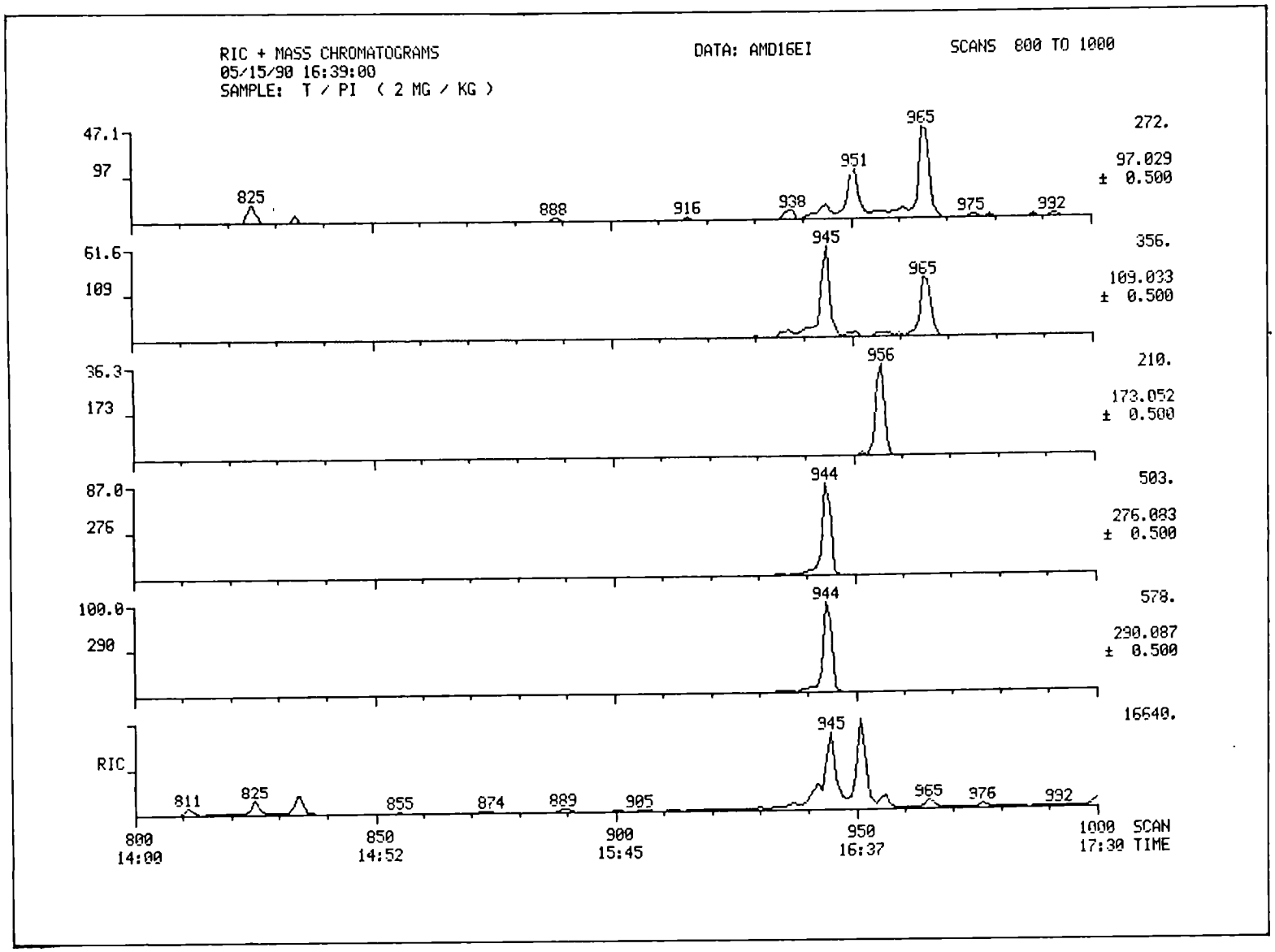


Figure 3.8 RIC of tomato sample (2mg/kg mixture of malathion and pirimiphos-methyl) analysed using full scan mode (40-450 amu); Comparing with the mass chromatogram for m/z 97, 109, 173, 276, and 290.

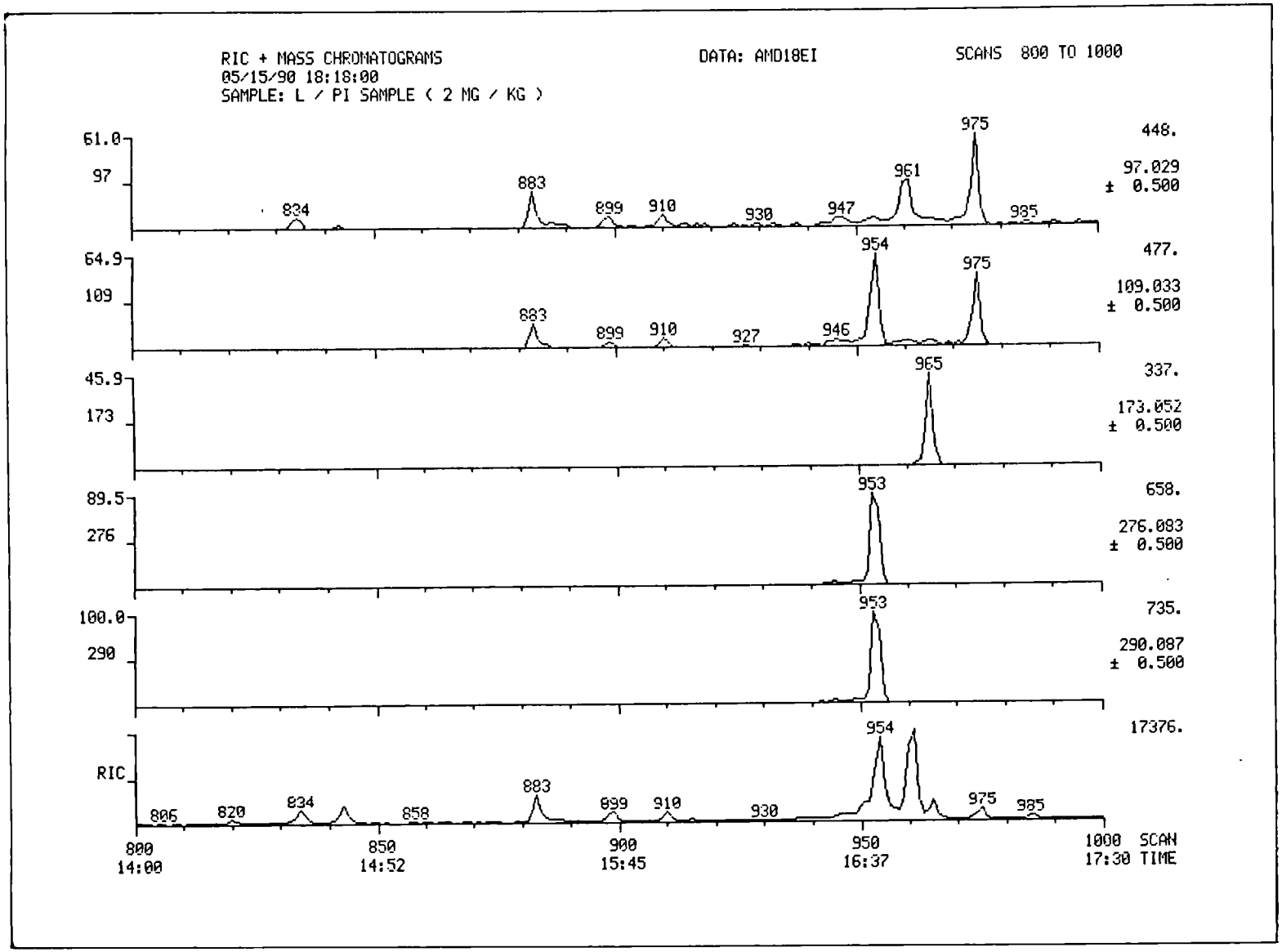


Figure 3.9 RIC of lettuce sample (2mg/kg mixture of malathion and pirimiphos-methyl) analysed using full scan mode (40-450 amu); Comparing with the mass chromatogram for m/z 97, 109, 173, 276, and 290.

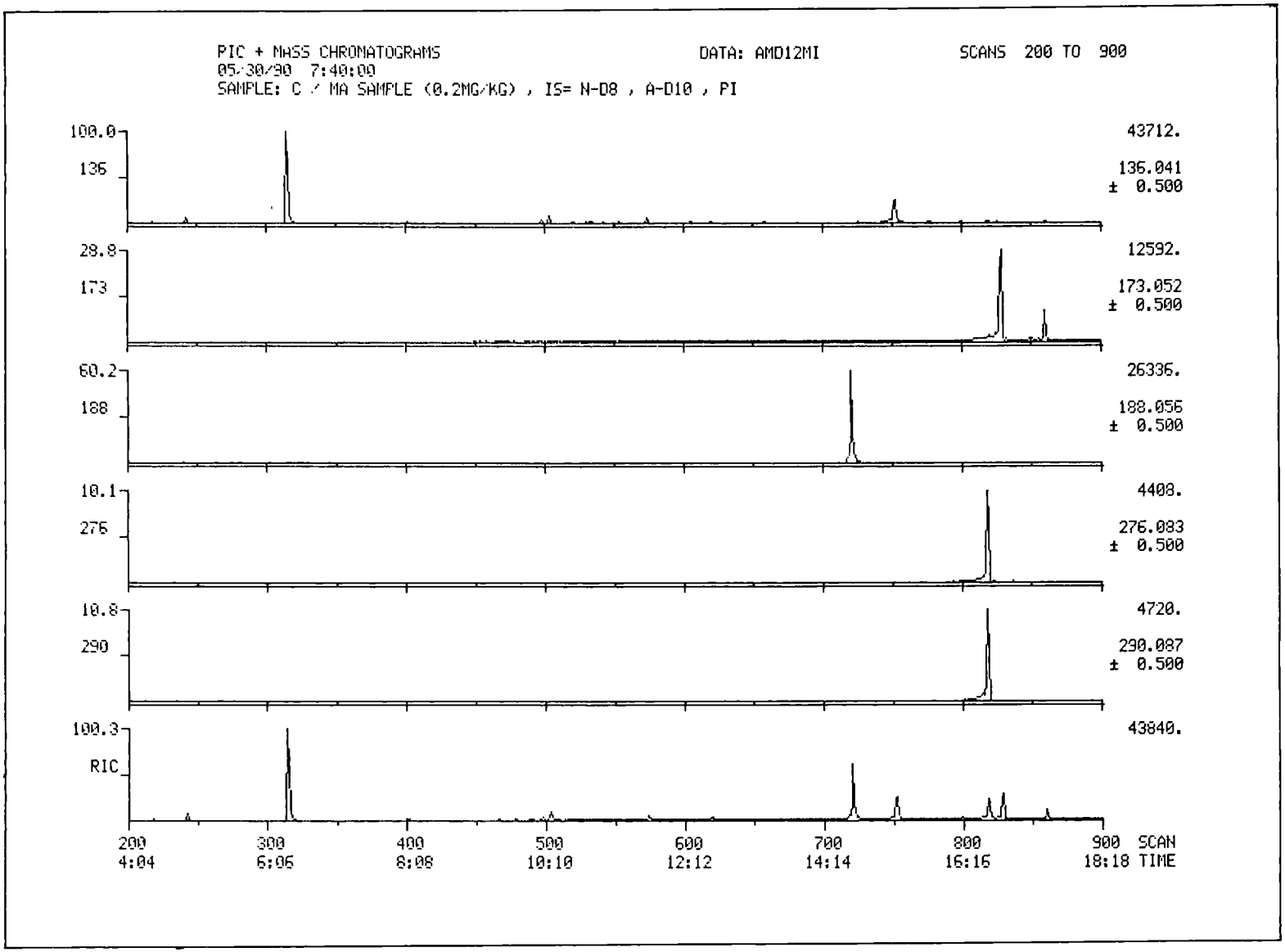


Figure 3.10 RIC of carrot sample (0.2mg/kg mixture of malathion and pirimiphos-methyl) analysed using full scan mode (40-450 amu); Comparing with the mass chromatogram for m/z 136, 173, 188, 276, and 290.

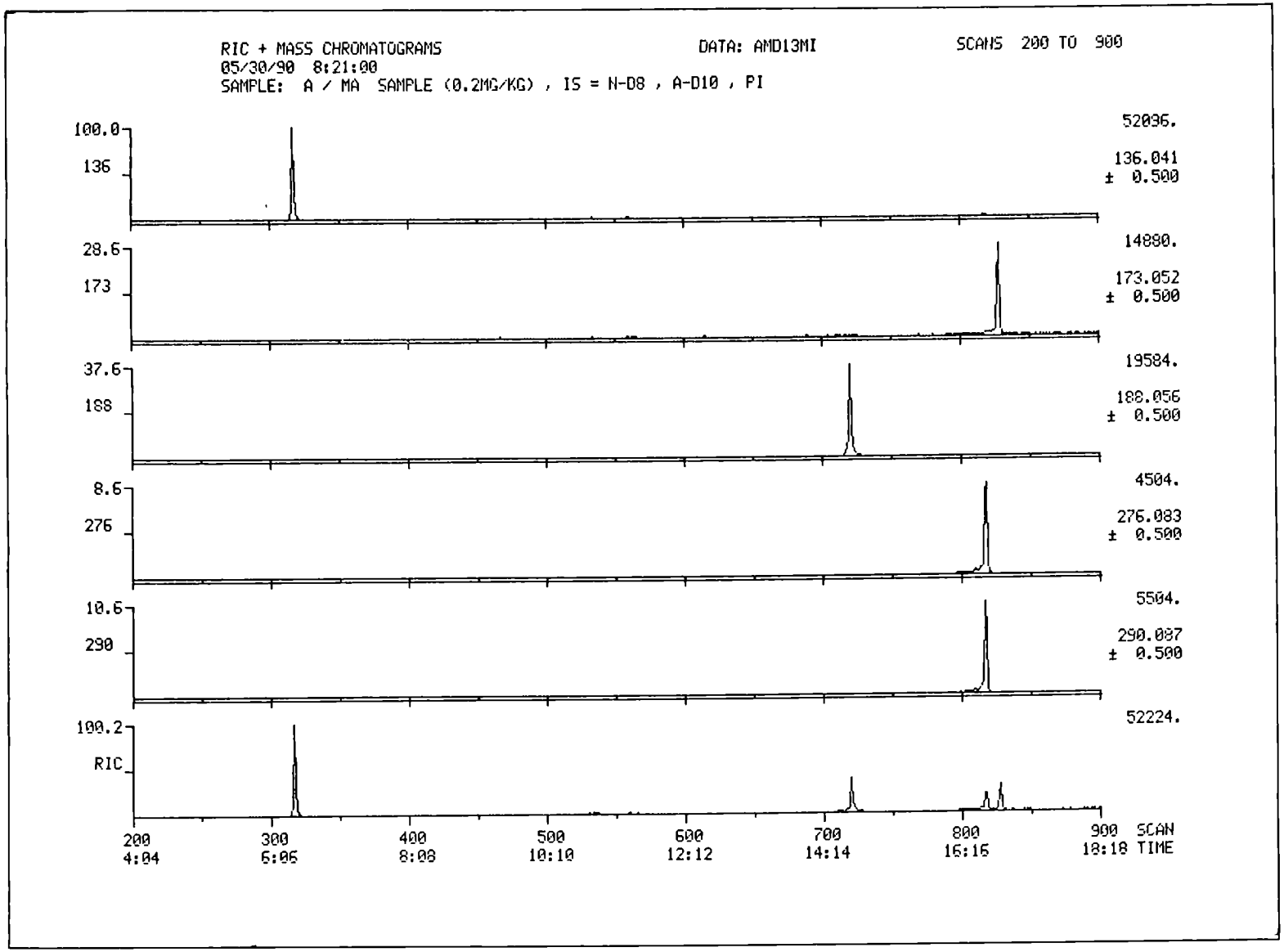


Figure 3.11 RIC of apple sample (0.2mg/kg mixture of malathion and pirimiphos-methyl) analysed using full scan mode (40-450 amu); Comparing with the mass chromatogram for m/z 136, 173, 188, 276, and 290.

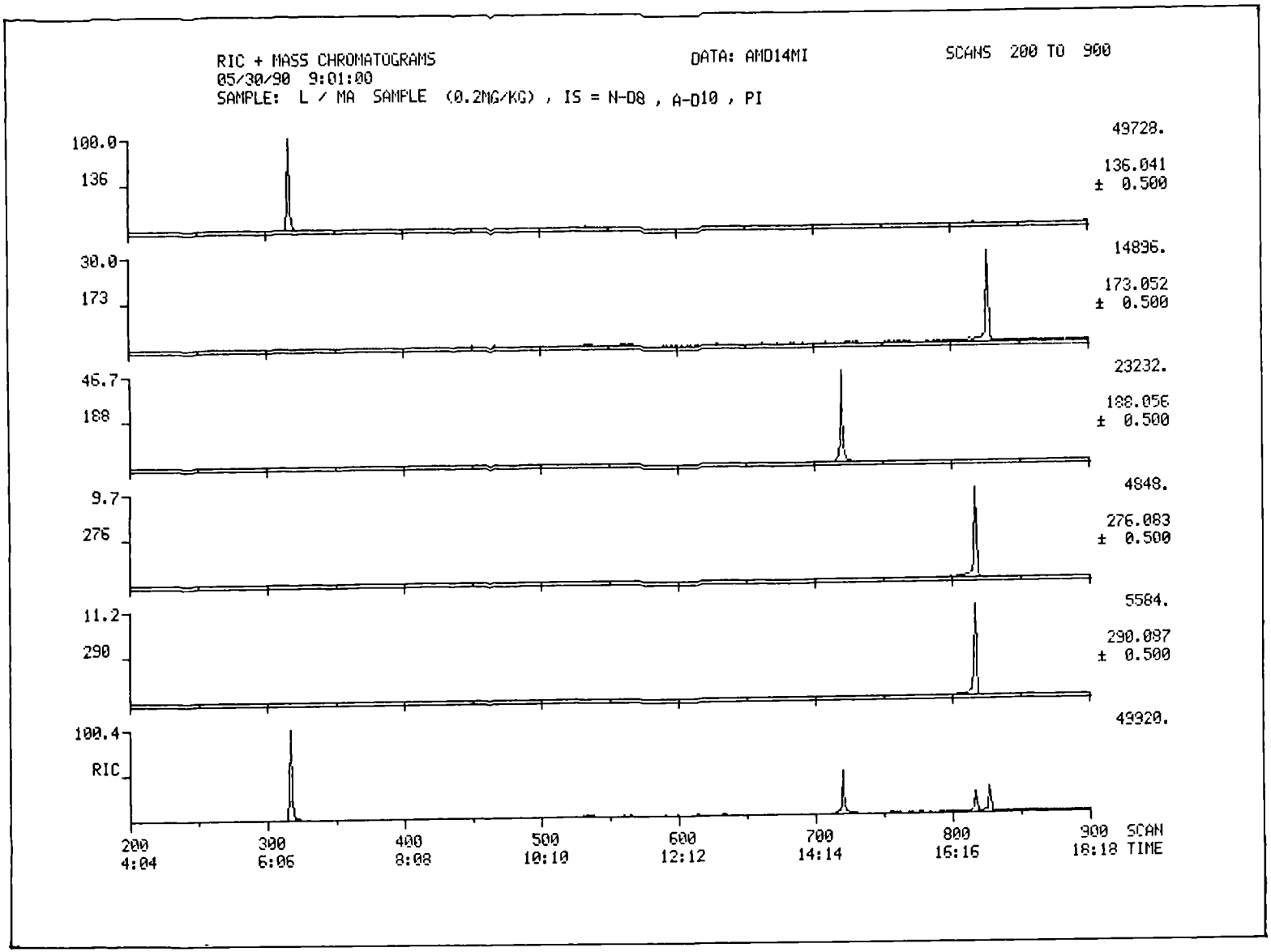


Figure 3.12 RIC of lettuce sample (0.2 mg/kg mixture of malathion and pirimiphos-methyl) analysed using full scan mode (40-450 amu); Comparing with the mass chromatogram for m/z 136, 173, 188, 276, and 290.

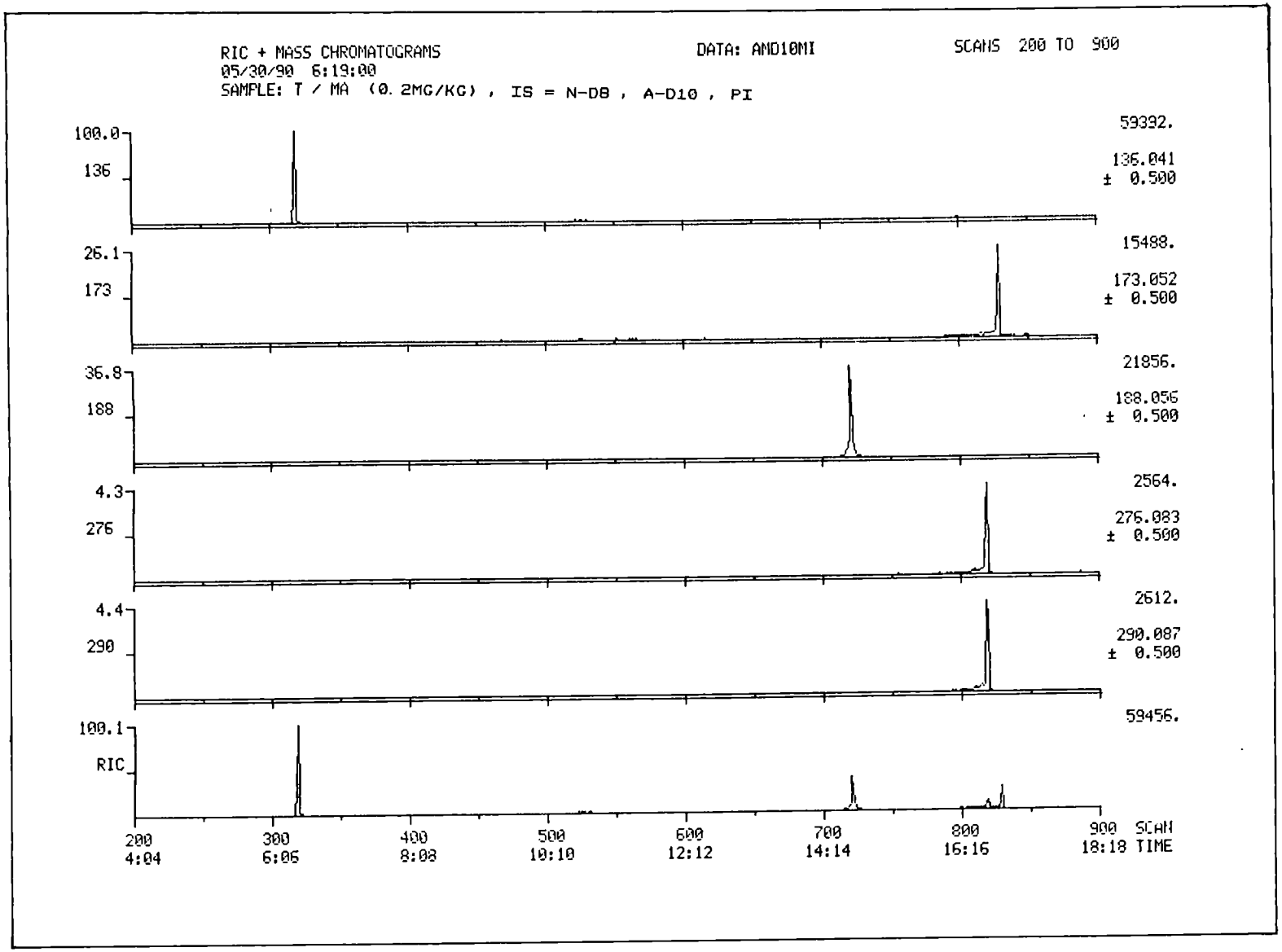


Figure 3.13 RIC of tomato sample (0.2mg/kg mixture of malathion and pirimiphos-methyl) analysed using full scan mode (40-450 amu); Comparing with the mass chromatogram for m/z 136, 173, 188, 276, and 290.

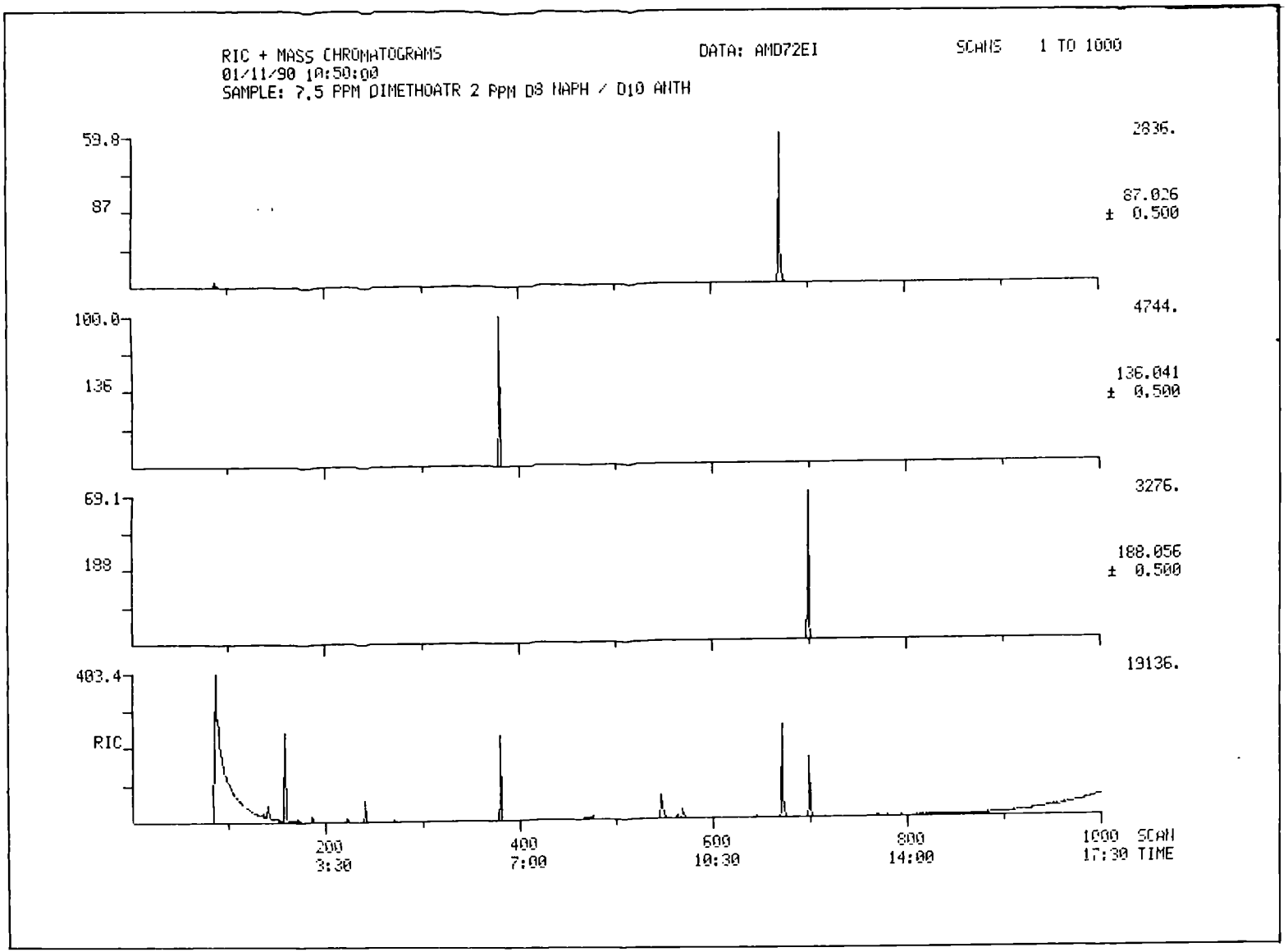


Figure 3.14 RIC of dimethoate (7.5 ppm) with internal standards d_8 naphthalene and d_{10} anthracene (2 ppm) using full scan mode (40-450 amu); Comparing with the mass chromatogram for m/z 87, 136, and 188.

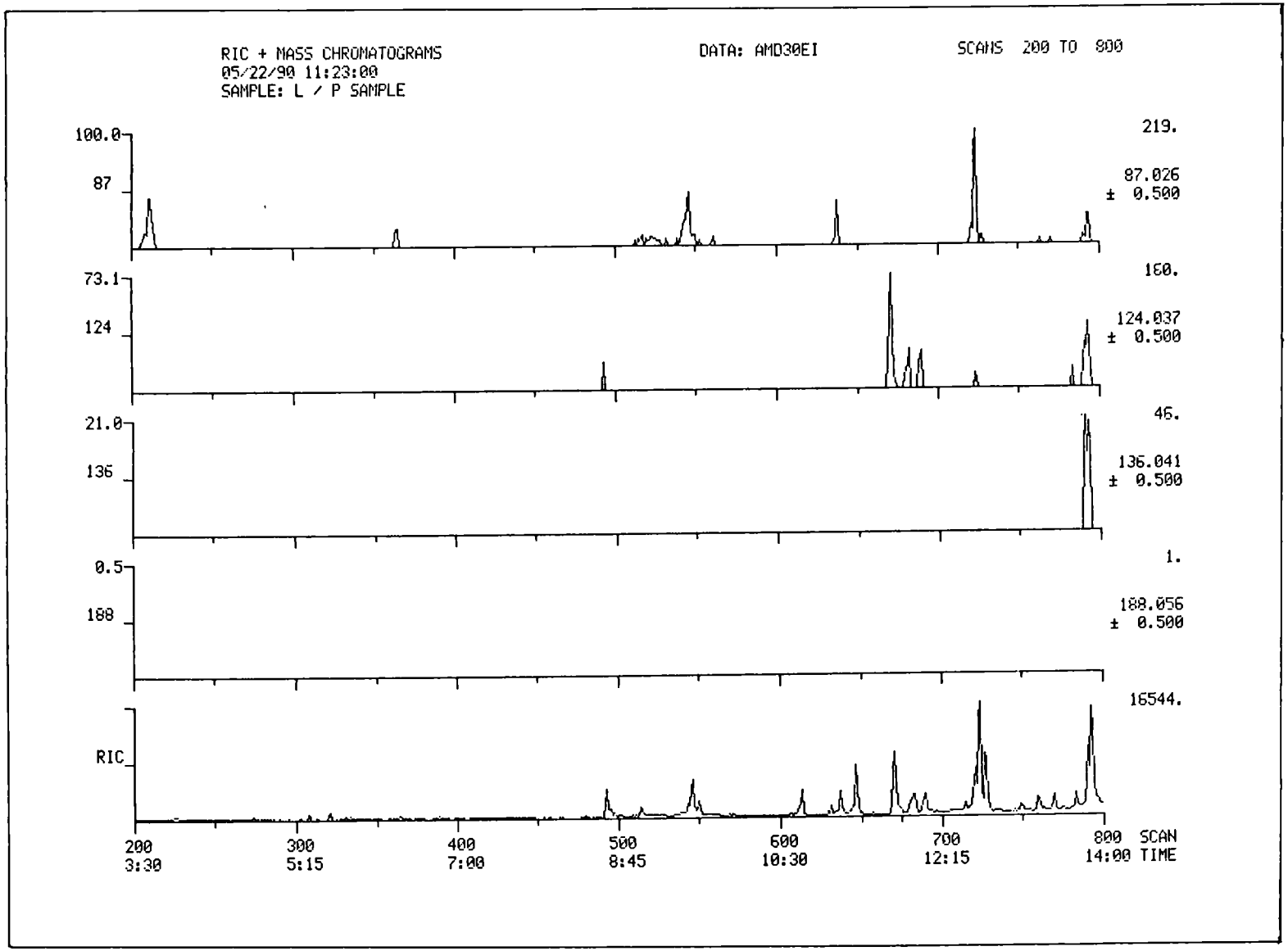


Figure 3.15 RIC of lettuce sample (without pesticides) analysed using full scan mode (40-450 amu); Comparing with the mass chromatogram for m/z 87, 124, 136, and 188.

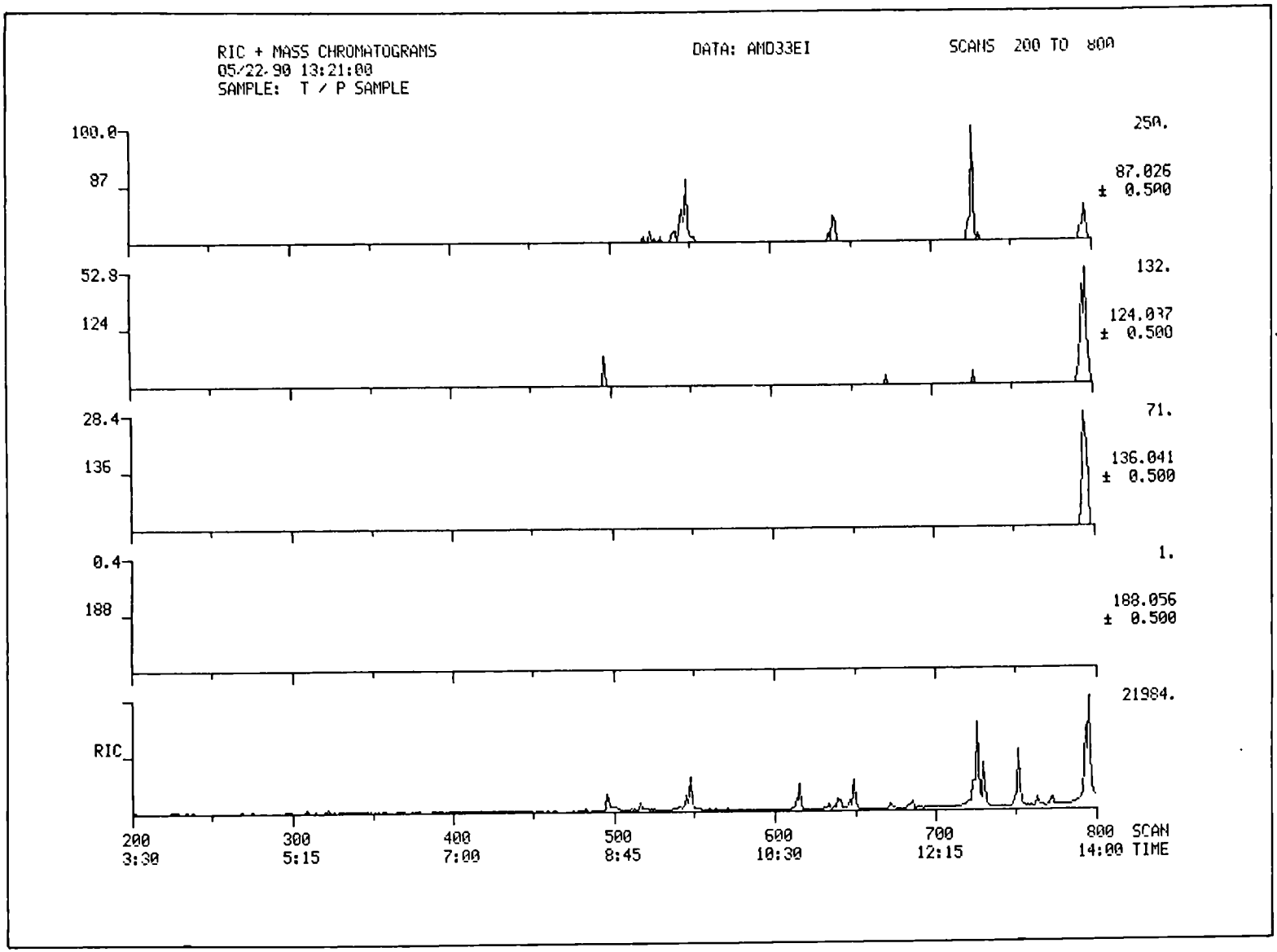


Figure 3.16 RIC of tomato sample (without pesticides) analysed using full scan mode (40-450 amu); Comparing with the mass chromatogram for m/z 87, 124, 136, and 188.

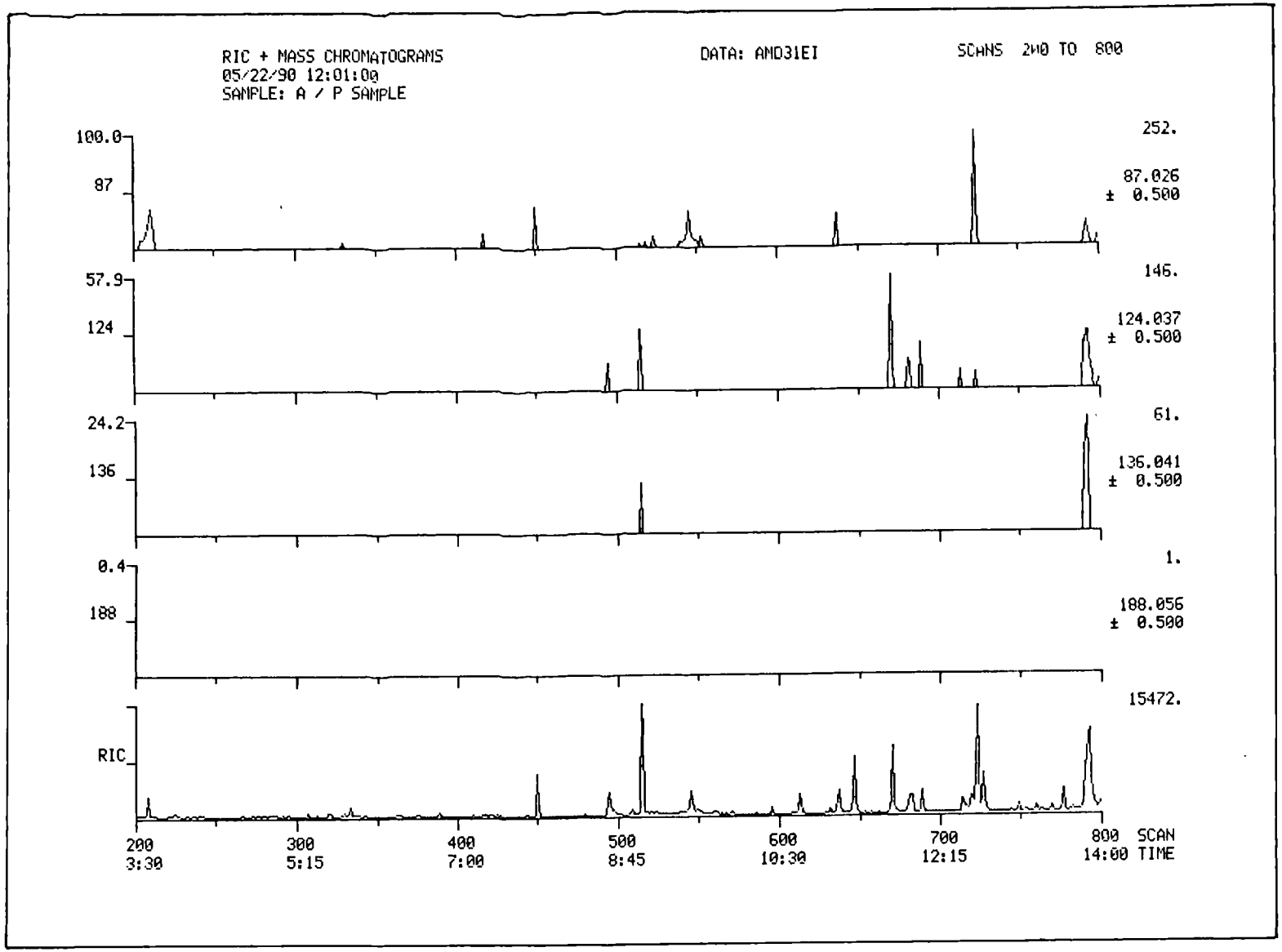


Figure 3.17 RIC of apple sample (without pesticides) analysed using full scan mode (40-450 amu); Comparing with the mass chromatogram for m/z 87, 124, 136, and 188.

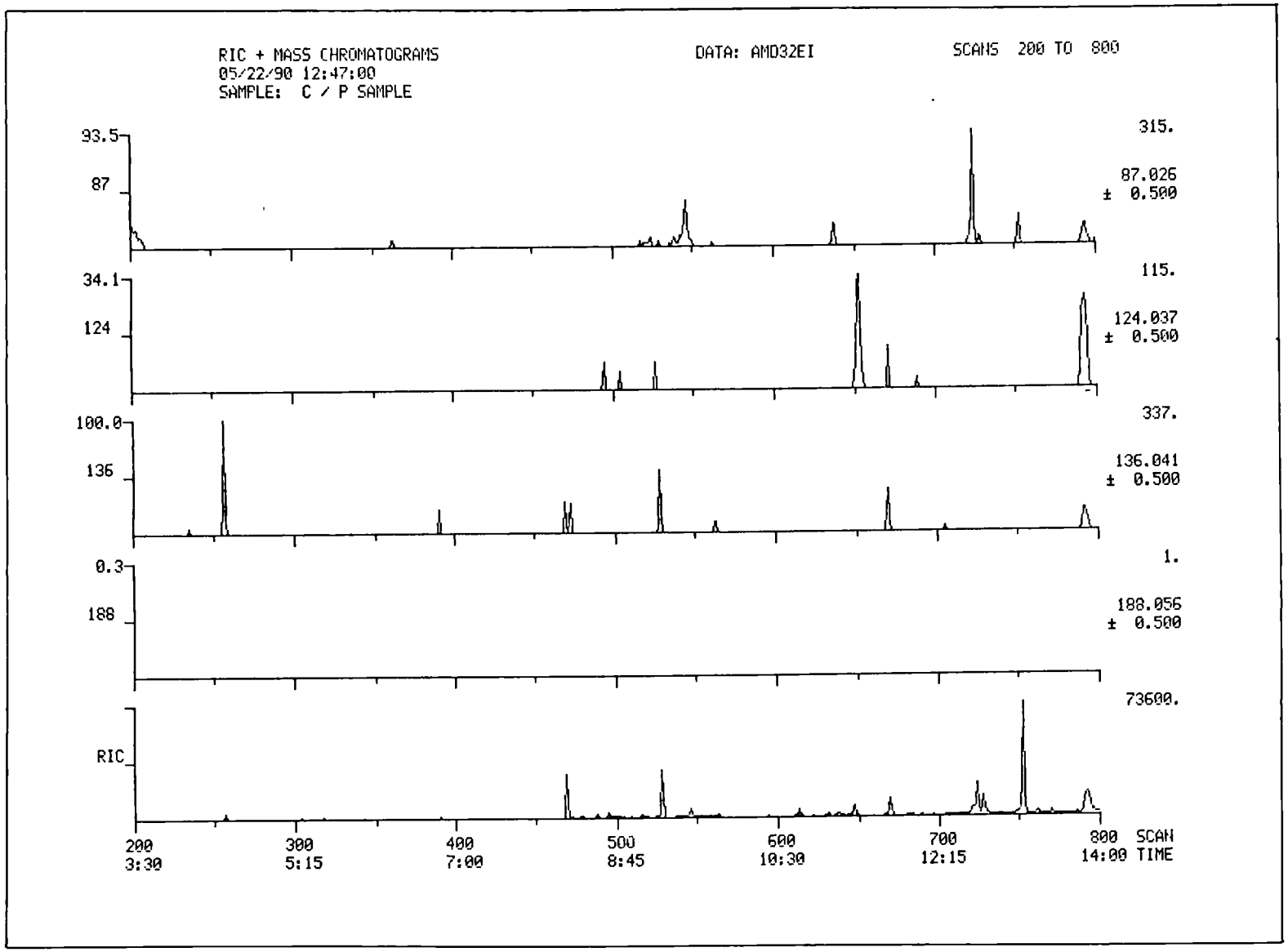


Figure 3.18 RIC of carrot sample (without pesticides) analysed using full scan mode (40-450 amu); Comparing with the mass chromatogram for m/z 87, 124, 136, and 188.

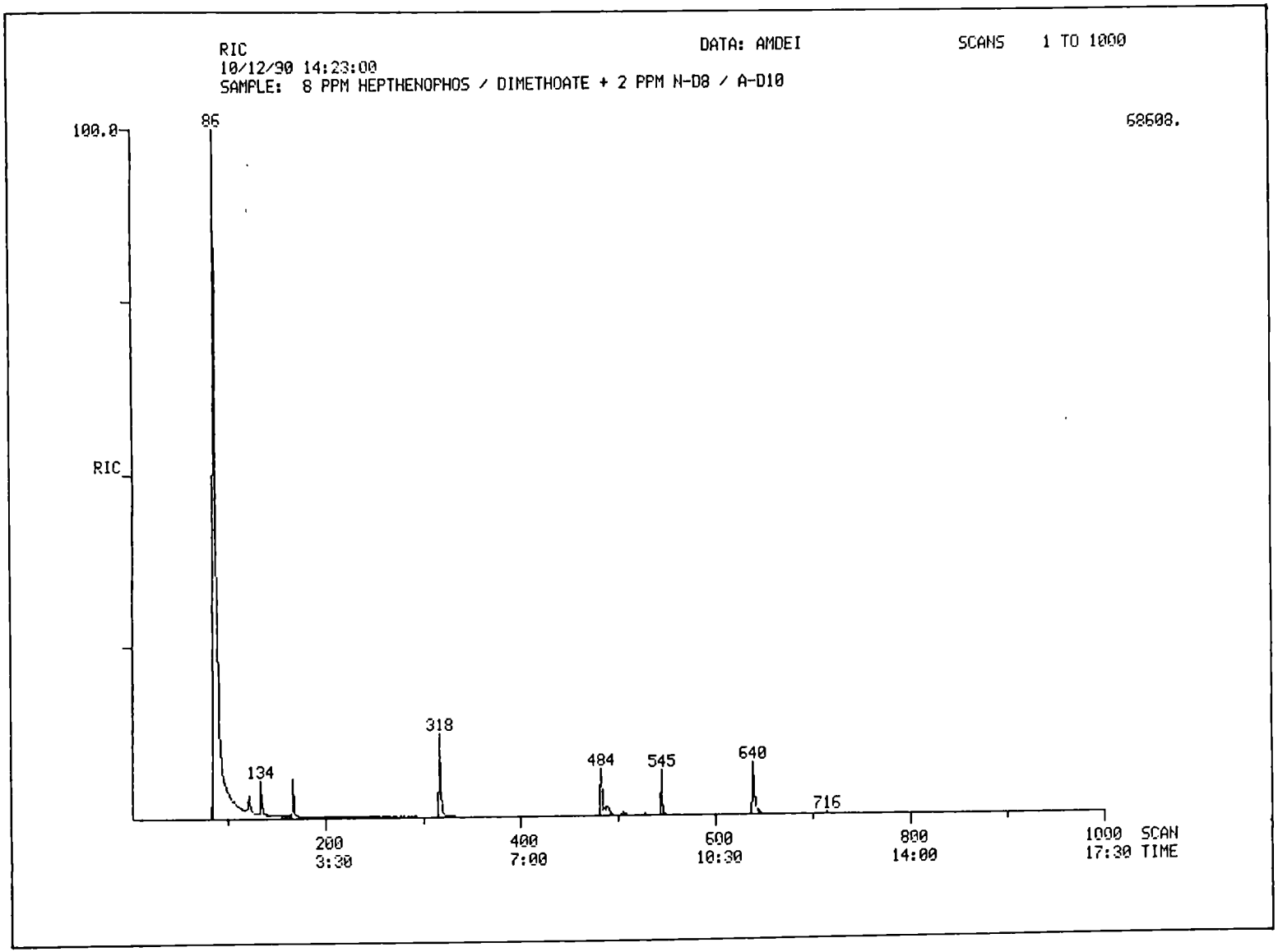


Figure 3.19 RIC of heptenophos (4 ppm) with internal standard d_8 naphthalene and d_{10} anthracene (2 ppm) using full scan mode (40-450 amu) GC-MS.

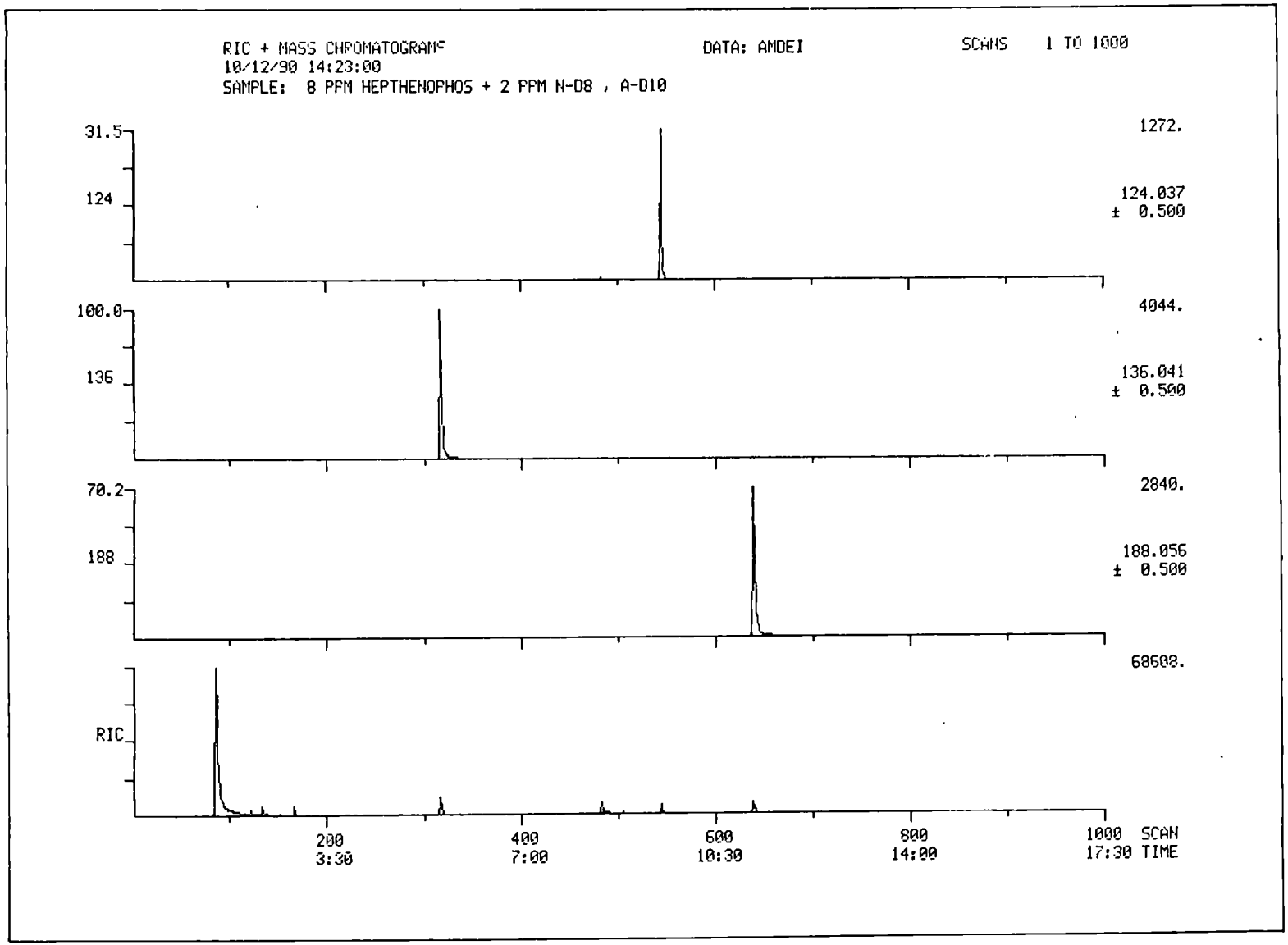


Figure 3.20 Mass chromatogram for m/z 124 (heptenophos), 136 (d_8 naphthalene) and 188 (d_{10} anthracene) relation to the RIC in figure 3.19.

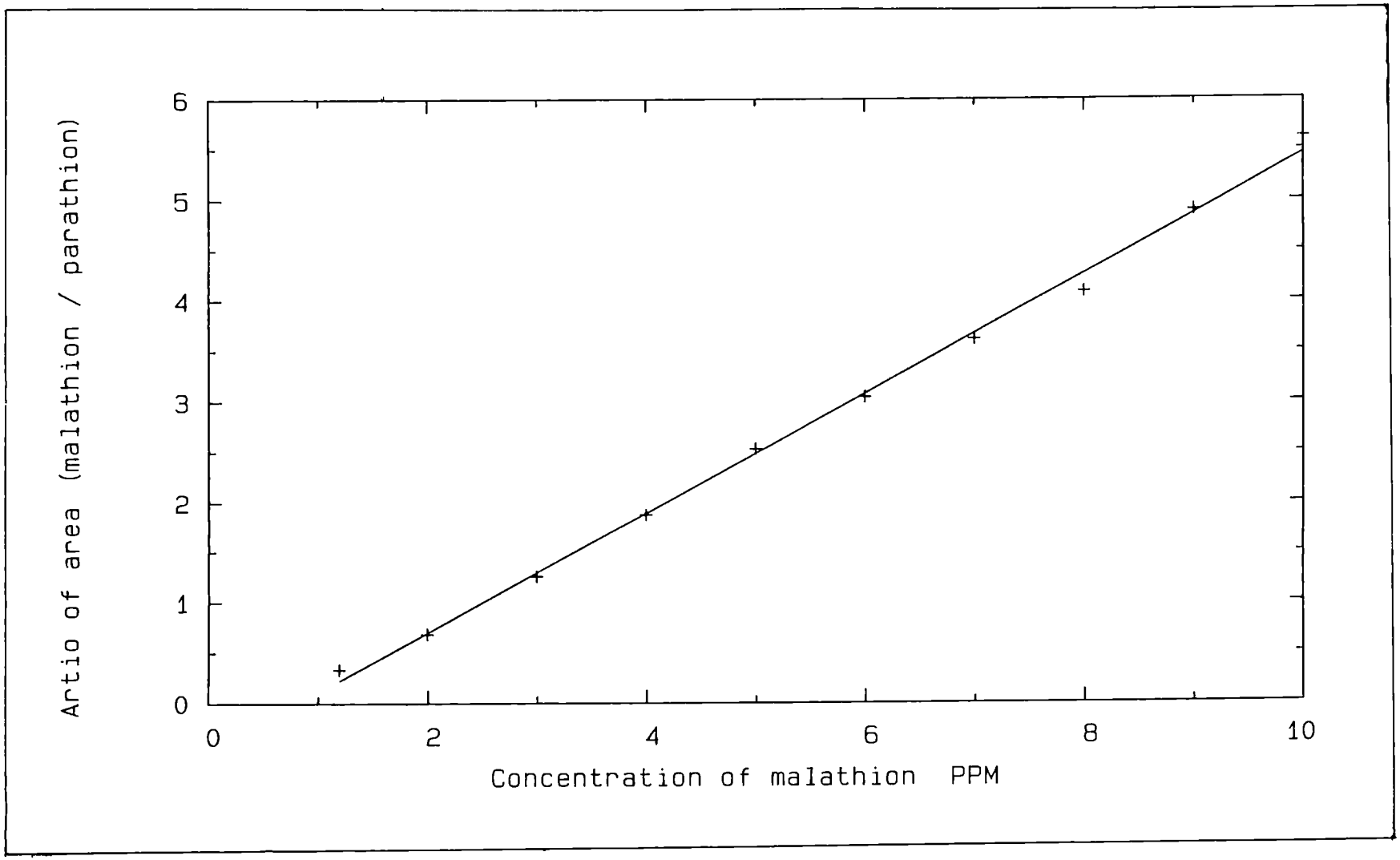


Figure 3.21 Internal standardisation calibration curve for malathion analysed using GC-MS.

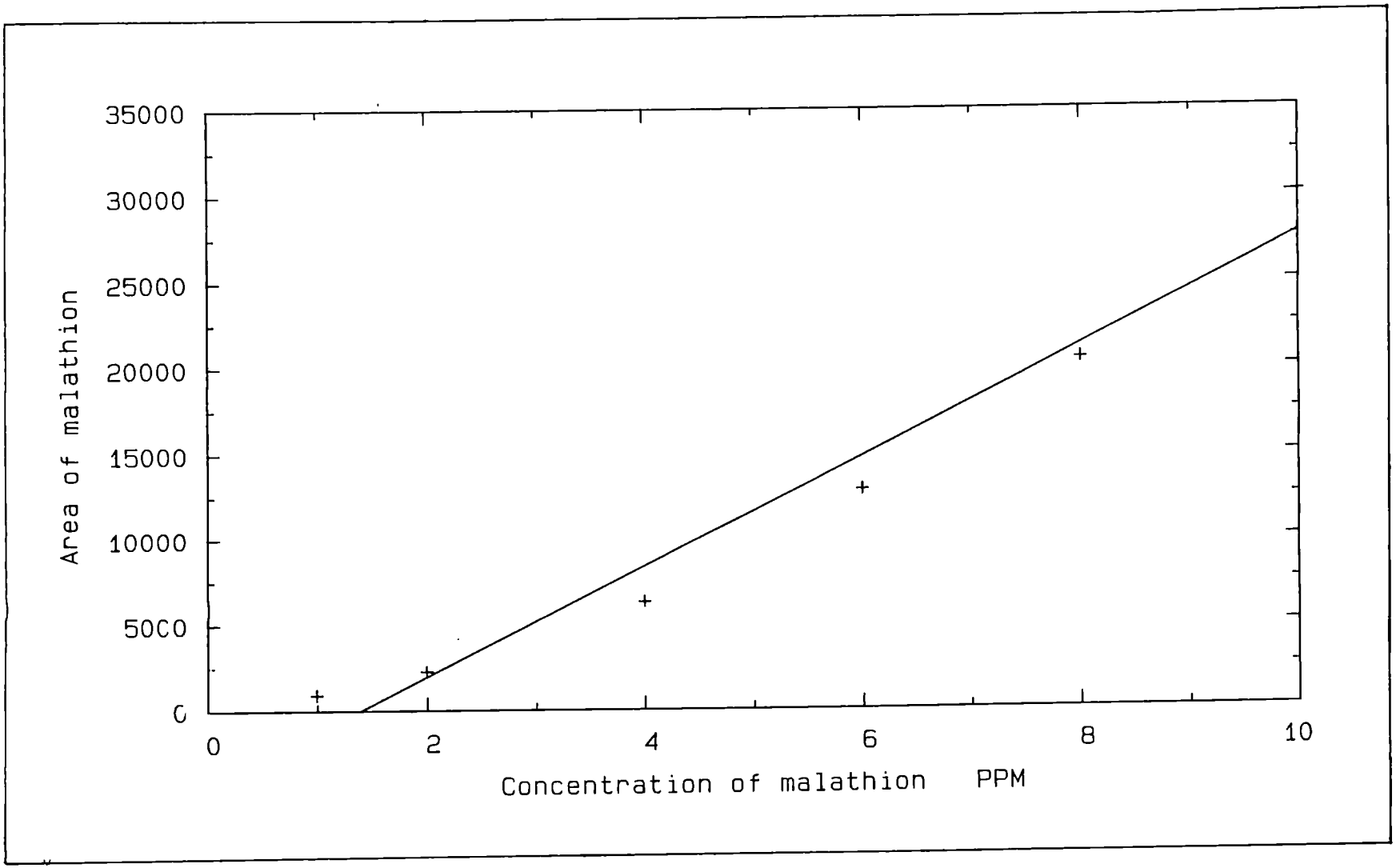


Figure 3.22 External standardisation calibration curve for malathion analysed using GC-MS.

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CHAPTER 4

Determination of organophosphorus pesticides in fruit and vegetable materials by Gas-Liquid Chromatography with electron-capture detection

4.1 EXPERIMENTAL

4.1.1 Introduction

The interest in the development of good methods for the determination of organophosphorus pesticides has increased in recent years because of the heavy use of orgnophosphorus compounds in agriculture and their resulting deposition and persistence in the environment and foodstuffs.

A number of authors have proposed methods for the analysis of various combinations of organophosphorus pesticides by gas-liquid chromatography [1-14]. Most of these workers used silicone liquid phases to achieve the desired separation of pesticides and have employed electron-capture, flame-photometric and flame ionisation detectors for detection and quantification.

The aims of this present study are to establish suitable conditions for a rapid and accurate gas chromatographic analysis of fruit and vegetable materials for the determination of certain selected organophosphorus pesticides widely used to crops.

GC wide-bore capillary and packed columns having a variety of internal diameters, length, and liquid phases are evaluated to determine the best chromatographic conditions.

Comparison of the variation in the sensitivity with temperature of the ECD detector and column is applied to obtain the best conditions for the column chromatography. The optimal GC-ECD conditions were investigated for application of pesticides to determine the lowest detection limits and good recoveries were obtained under these conditions.

4.1.2 Instrumentation

4.1.2.1 Gas Liquid Chromatography

The gas liquid chromatography used to obtain optimal conditions was a model 3700 Varian, facility fitted with a pen recorder, model IJ instrument CR 450 and a ^{63}Ni (8mCi) electron capture detector and employing Nitrogen as the carrier and make-up gas.

The optimum operation conditions were found to be:

A: Packed Column, 5% SE30 as stationary phase

Parameters	Malathion	Pirimiphos-methyl	Dimethoate	Heptenophos
Injection port temperature deg C	220	200	220	180
Column oven temperature deg C isothermal	190	175	190	150
Flow rate ml/min	65	63	62	64
Detector oven temperature deg C	360	390	390	370

B: Wide Bore Capillary Column, BP10 as stationary phase (14% cyano propyl phenyl dimethyl siloxane)

Parameters	Malathion	Pirimiphos-methyl	Dimethoate	Heptenophos
Injection port temperature deg C	220	200	220	180
Column oven temperature deg C isothermal	170	170	170	125
Flow rate ml/min	4.2	3.6	3.2	3.5
Detector oven temperature deg C	340	360	330	350

4.1.2.2 Columns

A. Packed Columns

Two packed columns of the same length but differing in type of liquid phase and phase thickness were used. The characteristics of the columns were as follows:

Table 4.1.2.2.1 The characteristics of packed columns used

Liquid phase	temperature deg C	solid support	internal diameter (mm)	polarity	length (m)
Apiezon L 10%	50 - 300	WAW-DMCS	4	N.P. *	4
Silicone SE-30 5%	50 - 300	WAW-DMCS	4	N.P. *	4

* N.P. = Non Polar

B. Wide-Bore Capillary Column

One wide-bore (fused silica) capillary column was investigated and the characteristics are as follow:

Table 4.1.2.2.2 The characteristics of the wide-bore capillary column used

length (m)	stationary phase	internal diameter (mm)	polarity	phase thick-ness (um)
12	14% cyanopropyl phenyl dimethyl siloxane PB10	0.53	S.P.*	1

* S.P. = Slightly Polar

4.1.3 Reagents

The reagents used were identical to those listed in Chapter 3, Section 3.3 to 3.3.6.

4.1.4 Apparatus

The apparatus is previously described in Chapter 3, Section 3.4.

4.1.5 Stock standard solutions

The stock solutions have been described in Chapter 3, Section 3.5.

4.1.6 Preparation of spiked samples

Preparation of spiked samples is described in Chapter 3, Section 3.6 to 3.6.3.

4.1.7 Procedure

The procedure used is identical to that described in Chapter 3, Section 3.7.1-4.

4.1.7.1 GC-ECD Analysis

The use of packed columns - 4 meter Apiezon L (10%), 4 meter SE30 (5%) - and wide-bore capillary columns was investigated to determine the optimal performance in terms of separation, peak shape, sensitivity and analysis time for the pesticides studied.

The average area for each pesticide at each column temperature was evaluated and comparisons of peak area versus temperature are presented in Section 4.2.

These tables indicate the chromatographic response for each column temperature range studied. The smaller the peak area obtained, the greater is the adsorptive effects or oxidation of the liquid phase, pesticides, etc. and vice versa. In this thesis, data in the tables were obtained under conditions given in a later section for experiments using the ECD detector. Also for optimum conditions, the detector temperature was investigated. Thus the average area (three injections) for each component at each detector temperature was evaluated and a plot of average peak area versus temperature was obtained. As a result a thermogram was produced. A thermogram indicates the response of the detector in the detector temperature range studied. The higher the area under the thermogram the more sufficient is the energy to cause attachment.

To find the optimal efficiency of the chromatographic column, optimisation of the flow rate for each samples was investigated.

For the above reasons the following procedures were determined:

4.1.7.1.1 Optimisation of column temperature

For the four organophosphorus pesticides, packed columns and wide-bore capillary columns were found to give the best conditions in the temperature ranges of 150 - 200 deg C for malathion, pirimiphos-methyl and dimethoate, and 120 - 150 deg C for heptenophos.

4.1.7.1.1.1 A typical chromatographic run

In this work, a run represents a temperature study of a column starting at a column temperature of 200 deg C (150 deg C for heptenophos), which was decreased at 10 or 5 deg C intervals to 150 deg C (120 deg C for heptenophos). A run had to be completed within the same day which took about 12 hours. The chromatographic conditions at the beginning and end of each run had to be the same to obtain reproducible results. Only the temperature of the column here is being varied, all other variables remaining constant. At each temperature in a run (200 to 150 and 150 to 120 deg C) one microlitre of sample was injected. The same amount (1ul, 20 ppm standard solution) of sample was injected at each temperature. Before the first injection was made, the instrument was stabilised for at least three hours to obtain a straight baseline and instrument destabilisation was indicated by random drift of the recorder baseline.

When the column temperature was changed to the next (lowest) temperature (10 or 5 deg C interval) to be studied about one hour was required for the instrument to be re-stabilised. With GC-ECD system, bleed of the column liquid phase caused the recorder baseline to drift down scale for a 10 or 5 deg C column interval temperature (one hour period of time). In these cases the baseline was returned to the original position with the

zeroing control of the recorder.

Optimum operating column temperature are listed in the previous section, 4.2.1.

4.1.7.1.2 Optimisation of the flow rate

To obtain optimum flow rates for each component for each column, the Van Deemter equation corresponding to HETP versus u is plotted.

In the Van Deemter equation, there is an optimum flow rate u_{Opt} for the system, at which H is a minimum. The optimum velocity will not be the same for the different components of a mixture, and should be selected for the components most difficult to separate. Chromatographers often choose to use a velocity greater than theoretical optimum in order to reduce the time of analysis. As the slopes of the left and right curve suggest, it is not good practice to used flow rates lower than the optimal flow rate.

Optimum flow rates for op pesticides with each column are presented in Section 4.1.2.1.

4.1.7.1.3 Electron capture detector temperature optimisation

The procedure was the same as for column temperature optimisation except for the following variation. To obtain optimum detector cell temperature, the run was started at a detector cell temperature of 390 deg C (^{63}Ni detector), and was decreased at 10 deg C intervals to 250°C. Each run had to be completed within the same day and took about 8 hours. The conditions at the beginning and the end of each run were required to be the same so as to obtain reproducible results, with only the temperature of the cell detector being varied.

When the detector cell temperature was changed to the next lower temperature (10 deg C interval) to be studied about 15 minutes were required for the cell detector temperature to be well settled down.

A total of fifteen temperatures were studied in each run, and the optimum cell detector temperatures are listed in Section 4.2.1 for both columns.

4.1.8 Injection port temperature

Several workers have used different injection temperatures (200 to 300 deg C) for op pesticides. In this project to ensure rapid vaporisation of the pesticides upon injection into the GC, injection port temperatures for each column were selected as listed in section 4.1.2.1.

4.1.9 Procedure for calibration

The procedure for calibration was the same as that described in Chapter 3, Section 3.7.9 except for the following:

Linearity is the range of sample concentration over which the detector responds in proportional increments within a certain arbitrary deviation. The peak area ratio (pesticide/internal standard) is plotted against a wide range of solute concentrations. Unfortunately, ECD has a small dynamic range of linearity, thus the analyst has to be very careful about concentration of the unknown sample.

There are two limits to the linearity curve: the lower concentration limit is set by the limit of detection, and the upper limit is defined by an arbitrary percentage deviation from the linearity curve, normally about 5%. The calibration curves for each op pesticides using GC-ECD are discussed later in Section 4.2.

4.1.10 Detection limit procedure

The detection limit is defined as the quantity of op pesticide in a sample injected at optimum conditions which will cause a response which is twice the noise level [17]. The units are normally given as gram of solute injected and the definition does represent a

reasonable criterion for detector comparison.

4.1.11 Pesticide residue recovery procedure

The recovery procedure used is identical to that described in Chapter 3, Section 3.7.1.4.

4.1.12 Support materials

All the supports were of the same particle size, 80 - 100 mesh (0.177 mm to 0.149 mm) and these materials were readily available in the laboratory.

4.1.13 Liquid phases

Silicone liquid phase SE30 and Apiezon L were used in this project. The SE30 column was prepared by coating the support with 5% (W/W) SE30 and the Apiezon L by coating with 10% (W/W). The Apiezon L column was only used for the study of malathion.

4.1.14 Preparation of column packing

The general requirements which need to be satisfied, regardless of the procedure used for making the packing, are the following [15]:

(A) Uniform distribution of the liquid phase on the surface of the particles must be assured

(B) Breakage of the support particles must be avoided. This is especially critical with Chromosorb W's which are the most friable (easily reduced to powder) of the supports used in this project. If the liquid phase is not distributed uniformly over the surface of the particles then active sites on the support will be exposed and will cause severe adsorption of the solute. This adsorption is indicated by tailing peaks. If the coated particles are broken during packing, active sites become exposed to the solutes tested, and, adsorption and tailing occur.

For preparation of 10 gram of support (e.g. 5% SE30), 0.5 gram of SE30 was dissolved in 150 ml of Chloroform with stirring, at room temperature overnight and to the SE30 solution the 9 grams of chromosorb WAW DMCS were added. Slowly, with delicate stirring, the mixture was allowed to stand at room temperature for 10 minutes with intermittent stirring. The mixture was transferred to a rotary evaporator flask. The rotary evaporator method was used to evaporate the Chloroform (it is necessary to avoid rapid rotation of the flask). After the solvent had been removed and the particles become free, the content of the flask was gently poured into a large watch glass, spread out, and left overnight (in an oven at 105°C) to remove any traces of solvent.

An 4 m x ¼ i.d. stainless steel tube was packed by the tap-and-fill method, aided by vacuum suction. One end of the column plugged with glass wool and packed with the prepared packing. During the packing of the column, tapping the side of the column with rod or with the aid of an electronic vibrator was necessary in order to make the particles settle compactly without leaving any spaces. The open end of the column was plugged with glass wool. The column was then fitted with ferrules and placed in the Varian 3700 Gas Chromatograph Oven and conditioned overnight at 220°C with carrier gas (Nitrogen) flowing through at a rate of 10 mls per minute. The conditioning period is completed when a stable detector baseline is obtained. After the conditioning for 24 hours, the column was ready for testing to ensure that the performance of the column is adequate.

A measure of column efficiency is made with a test sample. After testing the efficiency of the column, some test of column activity (site activity) and resolving power may be made.

4.2 RESULTS AND RESULTS ANALYSIS

4.2.1 Introduction

The effect of column and detector temperature and the nature of support (Chromosorb WAW-DMCS) on peak area response for four op pesticides (malathion, pirimiphos-methyl, dimethoate and heptenophos) were studied.

The temperature range and kind of column and stationary phase are described in section 4.1.

All of the columns were studied using a Varian 3700 gas Chromatograph employing electron capture detector (ECD), operated in the single column mode (packed or wide-bore capillary). The responses obtained in the study of the four pesticides by GC-ECD are discussed in terms of a comparison of the peak areas measured against column and detector temperature to obtain the best conditions for recovery studies of pesticides in sample analysis.

4.2.2 General considerations-spiking and sampling methodology

Spiking and sampling methodology have been described in Chapter 3, section 3.2.1.

4.2.3 Selection of column temperature

A) Packed Column

The column temperature which gave the highest detector response (peak areas for pesticides) were selected (see Table 4.1-4) for monitoring in GC-ECD analysis, although other column temperatures were investigated i.e.160 deg C, 170 deg C and 130 deg C for malathion, dimethoate and heptenophos respectively).

From the results (Table 4.1-3) decreasing the column temperatures reduces by 42%, 40% and 5% the sensitivity of analysis, thus increasing the detection limits. Furthermore, this will increase the analysis time but improve the shape of peak and provide the highest efficiency for separation.

Selection of 175 deg C as a column temperature for pirimiphos-methyl and to determine this pesticide as a residue in fruit and vegetable sample analyses provides the highest efficiency and peak area responses (see Table 4.4).

Table 4.1-4 shows that the same trend was observed in all pesticides i.e. with small peak areas at low temperatures, and a maximum response at some intermediate temperature. Daniel and Michel [16] found a similar pattern in the results (thermograms) for acetic and benzene and butanol, the only difference being that a plateau was reached at higher temperatures, rather than a decrease in response being observed.

The nature of the results obtained with GC-ECD could be explained by consideration of the following factors:

a) decomposition of the pesticides because of heating, together with catalytic effects of the column and supports
b) reaction of the pesticides with the liquid phase on the column
c) adsorption of the pesticides by the support and/or stationary phase
d) bleed of the stationary phase and its effect on the sensitivity of the electron-capture detector
e) priming by the pesticides of the exposed active sites on the supports.

Chromatography involving the WAW-DMCS column can generally be explained in terms of adsorption of the pesticides using a column temperature of 150 to 190 deg C and bleed of the liquid phase with a column temperature of 190 deg C and higher. As the column temperature is increased from 150 deg C to 190 deg C the pesticides are less adsorbed on the support and hence a higher response is observed with increasing column temperatures

(see Table 4.3) as a particular example). However, at a column temperature of 190 deg C the bleed of liquid phase from the column begins to compete with the pesticides for the electron population in the detector, hence reducing the response of the ECD towards the pesticides. Thus the response of the pesticides decreases from 190 deg C because of increasing column bleed as the column temperature is increased. However, in the case of both pirimiphos-methyl and malathion, the response of the pesticides between 175 and 190 deg C is constant and, therefore an increase in column bleed as the column temperature increases cannot be proposed as a reason for this effect.

Table 4.2shows the response for heptenophos as the column temperature is increased form 120 to 150 deg C. As the column temperature is increased from 120 to 140 deg C the heptenophose is adsorbed to a lesser extent on the support and hence a higher response is observed with increasing column temperatures. However at a column temperature of 140 deg C (for this pesticide) the decomposition of this may be attributed to catalytic effects of the support of reaction of the pesticide with the liquid phase on the column[19,31-33].

Only one pesticide sample was examined with 4 metre packed column, 10 % Apiezon L (WAW-DMCS). The malathion response on this column from 150 to 200 deg C is presented in Table 4.5. As the results show, from a column temperature of 150 to 190 deg C as the column temperature is increased the peak area increases but at a column temperature of 200 deg C the response is lower than at 190 deg C (comparing of Table 4.1 with Table 4.5) illustrating that the adsorption of the pesticides by the stationary phase (10% Apiezon L compared with 5% SE30) is more important at low column temperatures.

B) Wide-bore (fused-silica) capillary column

The material used to prepare wide-bore capillary column is synthetic silica or fused-silica, prepared by the flame hydrolysis of silicon tetrachloride ($SiCl_4$). The final product contains less than 1 pm of metallic impurities [18,19], such as Al, Ca, Cu, Fe, Mg ... [20].

The adsorptive and catalytic activity of the column materials can be attributed to the silica surface and to the presence of metal impurities at the surface which can function as lewis acid sites [21,22]. The metal impurities act as adsorption sites for lone-pair donor molecules such as ketones, amines, alcohol and π-bond-containing molecules.

Wide-bore capillary column chromatography of these pesticides can generally be explained in terms of adsorption of the pesticides (*malathion*, *dimethoate*, *pirimiphos-methyl*) between a column temperature of 150 to 170 deg C and catalytic degradation on the column from a column temperature of 170 deg and higher. As the column temperature was increased from 150 to 170 deg C the pesticides were less strongly absorbed on the column hence higher response is observed with increasing column temperature (see Table 4.7-9). However, at a column temperature of 170 deg C the catalytic degradation in the column began. Thus a lower response is observed with increasing column temperature. Table 4.6 shows the effect of column temperature in peak area for heptenophos analysed by wide-bore capillary. The same explanation can be proposed for malathion, pirimiphos-methyl and dimethoate, for which also only the column temperature is being varied.

From the results (Table 4.1-9), adsorptive losses vary inversely and catalytic losses vary directly with column temperature. Thus, as the column temperature increases, those pesticides whose relative peak areas increase are prone to adsorption interactions, and those solutes whose relative peak areas decrease are prone to catalytic degradation on the column.

In summary, to obtain optimal column temperatures, sensitivity tests with temperature must be utilized for any components which are in reality susceptible to acid-base interactions, hydrogen bonding attractions and catalytic influences.

4.2.4 Selection of temperature for the detector (ECD)

The detector temperature affects the number of electrons emitted from the radioactive source, their energies and the electron capture mechanism [19].

4.2.4.1 Electron attachment [23]

When an electron capturing solute enters the detector cell, an abundance of low-energy free electrons are available and this plasma is an ideal environment for electron attachment. Two main mechanisms have been proposed by several chromatographers

$$e^- + AB \text{ ==== } AB^- + \text{energy} \qquad \text{associative} \qquad (1)$$

$$e^- + AB \text{ ---> } A.+B^- \pm \text{energy} \qquad \text{dissociative} \qquad (2)$$

The solute molecule captures an electron to form a negative molecular ion (equation 1) which is stable or which dissociates to a radical (A.) and an ion fragment (B^-) (equation 2). In the first case (associative), the energy of electron attachment is thought to arise from the electron affinity of the molecule and is subsequently either liberated as radiation or translated to other molecules during collisions.

In the dissociative capture reaction, the energy balance is maintained by the relative kinetic energy of the reactants before and after collision, the energy released by the formation of a negative ion from a constituent atom or radical of the compound and the energy required or released during the dissociation of the molecule [24].

Wentworth and co-workers [24,25] described the kinetic theory for electron attachment (dissociative and non-dissociative) processes and showed that the Arrhenius equation can be used to derive a relationship between the temperature dependence of the equilibrium constant and heat of reactions. These workers established equation 3.

$$\ln KT^{3/2} = \ln z - \frac{\Delta E}{kT} \qquad (3)$$

where

K = capture coefficient

z = pre-exponential factor

ΔE = activation energy

k = Boltzmann constant

T = detector cell temperature.

The Arrhenius plot of $InKT^{3/2}$ versus 1/T may be linear with a positive slope for the capturing species undergoing a non-dissociative mechanism [25]. The slope $\Delta E/k$ is related to the activation energy for the mechanism under consideration, and therefore the capture response decreases with increasing cell temperature for non-dissociative capturing.

Attachment of an electron to a solute molecule is related to the electron affinity and to a requirement for sufficient energy to cause attachment at a given temperature. The larger the activation the greater the energy necessary for attachment and the slower is the attachment reaction (Fig. 4.2.1). The activated complex (AB^*) represents an intermediate formed when an electron is being accommodated by the solute molecule. The energy is absorbed by AB to form the activated complex, AB^*, represented by E_a.As $A.+B^-$ has a lower energy than AB, heat is evolved in the process and ΔE is the heat of the reaction. For the reverse reaction an endothermic process, the amount of activation energy becomes E'a.

A summary has been given by Wentworth and Chen [23] for the four electron capture mechanisms (Fig. 4.2.2) in terms of potential energy diagrams. Mechanism 1 (equation 1) shows the non-dissociative electron attachment, which is characterised by a positive slope and the formation of a stable negative ion. In this case, the overall change in internal energy for the process, ΔE, is equal to the electron affinity. Aromatic hydrocarbon are examples and representative of this type of mechanism.

Mechanism 2 (equation 2) can be exemplified by alkyl halogens (except C-F). A linear relationship is observed between E_a and ΔE with a slope of unity [26]. It is possible to differentiate between mechanism II and III, as E_a is larger in the former [27].

A positive slope in the $InKT^{3/2}$ versus 1/T plot may also occur if a solute undergoes capture by either mechanism III or IV. A negative molecular ion intermediate is believed to also occur in these cases [26]. In mechanism III, E_a is greater than ΔE, while they are equal in mechanism IV [26]. Aromatic halogens (Cl, Br, I) are assumed to undergo mechanism III, while acetic anhydride and ethyl acetate undergo mechanism IV [26]. Therefore the capture response increases with increasing temperature (up to optimal TEM) for dissociative capturing compounds, and after the optimal temperature capture the response may be constant or decrease with increasing temperature.

For organophosphorus compounds investigated in this work, the electron capture detector response is highly temperature dependent as the results show (see Table 4.10-11). In this case, the electron capture coefficients vary considerably with temperature for all the compounds. From data given in Table 4.10 the optimum cell detector temperature can be selected as described in Section 4.1.

Significant increases in sensitivity were observed as the temperature was increased to 340, 360, 330 and 350 deg C for malathion, pirimiphos-methyl, dimethoate and heptenophos, respectively. Analysis at these temperatures increased the sensitivity of detection of malathion, pirimiphos-methyl, dimethoate and heptenophos about 2, 3, 2 and 13 times respectively.

The decrease in area above 340 and 350 deg C for malathion and heptenophos respectively can be explained by kinetic theory for dissociative processes, mechanisms III and/or IV (see Figure 4.2.2) and a uniformity in response (area) above 350 and 330 deg C for pirimiphos-methyl and dimethoate, respectively, can be explained by the mechanism II.

Table 4.11shows the effect of detector cell temperature obtained on WAW-DMCS support using the conditions given in section 4.1. All pesticides, because of high flow rate, showed a higher optimal detector temperature and the increase in area with increasing temperature should be explained by mechanism II.

In summary, it is important for any chromatographer using GC-ECD that the temperature of the detector is specified and the optimum temperature for the analysis should be determined.

4.2.5 Selection of column flow rate

4.2.5.1 Packed column

To obtain the optimum flow rate, the van Deemter equation corresponding to HETP versus u^{-} is plotted, and flow rates of 65, 63, 69 and 64 ml $minute^{-1}$ (carrier gas) for malathion, pirimiphos-methyl, dimethoate and heptenophos, respectively, were determined.

From the results a carrier gas flow rate of 65 cm^3min^{-1} should be selected for a packed column of 4 metre having 5% SE30 as stationary phase with WAW-DMCS (80 to 100 mesh) as a solid support.

4.2.5.2 Wide-bore capillary column

The procedure used was the same as that for packed columns. The carrier gas flow rates of 4.2, 3.6, 3.2 and 3.5 cm^3min^{-1} for malathion, pirimiphos-methyl, dimethoate and heptenophos, respectively were determined. From these results a flow rate of 4.2 ml min^{-1} (for carrier gas with 26 ml min^{-1} for make up gas) can be selected for the wide-bore capillary column the characteristics of which are presented in table 4.1.2.2.2.

4.2.6 Qualitative analysis of pesticides

4.2.6.1 Conditions for routine analysis

The temperature limits for use of Ti^3H and SC^3H (in ECD), as set up by the US Atomic Energy Commission are 220 and 325 deg C, respectively. Above these maximum operating temperatures, Tritium leakage and contamination can occur. The ^{63}Ni source, however, allows analysis up to 350 deg C and heating up to 420 deg C for short periods[19].

From the results and results analysis in section 4.2.1-3 and for the above reason the optimal conditions for the wide-bore capillary column used in this work for the analysis of malathion, pirimiphos-methyl, dimethoate and heptenophos are as follows:

Parameter	Condition
detector cell temperature	340 deg C
(i) column oven temperature	125 deg C (only for heptenophos)
(ii) column oven temperature	170 deg C (for other pesticides in this work)
carrier gas flow rate (Nitrogen)	4.2 ml/min
make-up gas (Nitrogen)	26 ml/min

4.2.6.2 Selection of internal standard

The internal standard used for dimethoate and malathion was pirimiphos-methyl and those used for pirimiphos-methyl and heptenophos were malathion and dimethoate, respectively. These reference compounds were chosen because of their chromatographic properties, retention time, non-interference, and because of their availability and comparative sensitivity to detection by ECD.

4.2.6.3 Selectivity of ECD

Figure 4.2.3-6 shows the chromatograms obtained for the standard pesticides and figure 4.2.7-10 and 4.2.11-14 show the chromatograms obtained for samples (without pesticides) under the conditions used (see section 4.2.6.1).

Comparison of the chromatogram for heptenophos, figure 4.2.3 with the sample chromatograms figure 4.2.11-14 under the same conditions indicates that only the chromatogram obtained from carrot samples exhibited a very small peak close to heptenophos (this extraneous peak was considered not to be a major source of interference). Thus the heptenophos spiked sample can be determined without any clean-up method.

Comparison of figure 4.2.4 with figures 4.2.7-10 shows two small peaks close to that of dimethoate and thus dimethoate in the sample of carrot must be determined after a clean-up step.

4.2.7 Quantitative analysis

Quantitative GC-ECD (wide-bore capillary column) analysis of individual pesticides in extracts was performed by a technique of internal standardisation and this method has been described in section 3.2.5 and 3.7.4.

Standard solutions were made, as listed in Table 4.2.5.1, and the calibration curves were constructed by plotting peak height ratio (mean of three injections) against the weight of pesticides.

The peak heights were used rather than the peak areas because the results appeared more reproducible. Peak areas can be more variable because of the uncertainties in marking the start and end-points of the peaks. The calibration curves are shown in 4.2.13 (as an example), with 95% confidence limits indicated, and least squares analysis was used to

calculate the best straight line.

The statistical data are summarised in Table 4.13 . and the recoveries of the various compounds under the procedures used are given in Table 4.14-15

4.2.8 Detection limits

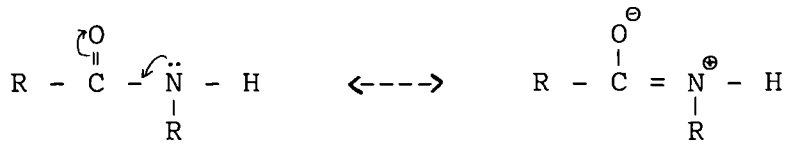
The detection limits were determined on the fused silica wide-bore capillary column under conditions of optimum instrument operation. The absolute detection limits of heptenophos malathion, dimethoate and pirimiphos-methyl obtained for injection of standards only are 7.81×10^{-14}, 4.03×10^{-14}, 3.91×10^{-14} and 6.84×10^{-13} gram per ul injection, respectively.

The level of sensitivity in electron capture detection is dependent upon compound properties, such as the isomeric form, the frequency of electrophore substitution and the position of the electrophore with respect to other functional moieties [23].

The difficulty in predicting relative capture coefficients on the basis of only electrophilic and nucleophic inductive effects is exemplified by the system moiety in phosphate pesticides [28,29]. The relative sensitivity changes in the order

$$\underset{\text{P-S}}{\overset{\text{S}}{\overset{\|}{}}} > \underset{\text{P-S}}{\overset{\text{O}}{\overset{\|}{}}} > \underset{\text{P-O}}{\overset{\text{S}}{\overset{\|}{}}} > \underset{\text{P-O}}{\overset{\text{O}}{\overset{\|}{}}}$$

An amide functionality which can resonate provides an electrophore region for capture[30]:



The compounds with ultrafast rate constants are $C Cl_4$, SF_6, $CFCL_3$ and CH_3I. The ECD is most responsive to these types of compounds and would normally be selected for their determination. This group includes most of the moderately strong and weak electrophores that make up the bulk of compounds determined by the ECD [22].

For the above reasons detection limits observed for op pesticides are reasonably low.

Column temperature deg C	Column efficiency N	HETP mm	Retention time min	PRE(%) ± S.D (c) [a]	PRA(%) ± S.D [b]
200	2416	1.62	1.23	56 ± 3.1	100 ± 4.9
190	2452	1.60	1.24	57 ± 4.6	100 ± 3.9
180	2770	1.41	1.33	64 ± 2.9	77 ± 5.1
170	3669	1.06	1.42	85 ± 6.1	68 ± 3.4
160	4328	0.94	1.52	100 ± 5	68 ± 4.2

Table 4.1 Effect of column temperature on peak area response, column efficiency and retention time for malathion, using 5% SE30 packed column

(a) percentage efficiency relative to 160 deg C (PRE)
(b) percentage peak area relative to 190 deg C (PRA)
(c) n = 3

Column temperature deg C	Column efficiency N	HETP mm	Retention time min	PRE (%) ± S.D.(c) [a]	PRA (%) ± S.D. [b]
190	2560	1.56	1.12	91 ± 4.7	100 ± 4.8
180	2577	1.55	1.13	92 ± 5.0	100 ± 4.2
175	2809	1.42	1.14	100 ± 3.9	100 ± 1.9
170	2708	1.48	1.20	96 ± 1.8	98 ± 7
165	2637	1.52	1.23	94 ± 6.1	98 ± 6.6
160	2593	1.54	1.48	92 ± 2.4	98 ± 3.9

Table 4.4 Effect of column temperature on peak area response, column efficiency and retention time for pirimiphos-methyl, using 5% SE30, 4 m, packed column.

(a) percentage efficiency relative to 175 deg C (PRE)
(b) percentage peak area relative to 175 deg C (PRA)
(c) n = 3

Column temperature deg C	Column efficiency N	HETP mm	Retention time min	(a) PRE (%) ± S.D. (c)	(b) PRA (%) ± S.D.
200	2432	1.64	0.84	80 ± 2.9	97 ± 5.3
190	2659	1.51	0.85	88 ± 3.8	100 ± 7
180	2880	1.39	0.86	95 ± 4.8	80 ± 5.2
170	3029	1.32	0.90	100 ± 3	60 ± 4.1
165	2769	1.44	0.93	91 ± 6.5	55 ± 3.9
160	1894	2.11	1.11	63 ± 4.1	53 ± 2.8
150	1361	2.94	1.25	45 ± 2.4	45 ± 1.9

Table 4.3 Effect of column temperature on peak area response, column efficiency and retention time for dimethoate, using 5% SE30, 4 m, packed column.

(a) percentage efficiency relative to 170 deg C (PRE)
(b) percentage peak area relative to 190 deg C (PRA)
(c) n = 3

Column temperature deg C	Column efficiency N	HETP mm	Retention time min	(a) PRE (%) ± S.D. (c)	(b) PRA (%) ± S.D.
150	2762	1.45	0.43	88 ± 3.7	88 ± 4.4
140	2991	1.34	0.45	95 ± 6	100 ± 5
130	3145	1.27	0.47	100 ± 5.1	95 ± 3.1
125	2875	1.39	0.56	91 ± 4.4	85 ± 2.9
120	1973	2.03	0.63	63 ± 2.2	71 ± 1.9

Table 4.2 Effect of column temperature on peak area response, column efficiency and retention time for heptenophos, using 5% SE30, 4 m, packed column

(a) percentage efficiency relative to 130 deg C (PRE)
(b) percentage peak area relative to 140 deg C (PRA)
(c) n = 3

Column temperature deg C	Column efficiency N	HETP mm	Retention time min	(a) PRE (%) ± S.D. (c)	(b) PRA (%) ± S.D.
200	2438	1.44	1.35	75 ± 3	95 ± 5.2
190	2582	1.36	1.37	78 ± 2.4	100 ± 6
180	2892	1.21	1.47	88 ± 6	85 ± 3.3
170	3286	1.07	1.58	100 ± 7.1	37 ± 4.7
160	3296	1.06	1.68	100 ± 4.4	30 ± 2.2
150	2158	1.62	1.62	66 ± 3.6	19 ± 1.1

Table 4.5 **Effect of column temperature on peak area response, column efficiency and retention time for malathion, using 10% Apiezon L, 4 m, packed column**

(a) percentage efficiency relative to 160 deg C (PRE)
(b) percentage peak area relative to 190 deg C (PRA)
(c) n = 3

Column temperature deg C	Column efficiency N	HETP mm	Retention time min	(a) PRE (%) ± S.D. (c)	(b) PRA (%) ± S.D. (c)
150	4933	2.43	2.80	59 ± 3.2	39 ± 2.4
140	4988	2.41	4.35	60 ± 2.9	64 ± 6.2
130	5868	2.04	6.94	71 ± 4.0	64 ± 3.8
125	8321	1.44	8.76	100 ± 4.3	100 ± 5
120	7845	1.53	11.56	94 ± 6.1	88 ± 4.8

Table 4.6 **Effect of column temperature on peak area response, column efficiency and retention time for heptenophos, using wide-bore capillary column, 12 metre, BP10**

(a) percentage efficiency relative to 125 deg C (PRE)
(b) percentage peak area relative to 125 deg C (PRA)
(c) n = 3

Column temperature deg C	Column efficiency N	HETP mm	Retention time min	(a) PRE (%) ± S.D. (c)	(b) PRA (%) ± S.D. (c)
200	1627	7.38	1.37	38 ± 3.0	70 ± 3.3
190	2093	5.73	1.87	48 ± 2.4	71 ± 3.2
180	3118	3.85	2.79	72 ± 3.2	82 ± 2.0
175	3444	3.48	3.34	80 ± 4.4	86 ± 4.1
170	4322	2.78	4.18	100 ± 5.4	100 ± 4.3
165	4259	2.82	5.04	99 ± 7.1	97 ± 6.1
160	3965	3.03	6.15	92 ± 6.3	96 ± 5.5

Table 4.7 **Effect of column temperature on peak area response, column efficiency and retention time for dimethoate, using wide-bore capillary column, 12 meter, BP10**

(a) percentage efficiency relative to 170 deg C (PRE)
(b) percentage peak area relative to 170 deg C (PRA)
(c) n = 3

Column temperature deg C	Column efficiency N	HETP mm	Retention time min	(a) PRE (%) ± S.D. (c)	(b) PRA (%) ± S.D. (c)
190	6042	1.91	2.69	66 ± 4.3	67 ± 5.2
180	7912	1.52	3.77	86 ± 6.6	92 ± 7.3
170	9101	1.32	5.81	99 ± 5.6	100 ± 4.9
160	9179	1.31	8.82	100 ± 2.9	98 ± 5.7
150	9092	1.32	12.32	99 ± 7.8	88 ± 6.1

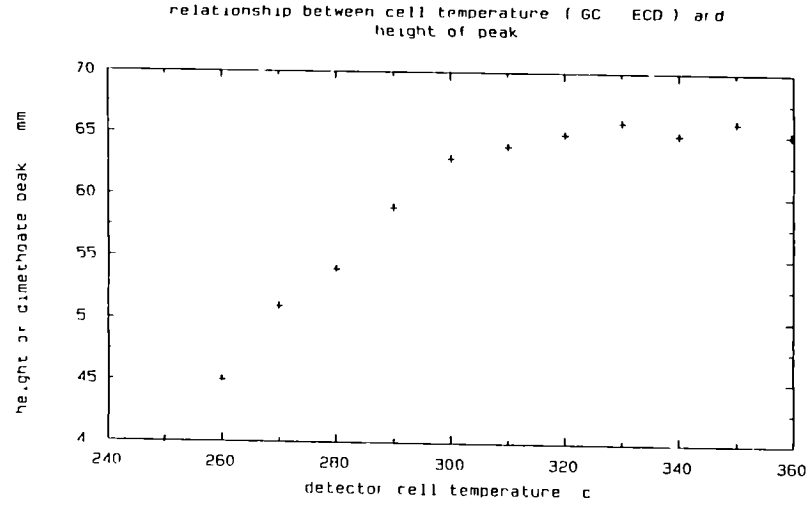
Table 4.8 **Effect of column temperature on peak area response, column efficiency and retention time for malathion, using wide-bore capillary column, 12 metre, BP10**

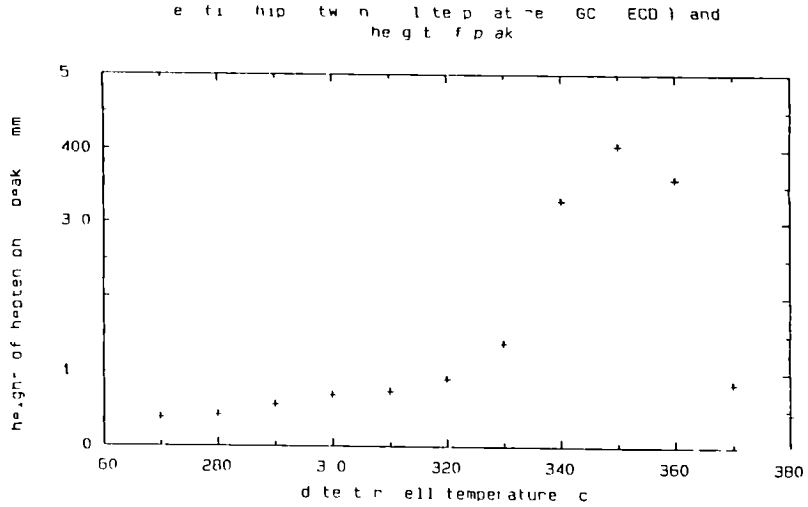
(a) percentage efficiency relative to 160 deg C (PRE)
(b) percentage peak area relative to 170 deg C (PRA)
(c) n = 3

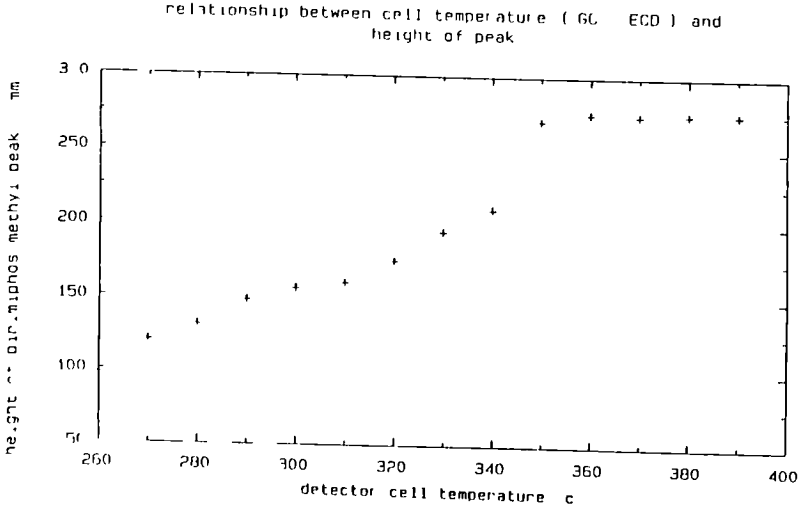
Column temperature deg C	Column efficiency N	HETP mm	Retention time min	(a) PRE (%) ± S.D. (c)	(b) PRA (%) ± S.D. (c)
200	3426	3.50	1.58	33 ± 4.8	84 ± 7.7
190	7266	1.65	2.32	70 ± 2.2	82 ± 8.1
185	10389	1.16	2.87	100 ± 4.2	88 ± 4.9
180	6648	1.81	3.4	64 ± 1.9	90 ± 5.8
175	7710	1.56	4.2	74 ± 8.1	98 ± 6.9
170	7869	1.52	5.44	75 ± 6.8	100 ± 1.7
160	8670	1.38	8.45	84 ± 7.8	94 ± 2.6

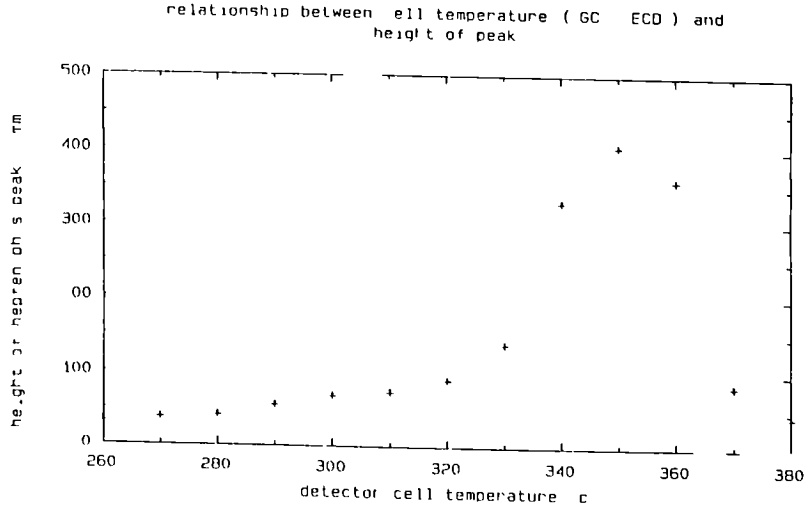
Table 4.9 **Effect of column temperature on peak area response, column efficiency and retention time for pirimiphos-methyl, using wide-bore capillary column, 12 meter, BP10**

(a) percentage efficiency relative to 180 deg C (PRE)
(b) percentage peak area relative to 170 deg C (PRA)
(c) n = 3









Effect of detector cell temperature on peak area response, using a 12 meter wide-bore BP10 capillary column under conditions as described in Section 4.1

Detector cell temperature (ECD) deg C	peak area response (mm^2), mean of three injections			
	malathion	pirimiphos-methyl	dimethoate	heptenophos
250	80	-	40	-
260	81	97	45	32
270	96	120	51	38
280	96	131	54	42
290	112	148	59	56
300	120	156	63	69
310	124	160	64	74
320	132	175	65	91
330	136	195	66	139
340	140	210	65	330
350	140	270	66	405
360	128	275	65	360
370	96	274	-	85
380	-	275	-	45
390	-	275	-	-

Table 4.10 **Effect of detector cell temperature on peak area response, using a 12 metre wide-bore BP10 capillary column under conditions as described in Section 4.1**

Detector cell temperature (ECD) deg C	Peak area response (mm^2), mean of three injections			
	malathion	pirimiphos-methyl	dimethoate	heptenophos
250	70	23	78	35
260	-	23	-	36
270	69	24	78	38
280	-	24	-	45
290	70	25	78	-
300	-	25	-	60
310	70	27	78	70
320	-	29	-	-
330	75	32	92	79
340	80	34	108	80
350	83	37	125	120
360	*90*	*39*	*137*	*280*
370	90	41	153	362
380	90	42	165	361
390	90	43	175	362

Table 4.11 **Effect of detector cell temperature on peak area response, using a 4 metre packed column, 5% SE30, under conditions described in Section 4.1**

Name of Pesticide	working concentration range for sample (ppm) internal standard	internal standard used
malathion	(2, 3, 4, 5, 6, 7, 8)/5	pirimiphos-methyl
pirimiphos-methyl	(1, 2, 4, 6, 7, 8)/5	malathion
dimethoate	(0.8, 1, 2, 3, 4, 5, 6)/4	pirimiphos-methyl
heptenophos	(1, 2, 3, 4, 6, 7, 8)/4	dimethoate

Table 4.12 Working concentration range for pesticides

Name of Pesticide	straight time equation y = ax + b	correlation coefficient (R)
malathion	y = 5.59x - 1.82	0.9995
pirimiphos-methyl	y = 3.32x - 0.51	0.9989
dimethoate	y = 2.4x - 0.90	0.9978
heptenophos	y = 1.21x - 0.23	0.9988

Table 4.13 Linear regression analysis data obtained from internal standardisation method for pesticides determination

sample	Recovery (%)			
	apple	carrot	tomato	lettuce
pesticides	mean ± RDS	mean ± RDS	mean ± RDS	mean ± RDS
malathion	96.58 ± 5.14	99.24 ± 4.92	91.81 ± 4.31	97.82 ± 4.95
pirimiphos-methyl	97.13 ± 2.75	96.43 ± 4.26	96.37 ± 2.98	97.15 ± 5.89
dimethoate	95.27 ± 3.03	94.53 ± 3.25	93.11 ± 4.38	96.48 ± 4.06
heptenophos	96.23 ± 3.91	97.69 ± 2.82	93.86 ± 3.22	95.73 ± 2.89

Table 4.14 Recovery of pesticides from spiked sample, 2mg/kg

sample	Recovery (%)			
	apple	carrot	tomato	lettuce
pesticides	mean ± RSD	mean ± RSD	mean ± RSD	mean ± RSD
malathion	98.85 ± 3.11	100.78 ± 4.26	90.18 ± 4.61	99.77 ± 4.98
pirimiphos-methyl	97.59 ± 4.86	99.21 ± 3.49	98.01 ± 3.12	99.30 ± 3.83
dimethoate	97.99 ± 3.74	93.23 ± 5.01	94.35 ± 4.01	98.63 ± 5.02
heptenophos	97.16 ± 2.99	101.05 ± 2.99	99.81 ± 3.22	100.86 ± 2.97

Table 4.15 Recovery of pesticides from spiked sample, 0.2 mg/kg

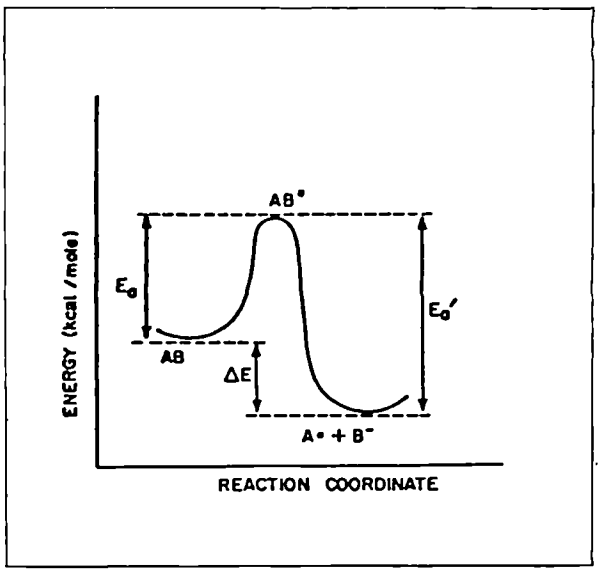


Figure 4.2.1 Relative energies of a molecule undergoing electron attachment.

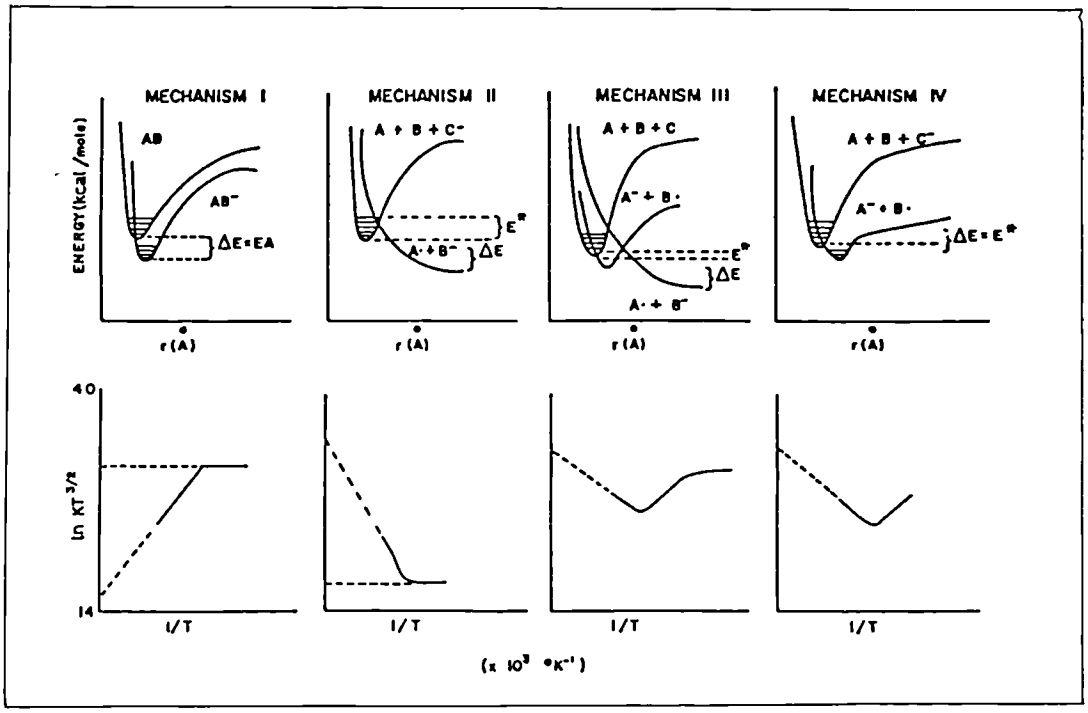


Figure 4.2.2 Summary of electron attachment mechanisms.

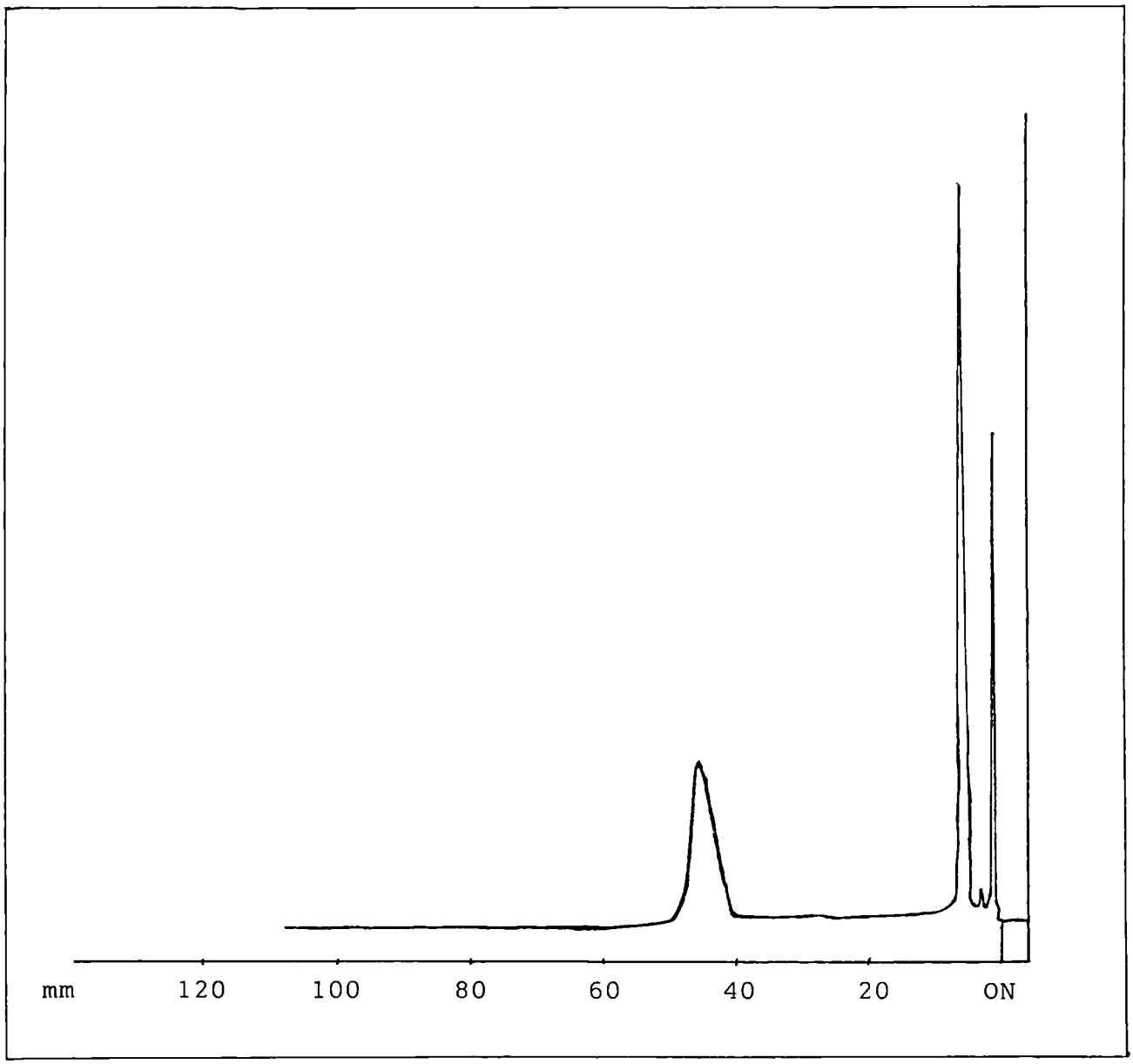


Figure 4.2.3 GC-ECD of heptenophos.

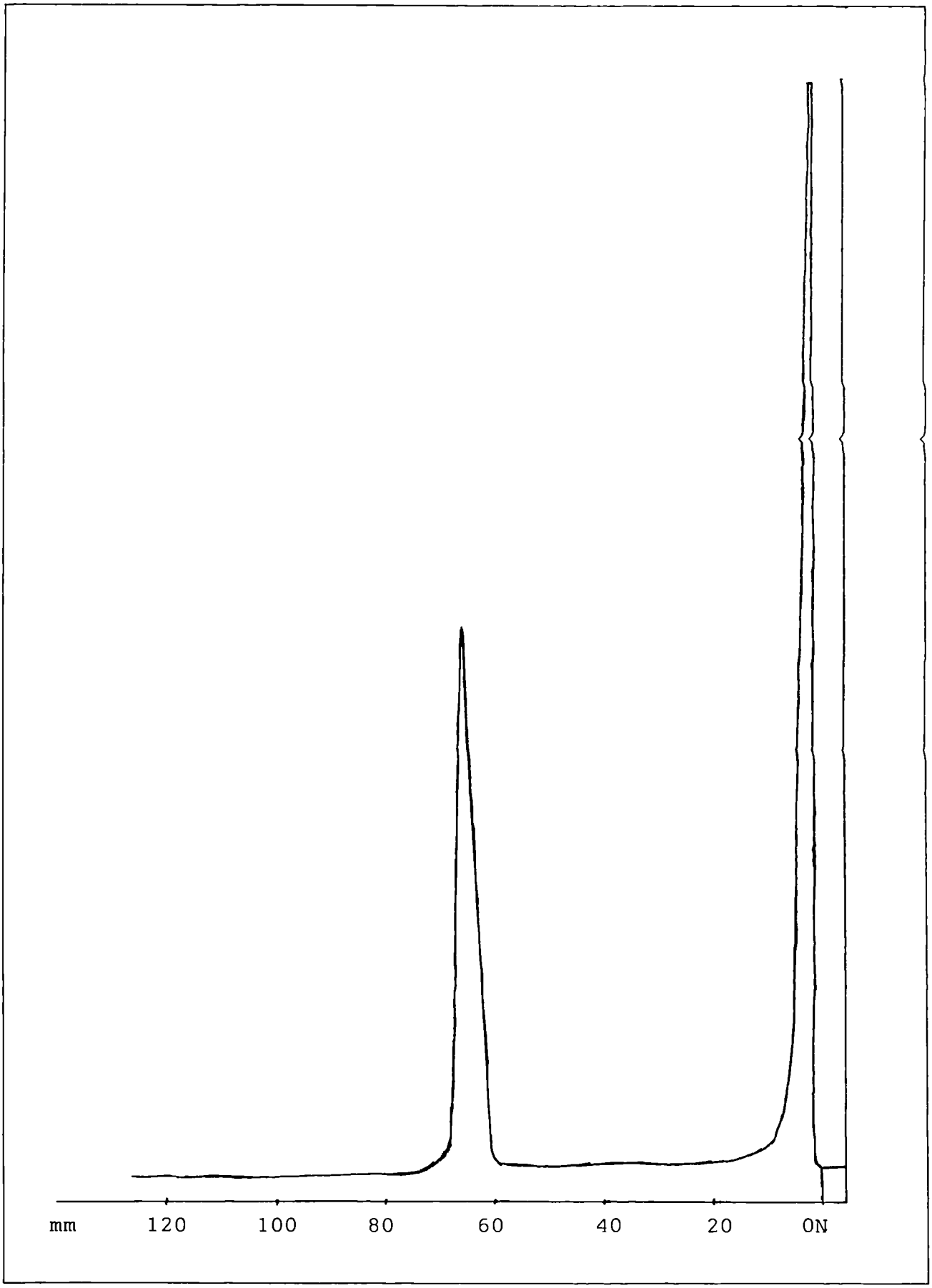


Figure 4.2.4 GC-ECD of dimethoate.

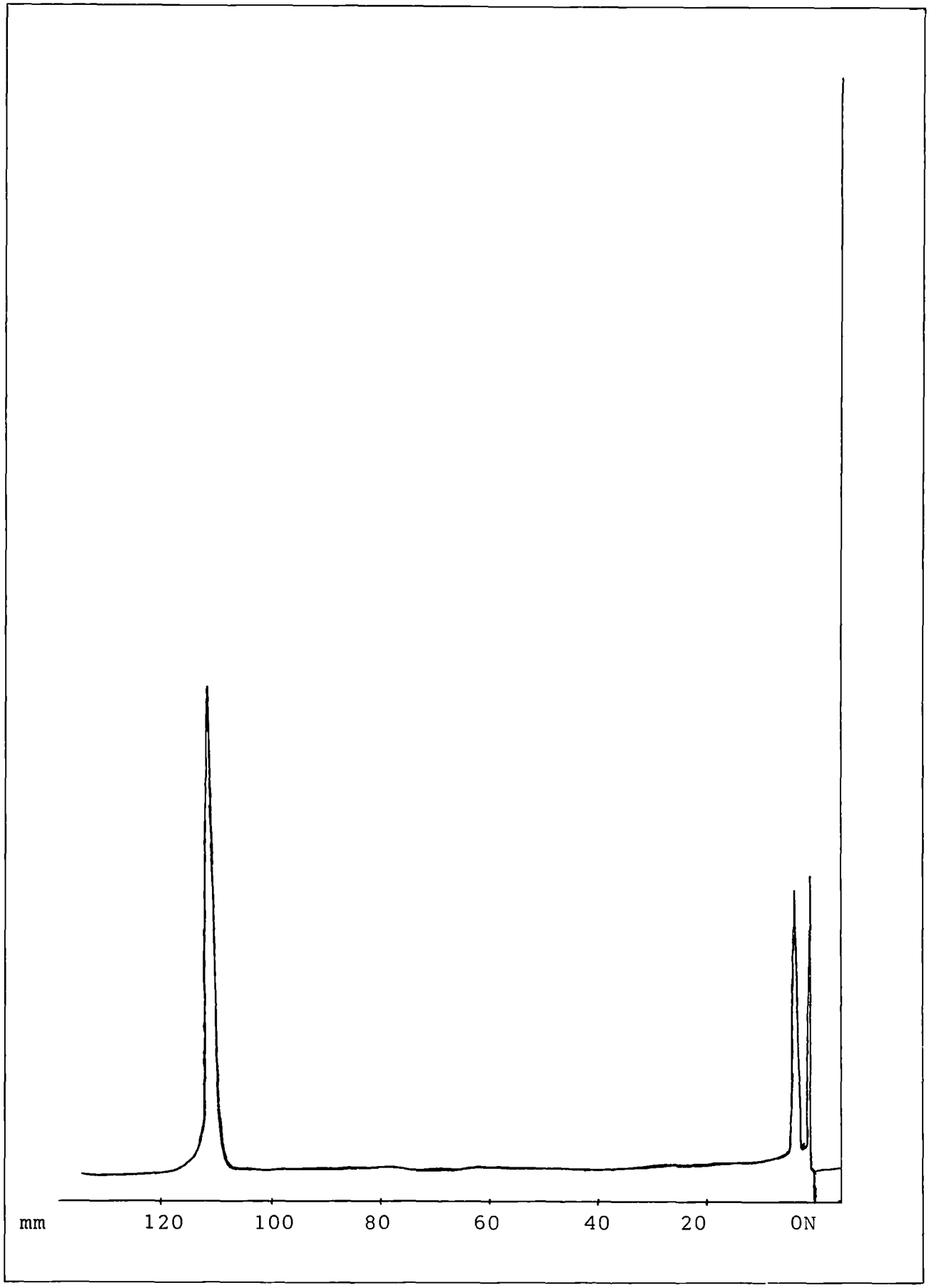


Figure 4.2.5 GC-ECD of malathion

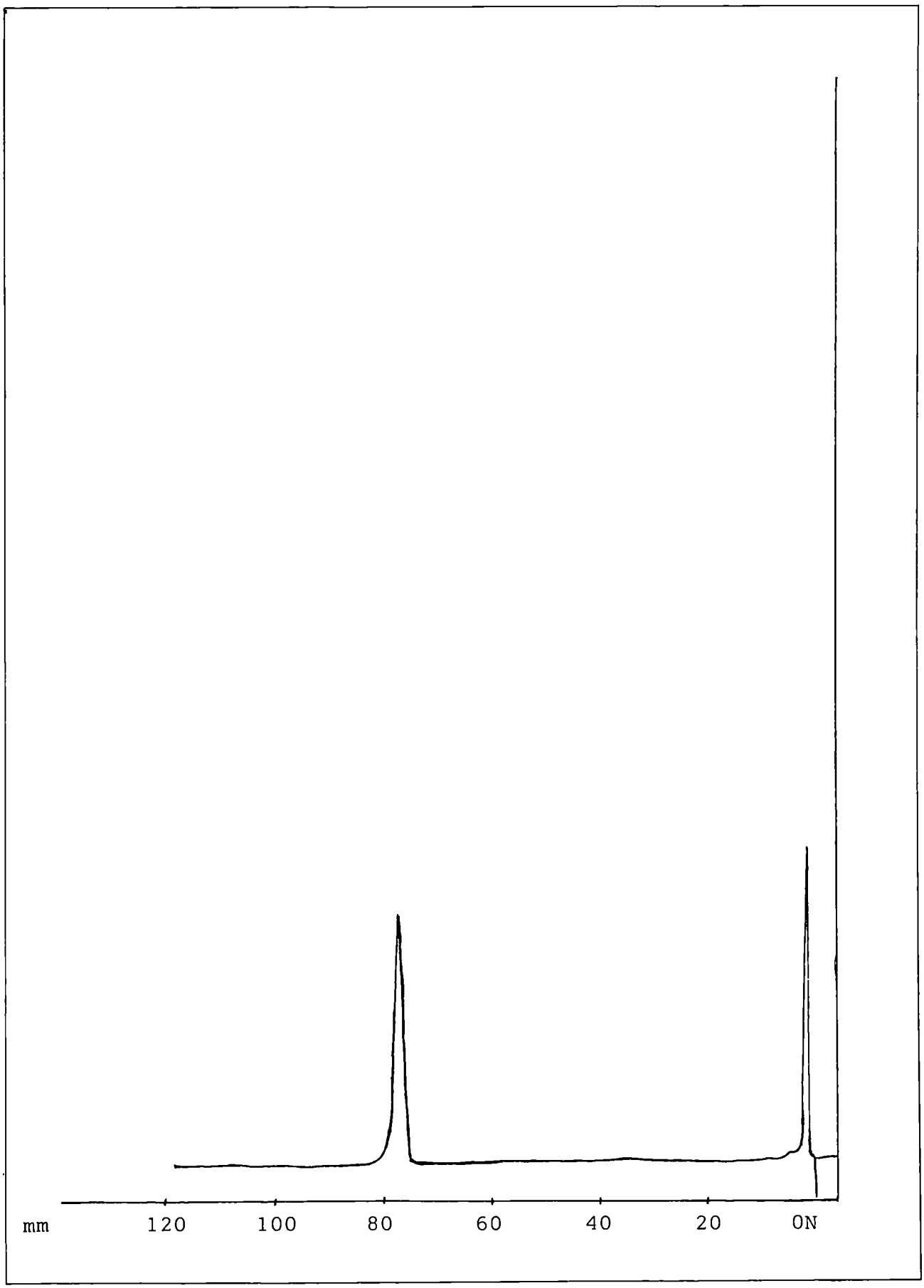


Figure 4.2.6 GC-ECD of pirimiphos-methyl

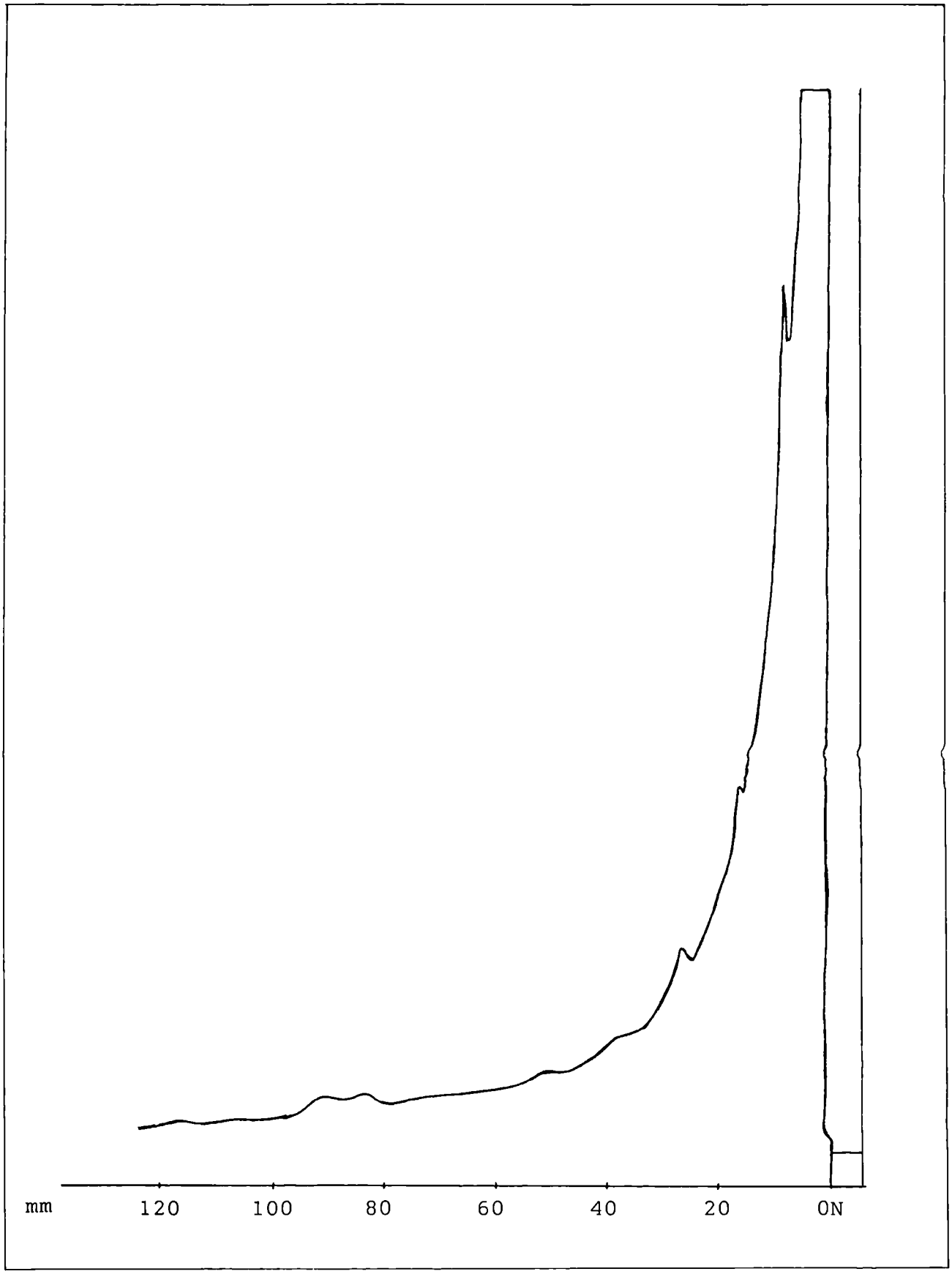


Figure 4.2.7 GC-ECD of tomato sample (without pesticides) under the conditions as described in Section 4.2.6.1

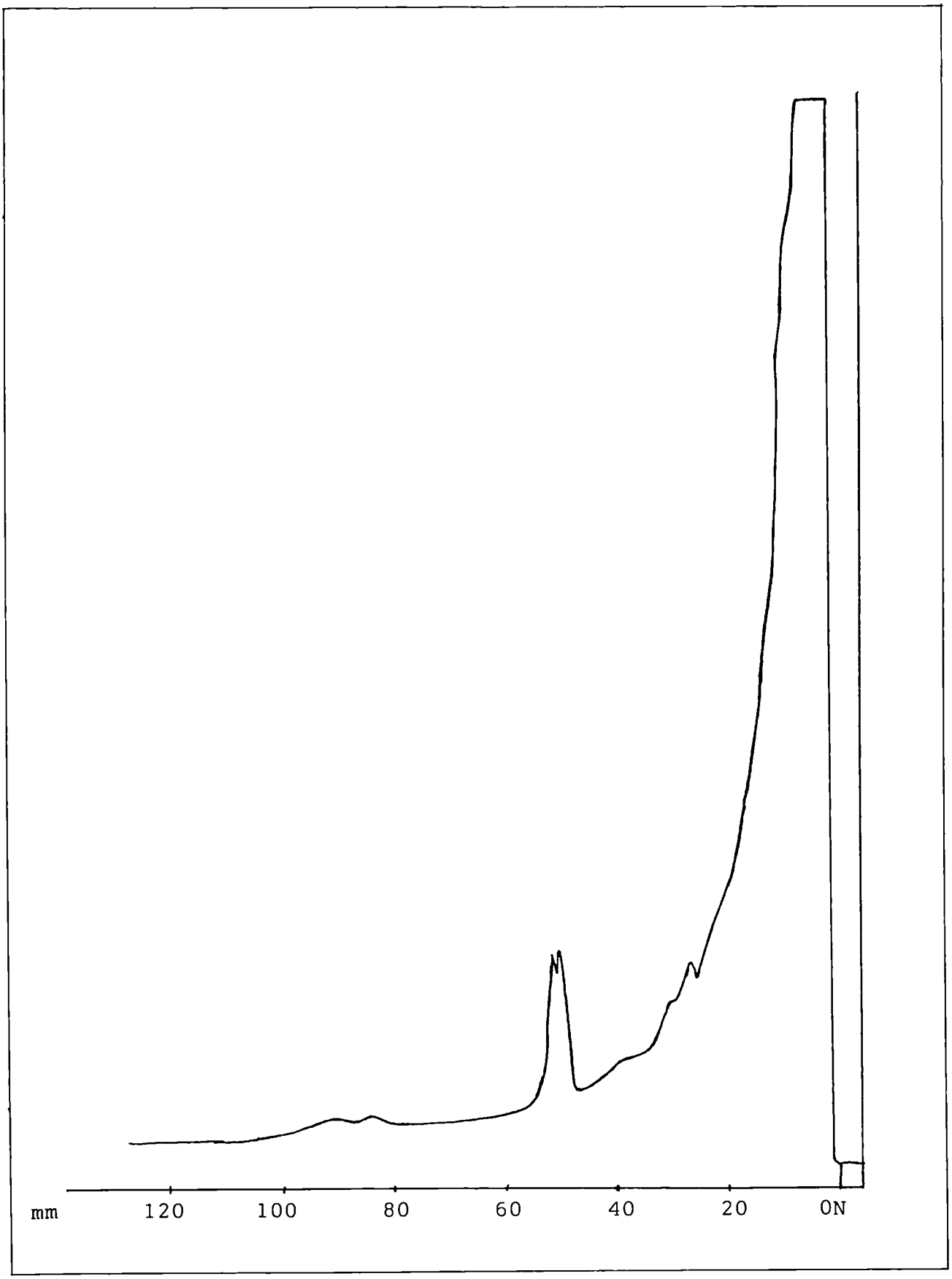


Figure 4.2.8 GC-ECD of apple sample (without pesticides) under the conditions as described in Section 4.2.6.1

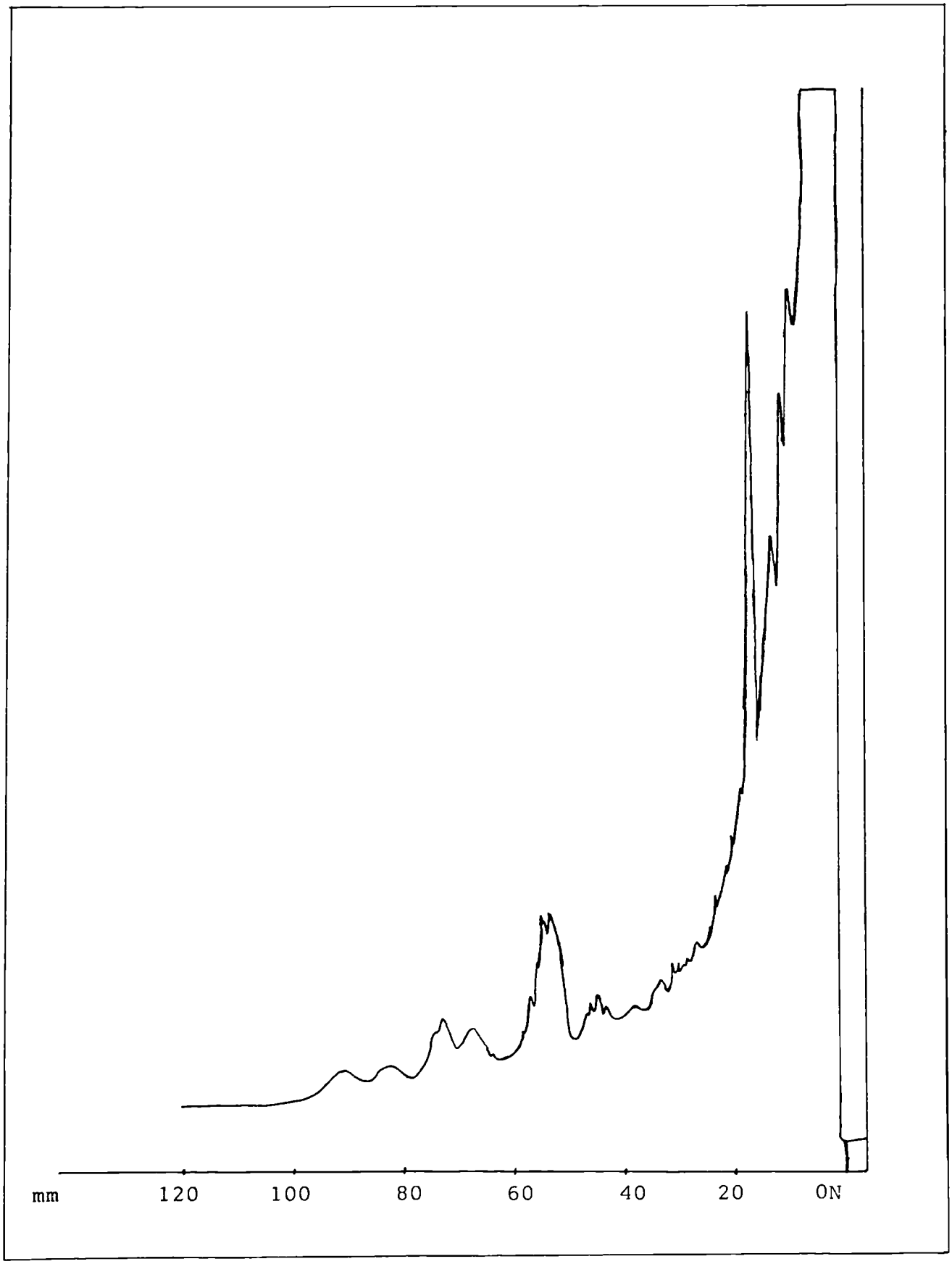


Figure 4.2.9 GC-ECD of carrot sample (without pesticides) under the conditions as described in Section 4.2.6.1

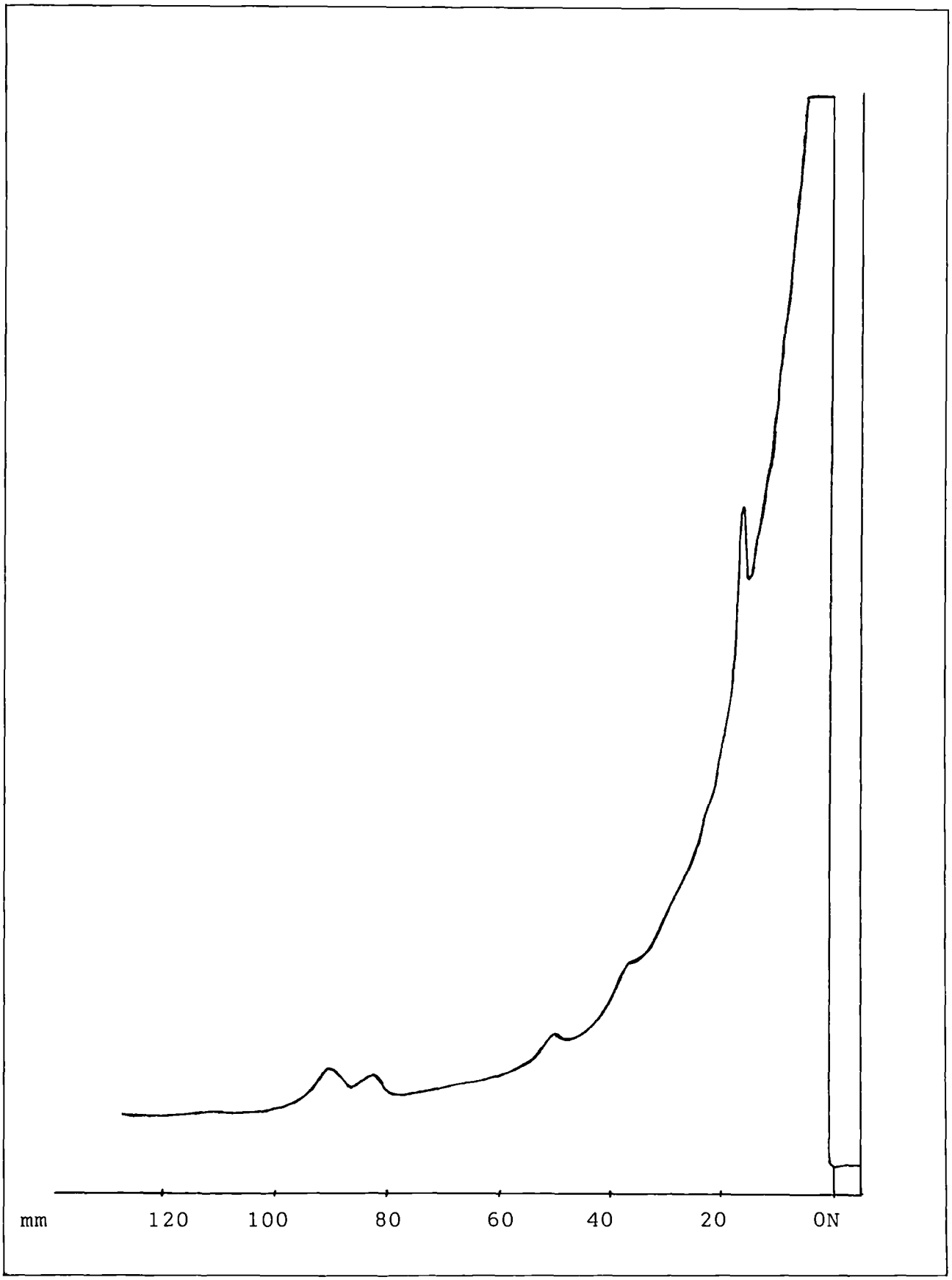


Figure 4.2.10 GC-ECD of lettuce sample (without pesticides) under the conditions as described in Section 4.2.6.1

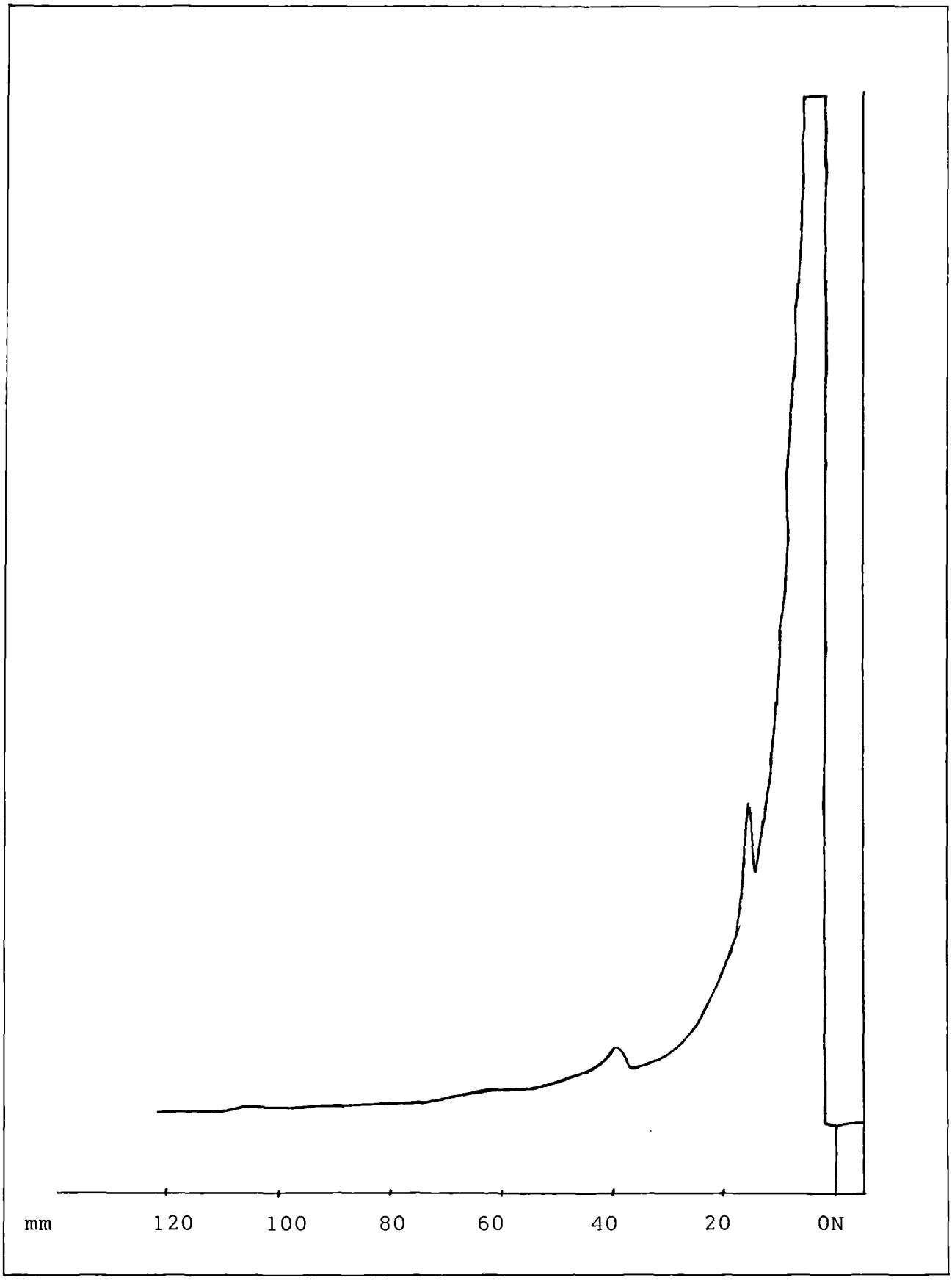


Figure 4.2.11 GC-ECD of apple sample (without heptenophos) under the conditions as described in Section 4.2.6.1

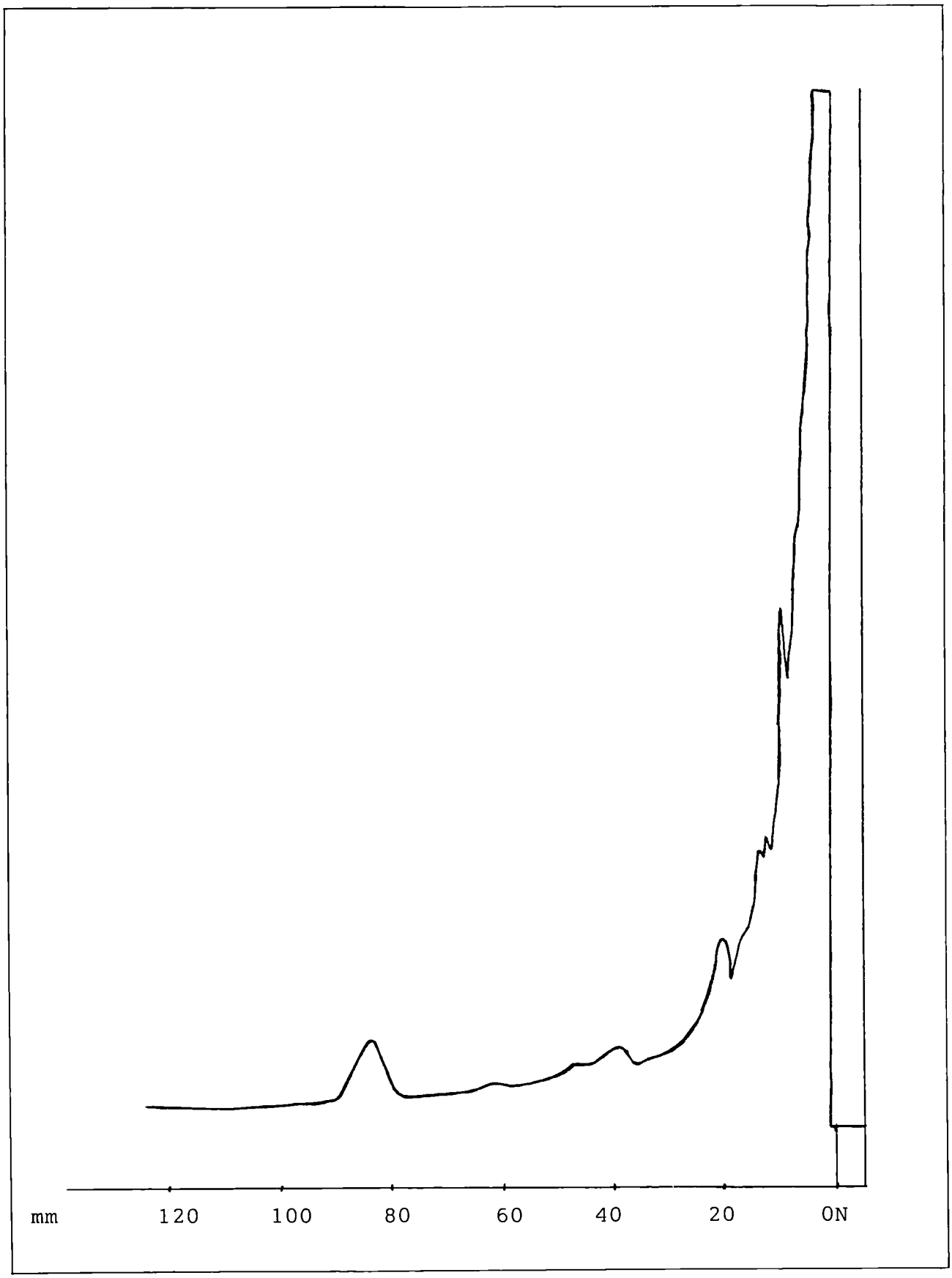


Figure 4.2.12 GC-ECD of carrot sample (without heptenophos) under the conditions as described in Section 4.2.6.1

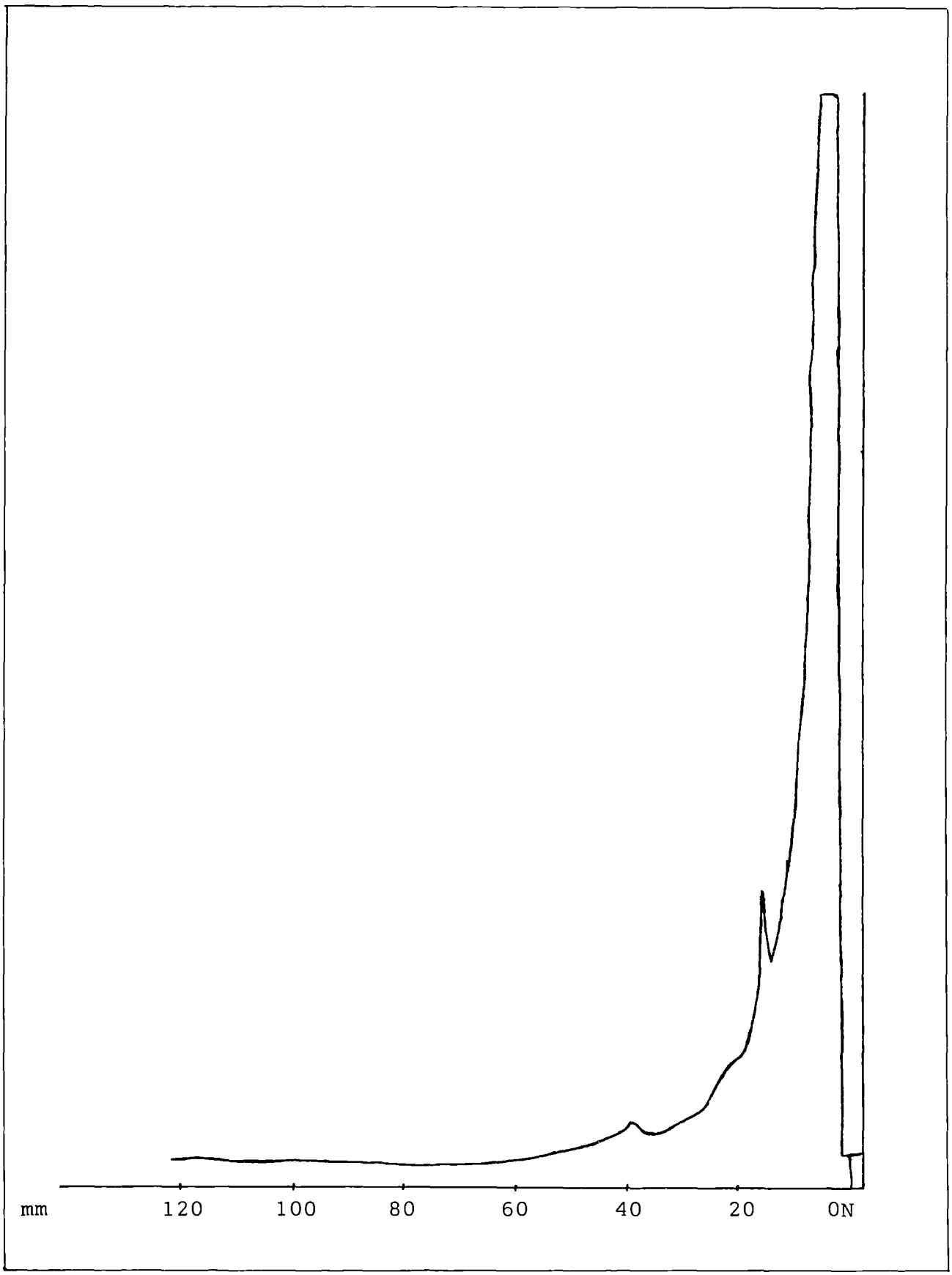


Figure 4.2.13 GC-ECD of tomato sample (without heptenophos) under the conditions as described in Section 4.2.6.1

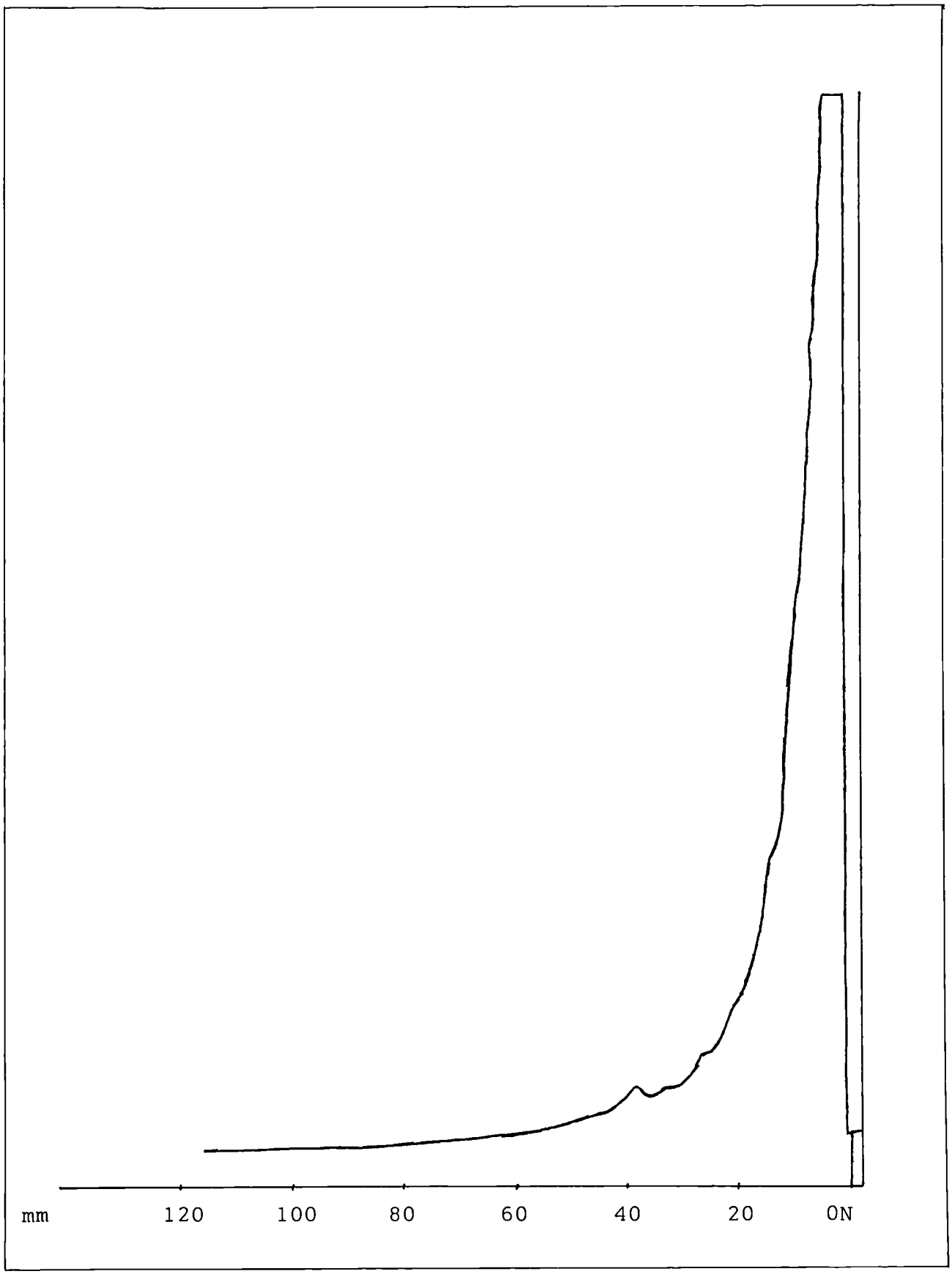


Figure 4.2.14 GC-ECD of lettuce sample (without heptenophos) under the conditions as described in Section 4.2.6.1

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CHAPTER 5

General Discussion

GENERAL DISCUSSION

5.1 The application of GC-MS

5.1.1 Introduction and principles

Estimation of quantities of substances by mass spectrometry is not straight forward because mass spectrometric measurements are not exactly reproducible. The detector response (for a sample) depends on several parameters that are difficult or impossible to control, including the condition, temperature and pressure of the ion source and the condition of the detector.

To obtain maximum response and accurate quantification for GC-MS all operational parameters must be optimised. This requires the consideration of both chromatographic performance and mass spectrometer sensitivity, and quality of mass spectra.

In this project for the application of GC-MS the following operational parameters were investigated, and the optimal conditions are described in Chapter 3, Section 3.2.6, 3.2.7, and 3.2.8.

1. Optimisation of electrometer zero
2. Electron multiplier voltage optimisation
3. Optimisation of selected mass interval
4. Optimisation of the quadrupole GC-MS
5. Column performance and selection for GC-MS analysis

5.1.2 Qualitative aspects of analysis

Non-polar capillary columns such as DB5 provide a good resolution and peak shape for most organo-phosphorus compounds. This column also provide an efficient separation for most of op pesticides.

By employing GC-MS, it is possible to extract quantitative information via mass chromatogram analysis at selected m/z values, without the necessity of the clean-up step (see Chapter 3, Section 3.2.4). The ability of the GC-MS to operate efficiently using direct coupling is of particular importance for analysis of environmental components where 100% sample transfer is required.

5.1.2.1 Quantitative selected ion monitoring

The mass spectrometer can be operated as a very sensitive, mass selective chromatographic detector. The mass spectrometer can be tuned to monitor a single ion or to switch at high frequency between several selected m/z values. Selectivity is obtained if the mass is a substance-characteristic parameter. Specificity is increased when several m/z values are selected because the probability of two different compounds having the same retention time (co-eluting particularly with HRGC) and the same characteristic mass ion ratios is very small. High sensitivity is obtained because the dwell time for each ion is increased compared to the normal scan mode (full scan mode).

The sensitivity of selected ion monitoring is increased by the order of 100 times compared to the full scan mode, providing detection limits as low as 10^{-13} g. Selected ion monitoring is now a widely used technique in the areas of environmental [1-4] and biomedical analysis [5-9]. The advent of relatively inexpensive quadrupole mass analyser for gas chromatography has enabled the greater capability of the selected ion monitoring technique in many laboratories. Furthermore, selected ion monitoring allows the use of stable isotope substances as internal standards [3,4,10]. These analogous can be added to the sample at an early stage to correct for losses at all stages of sample work up and analysis [11].

5.1.3 Quantitative aspects of analysis

Two approaches are usually used in quantitative analyses, namely external and internal standard methods. The external standard method is usually used in quantitative analysis

employing conventional GC. Great care should be taken to ensure the consistent injection of precisely equivalent volumes of sample and in reduction of the final volume to fixed (1 or 5) ml. However, this approach was not demonstrated to be reproducible in GC-MS analysis for which the MS acts as a detector because of the difficulty in maintaining optimum and linear sensitivity. The external standard was shown to be only reproducible for short periods of analyses, e.g. in the period of one working day. When the analyses were extended to longer periods, say 1-2 weeks, the correlation coefficient of the calibration graph obtained is very poor. Unless great care is taken to ensure that the GC-MS is working at a linear and optimum sensitivity, this method will produce a low precision and reproducibility of data.

On the other hand, application of internal standard methods can improve reproducibility of the quantitative data since this approach is clearly not influenced by the changing sensitivity of the MS but it is rather dependent on the stability of MS to consistently monitor mass ions in the range acquired. This technique still provides good results even when the MS is not working at its optimum sensitivity. It is also interesting to note that in the construction of the internal standardisation calibration graph a plot of the area of the mass chromatogram for a selected ion (representing the sensitivity) against the concentration ratio is preferred rather than a plot versus concentration of analyte. However, it is recommended that the same concentrations of internal standards are maintained for construction of the calibration graph.

In GC-MS analysis, two methods of data manipulation during the analysis can be carried out, namely full scan mode (40 to 450 amu), and SIM (at selected m/z value). SIM provides the most sensitive method, while the full scan mode provides the most useful qualitative information. Mass chromatogram data analysis during the post-analysis of a GC-MS run demonstrates a good reproducibility compared to an approach using RIC data analysis. Application of the area of RIC for quantification is susceptible to error because of chromatographic peak heterogeneity. The advantages of this method can be demonstrated in the quantification of organophosphorus employing d_8-naphthalene, d_{10}-anthracene and selected op pesticides as internal standards. The quantification can be

performed using mass chromatogram analysis as described in Chapter 3, Section 3.2.3-5.

SIM is usually employed in trace analysis of environmental samples. In this technique, practically up to 18 mass ions can be monitored in general. The less the number of ions monitored the more sensitive is the response that can be obtained. For qualitative work, SIM is too restrictive because it is designed for identification and quantification of target compounds only. Thus, for comprehensive qualitative work, the analysis should be carried out using the full scan mode. If the sensitivity using full scan mode is not adequate enough for qualitative purposes, the sample can be concentrated to increase the sensitivity.

In the SIM technique, however, it is necessary to have fore-knowledge of exactly what is to be quantified. Thus, the analyst should know the expected retention time of components to be quantified. In this thesis analyses of op pesticides in fruit and vegetable samples were carried out by monitoring the characteristic ions as described in Section 3.2.3. This approach provided a detection limit of malathion, pirimiphos-methyl, heptenophos and dimethoate are 1×10^{-13}, 0.75×10^{-13}, 1.5×10^{-13} and 3×10^{-13} gram per injection respectively.

5.1.4 Analysis of organophosphorus pesticides

There is a wide application of packed column GC with various detectors for the analysis of organophosphorus pesticides [9,12-17]. There are no reports on the use of GC-MS-SIM for identification and quantification of malathion, pirimiphos-methyl, dimethoate and heptenophos pesticides using high performance capillary column GC.

In this thesis, with the application of capillary GC-EIMS-SIM good separation and good peak shape was achieved even at high ramp rate (10-15 degC min^{-1}).

The usefulness of SIM can be demonstrated in analysis of pesticides using at least two internal standards. This technique is considered for the identification and quantification

of pesticides present in real vegetable and fruit, without the necessity of a clean-up step (data reported in Chapter 3). Thus, analysis time can be saved and the use of small volume of solvent, minimise the loss of pesticides during concentration. The whole preparation of sample, i.e. extraction to concentration stages, requires only two hours to complete. The recovery from spiked samples are between 91 and 101%. However, the recoveries of these four components are reasonable by using SIM. The results for these recovery are presented in Table 3.15 and 3.16 in Chapter 3.

5.1.5 Conclusion

Mass spectrometric analytical procedures are in constant state of development and refinement for the analysis of particular groups of compounds, and improvements in precision and limit of detection are continually being sought.

In the food contamination area, particularly for pesticides analysis at the present time, advantage should be taken of the widespread availability of stable isotope materials as internal standards.

In view of the advantages of quantitative mass spectrometry in the analysis of pesticide residues, much more work on method development would appear to be warranted.

5.2 The application of GC-ECD

5.2.1 Introduction and principles

Although efficient sample introduction devices, sensitive and selective detectors, high speed recorder, very good electronically-controlled ovens and other devices are essential components in modern high resolution GC systems, the column remains the heart of the analytical instrument. The quality of any separation cannot be any better than the column itself. The widespread use of capillary columns in GC has paralleled the development of column technology to realise the full potential available for the resolution of complex

mixtures into individual components. The preparation of a highly efficient, well de-activated and thermally stable column is imperative.

To achieve a good separation efficiency with a column, a uniform and homogeneous film of stationary phase must be applied to the column. Furthermore, this thin film must maintain and not rearrange to form droplets as the temperature is varied.

To obtain a maximum response from a column, the active surface sites must be modified by deactivation. When surfaces are deactivated they are rendered chemically inert to sample solutes, the original high energy surface is converted to a lower energy surface. Consequently, only non polar stationary phases can be coated easily on the de-activated surface. As the stationary phase polarity increases, so it is more difficult to coat the column.

Open tubular column GC overcomes five major difficulties of conventional packed columns, namely: surface adsorption; catalytic decomposition; limited column resolving power; peak broadening, and high stationary phase bleed rates. However, because of the low column sample capacity and the complexity of sampling techniques, quantitative analysis using open tubular column is not practised routinely in many laboratories.

Gas chromatography is an analytical method which includes the separation, identification, and quantitative determination of the individual compounds in the mixture analyzed. The applications of gas chromatography have reached the stage where information on the character of the individual components cannot be based on the interpretation of retention characteristics alone [18], a selective detector ignores unwanted components in a mixture and only responds to those which are of interest [19,20]. This is why a considerable changeover to the use of more selective rather than non-selective detectors can be seen.

In this part of the project for the application of GC-ECD the following operational parameters were investigated and the optimal conditions were described in Chapter 4.

1. Selection of temperature for the column used
2. Selection of temperature for the detector
3. Selection of flow rate for the column used

5.2.2 Qualitative aspects of analysis

Slightly polar wide-bore capillary columns coated with the stationary phase such as BP10, provide reasonable resolution and peak shape for organophosphorus compounds. This column also provides an efficient separation for most pesticides.

It has been clearly demonstrated that, under the condition of our experiments, the peak area response factors for pesticides vary with column and detector temperature, while this effect has not been reported previously in the pesticides analysis literature. It must be a common occurrence considering the frequency of use of non-polar and slightly polar type stationary phases, and should, therefore, be taken into account. The analysis of pesticides should be carried out under optimised column conditions and detector temperatures in order to obtain optimal area responses.

The adsorption of the pesticides by the support and/or stationary phase in packed columns is higher than for the wide-bore fused silica capillary column and is the reverse for the catalytic decomposition.

Gosselin [21] and Pellizzari [22] have reported that the bleed of the stationary phase can adversely affect the sensitivity of the ECD especially if the ECD happens to respond well to this function at elevated temperatures.

The organophosphorus compounds react with silicon compounds to give - P - O - Si compounds [23,24]. The $-\overset{\overset{\|}{P}}{\underset{|}{}}-O-Si-$ compounds are much more common than the $-\overset{\overset{|}{P}}{\underset{|}{}}-O-Si-$ compounds.

The organophosporus pesticides can also be oxidised and the sulfur atom of thiophosphoryl and thioether groups are replaced with oxygen [25]. Isomerisation can also occur at elevated temperatures [25].

5.2.3 Quantitative aspect of analysis

Gas chromatography with selective detectors is the analytical method generally used for determination of pesticide residues in food, fruit and vegetables [12,16,26]. A major problem arises from the great number of compounds to be analyzed which has increased to the point where it is almost impossible to separate the components in a single chromatogram. This situation applies even to high performance capillary columns. Measurements of retention time and peak area on one single column is definitely not sufficient for identification and quantification of a pesticide. It, therefore, becomes necessary to re-run the sample on two columns having a different polarity of stationary phase.

Several fundamental problems and practical considerations, such as sampling mode selection and technique, column stability, injector reproducibility, column temperature and gas flow stability, detector sensitivity, stability and linearity, are all important in GC quantitative analysis.

5.2.4 Analysis of organophosphorus pesticides

One of the most frequently performed determinations in environmental chemistry is gas chromatographic (GC) analysis of pesticides occurring as trace residues in various substrates. There are no reports of the use of GC-ECD for quantification of malathion, pirimiphos-methyl, dimethoate and heptenophos (ops), using packed column (5% SE30) and wide-bore capillary column for analysis.

The application of wide-bore capillary GC-ECD was found to be suitable here and reasonable separation and good peak shape was achieved under isothermal condition.

Electron capture detection can be used in analysis of pesticides using at least one internal standard. This detector is considered for the identification and quantification of real fruit and vegetable samples, without the necessity for a clean-up step (except for heptenophos and dimethoate in carrot). The data are presented in Chapter 3.

Quantification of the extracts analyzed by GC-ECD and GC-MS gave results with an excellent correlation (see Section 5.3).

5.2.5 Conclusion

The screening for pesticide residues in foods should unquestionably be carried out using relatively inexpensive chromatographic techniques. The reliable data can be obtained by an experienced analyst using gas chromatographic (GC) retention times on different stationary phases combined with information concerning the responses from various specific GC detectors such as electron capture. However, the difficulties of quantification of individual members of complex pesticide mixtures present at low level in often intractable food matrices should not be underestimated.

It is concluded here that analyses of pesticides should be carried out under optimised column and detector temperatures in order to obtain the full benefit of the optimum sensitivity of the ECD and it is preferable to work with non-electron affinic stationary phases.

5.3 Comparison of the GC-ECD and the GC-MS methods

Pesticide samples were investigated by both methods, and the peak areas and peak heights of organophosphorus were determined. The amounts of op compound present in the samples were estimated from the calibration graphs.

The GC-ECD results plotted against the GC-MS results are presented in Figure 5.1-8*. GC-MS recovery is higher than GC-ECD for malathion, pirimiphos-methyl and

dimethoate samples, but it is the inverse for heptenophos samples.

The two quantitative procedures of GC-MS and GC-ECD subsequent to solvent extraction gave results in excellent agreement.

It should be noted, however, that solvent extraction of dimethoate and heptenophos in carrot samples shows interference with determination by GC-ECD and necessitated a prior clean-up of the extract. No such interference problem arises via GC-MS determination because of the selectivity of the mass spectrometer as a detector.

5.3.1 Conclusion

Quantitation of the extract by GC-ECD and GC-MS gave results in excellent agreement. However, the selectivity of the detector involved in the GC-MS techniques renders it superior when contaminants such as dimethoate are present in carrot samples.

A combination of solvent extraction (without clean-up step) and GC-ECD determination may thus be used for samples where no contaminants are co-extracted.

* m = malathion
d = dimethoate
p = pirimiphos methyl
h = heptenophos

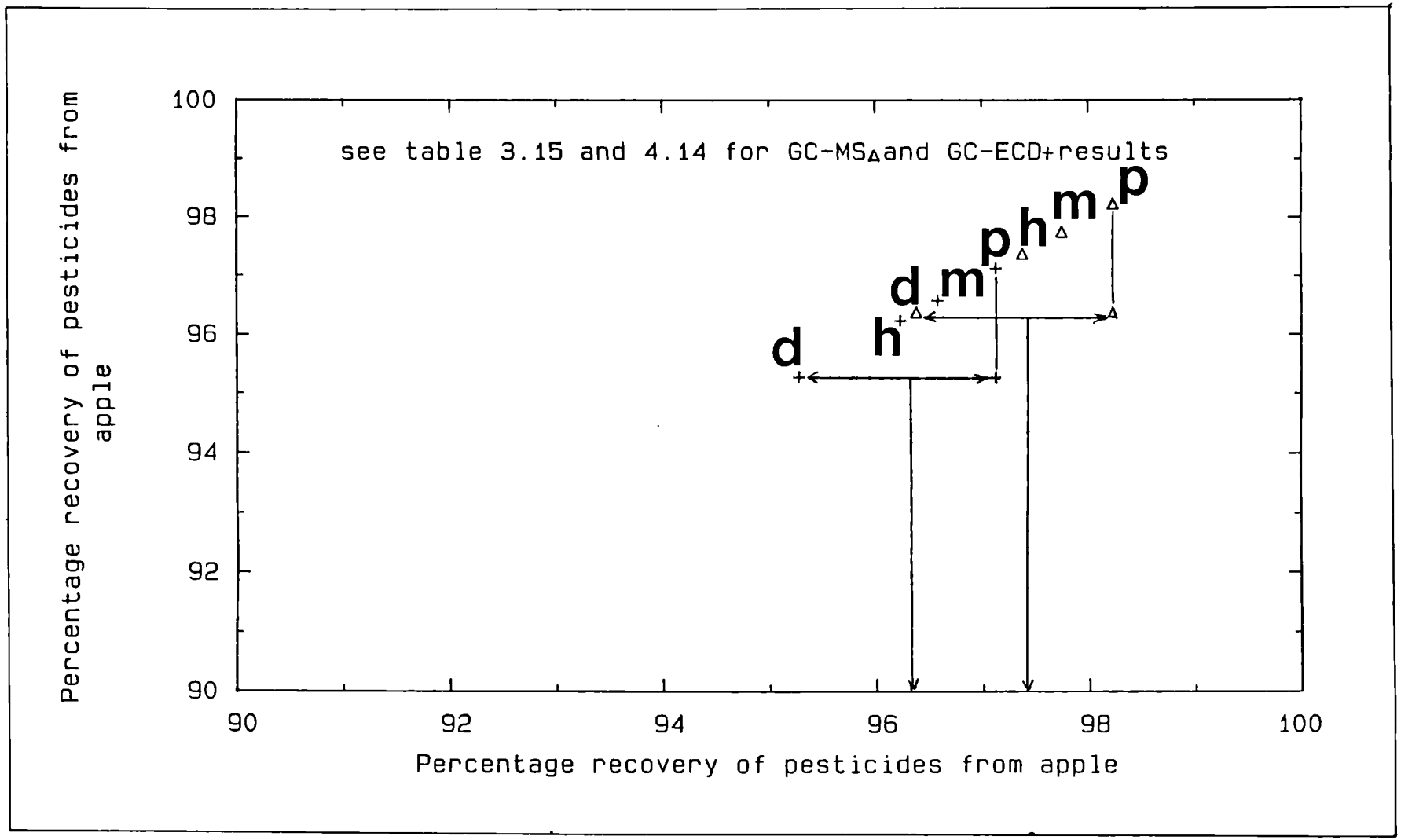

Figure 5.1 GC-MS results plotted against GC-ECD results for op pesticides sample (2mg/kg)

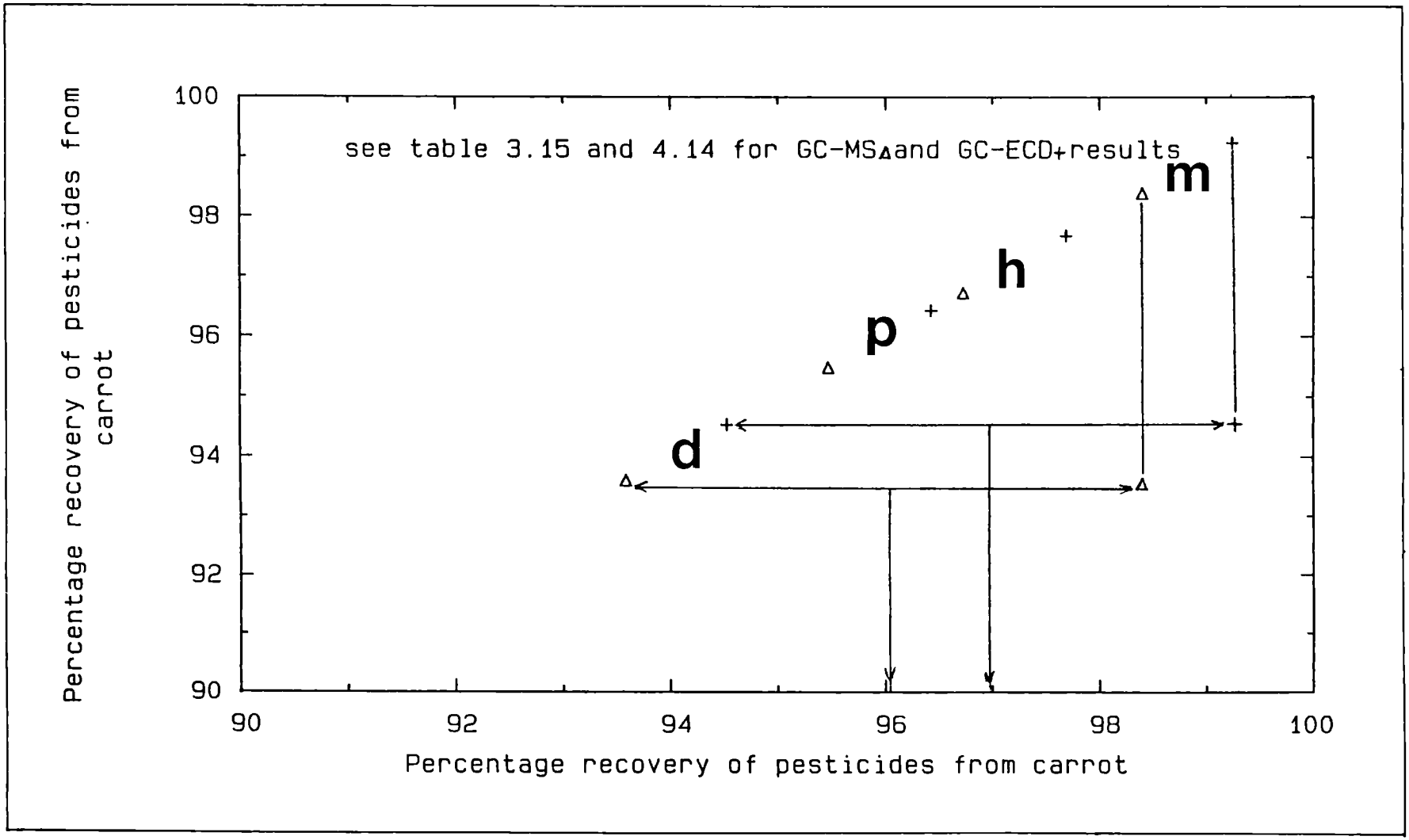

Figure 5.2 GC-MS results plotted against GC-ECD results for op pesticides sample (2mg/kg)

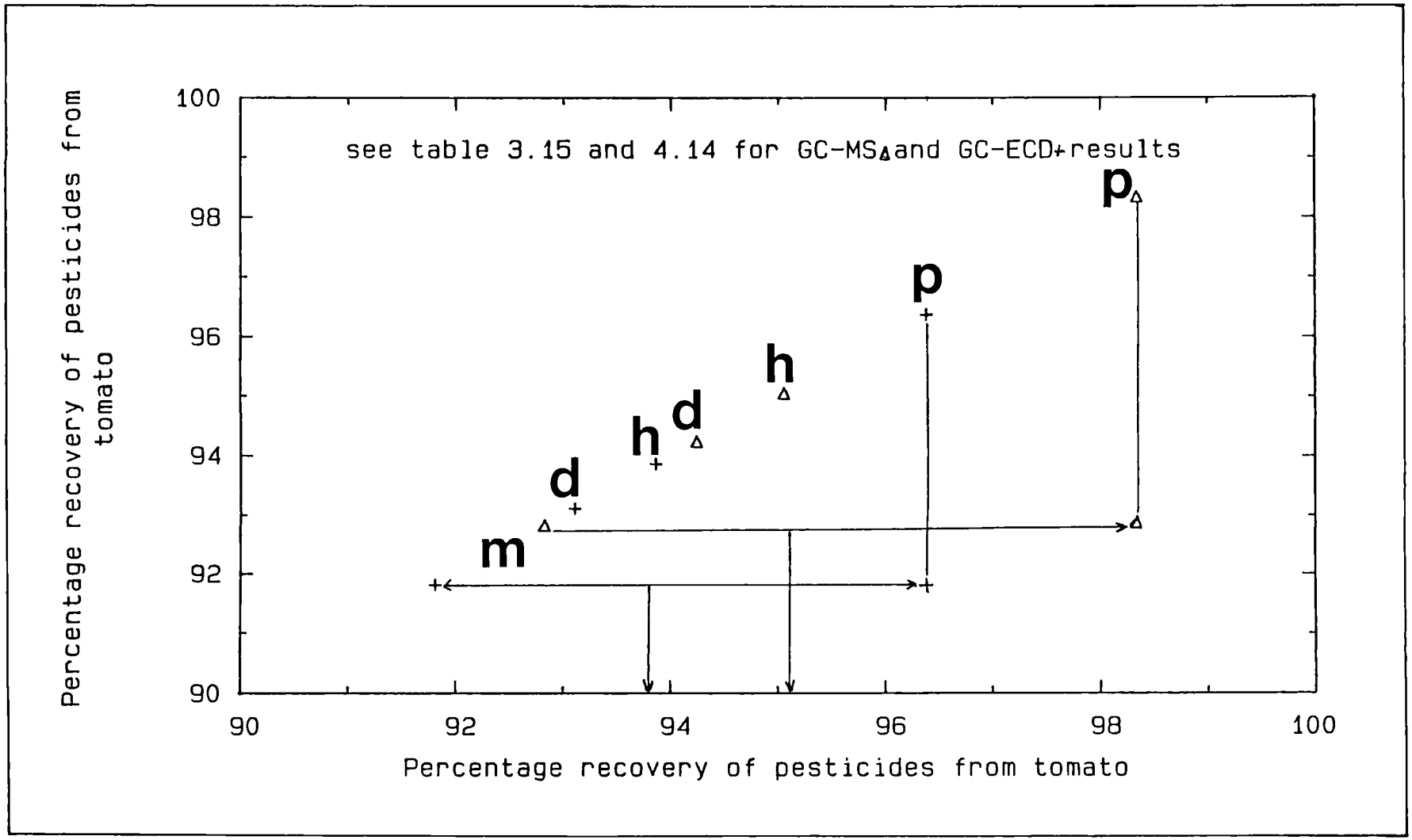

Figure 5.3 GC-MS results plotted against GC-ECD results for op pesticides sample (2mg/kg)

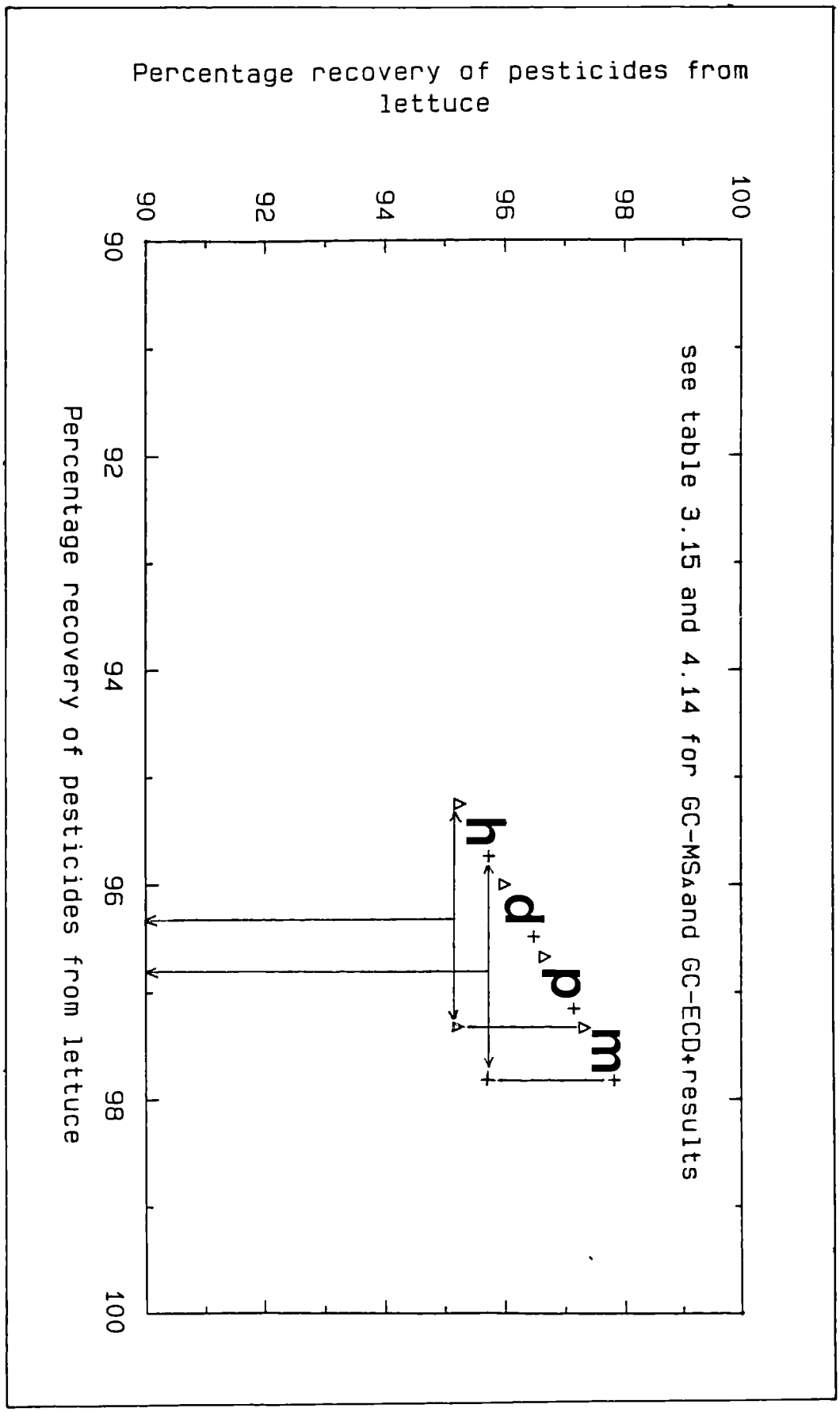

Figure 5.4 GC-MS results plotted against GC-ECD results for op pesticides sample (2mg/kg)

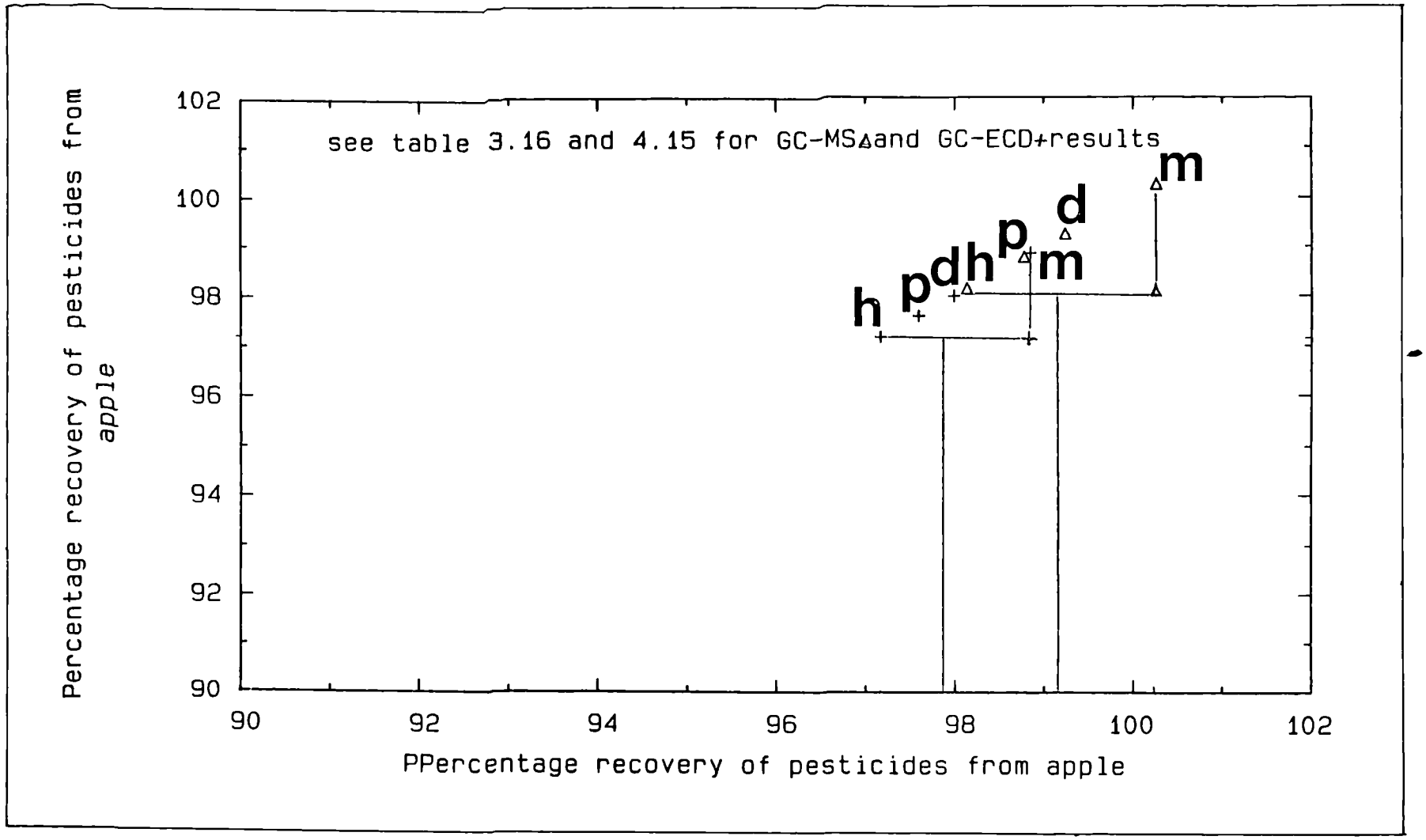

Figure 5.5 GC-MS results plotted again st GC-ECD results for op pesticides sample (0.2mg/kg)

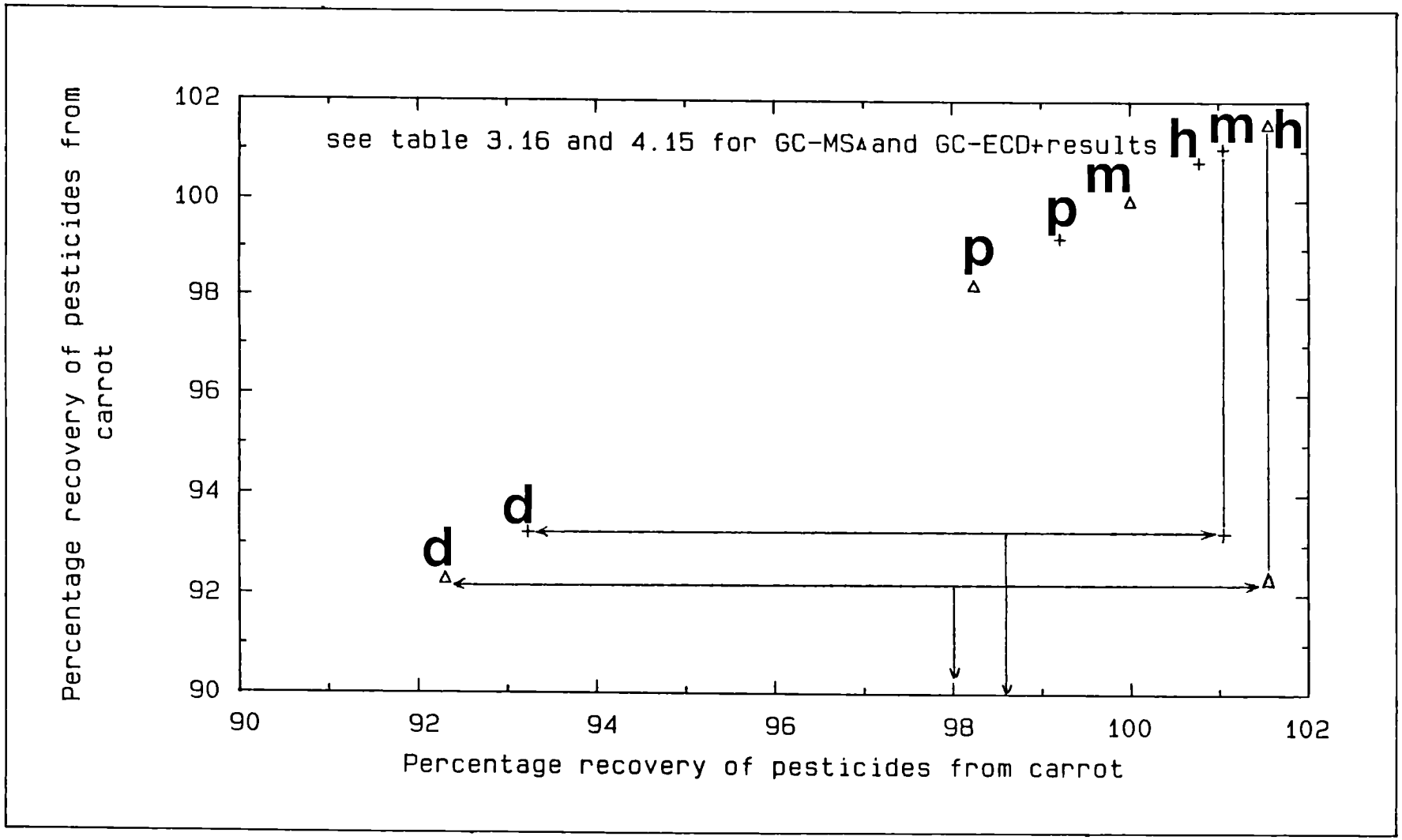

Figure 5.6 GC-MS results plotted against GC-ECD results for op pesticides sample (0.2mg/kg)

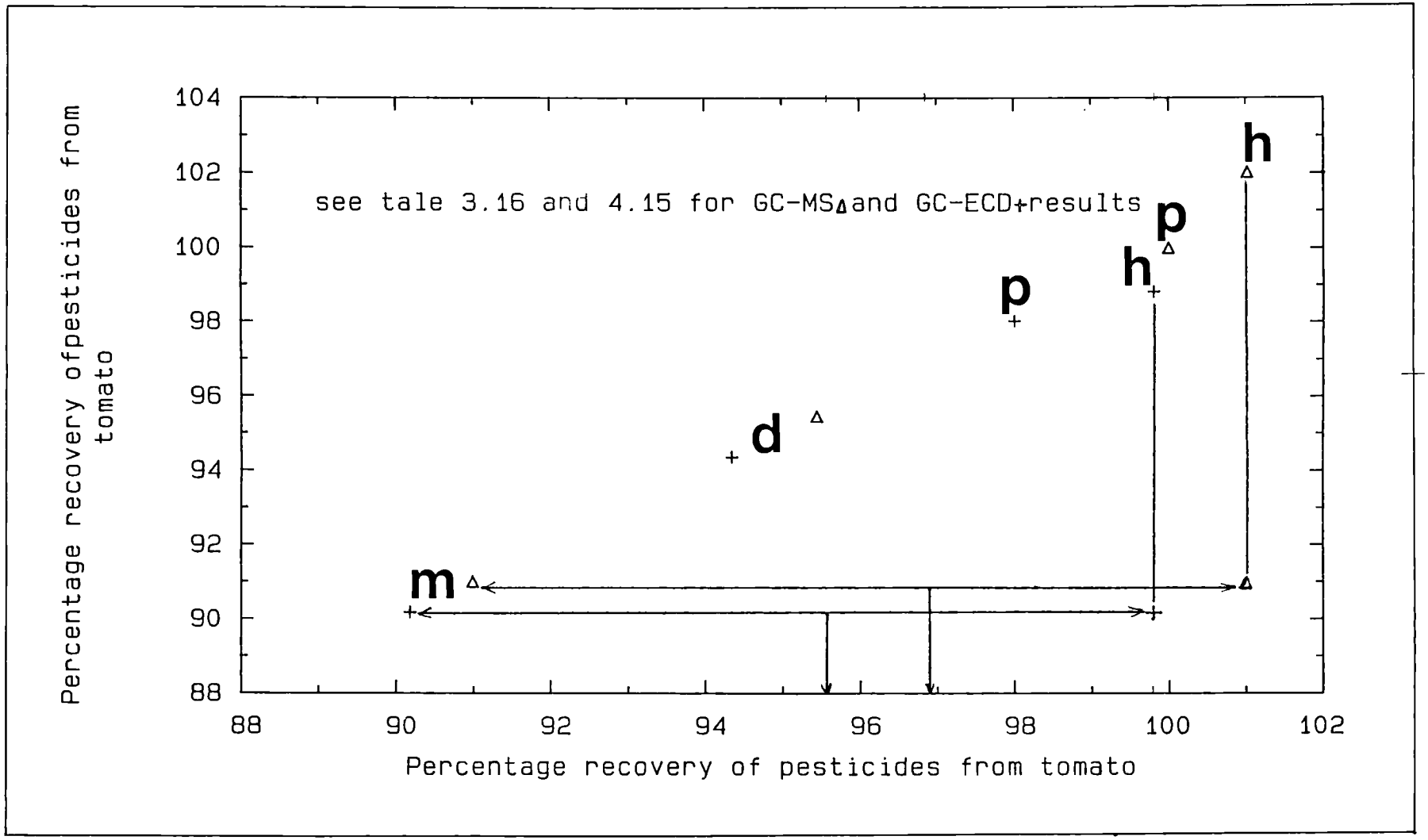

Figure 5.7 GC-MS results plotted against GC-ECD results for op pesticides sample (0.2mg/kg)

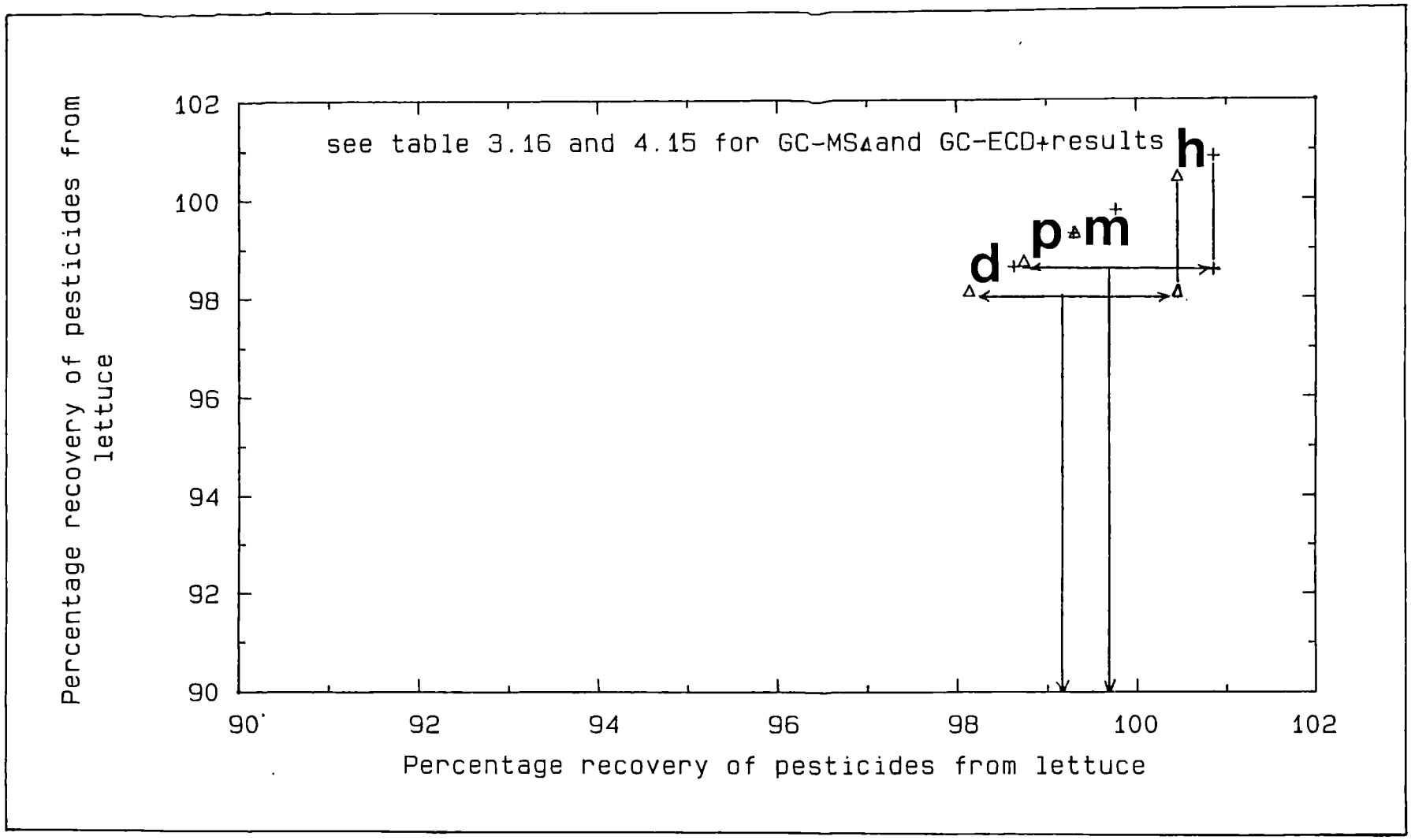

Figure 5.8 GC-MS results plotted against GC-ECD results for op pesticides sample (0.2mg/kg)

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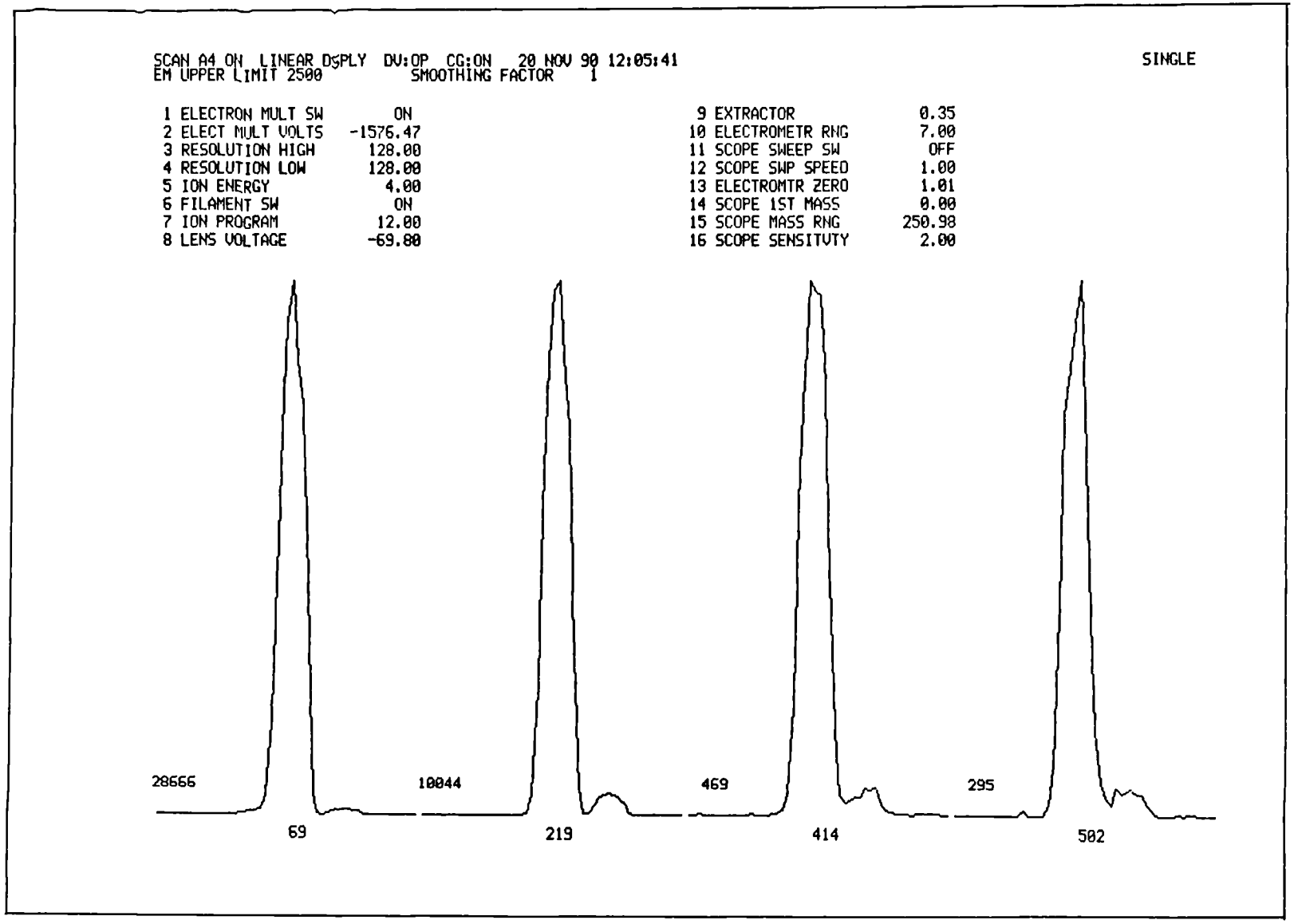
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7 ION PROGRAM 12.00
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12 SCOPE SWP SPEED 1.00
13 ELECTROMTR ZERO 1.01
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Figure A.3.1

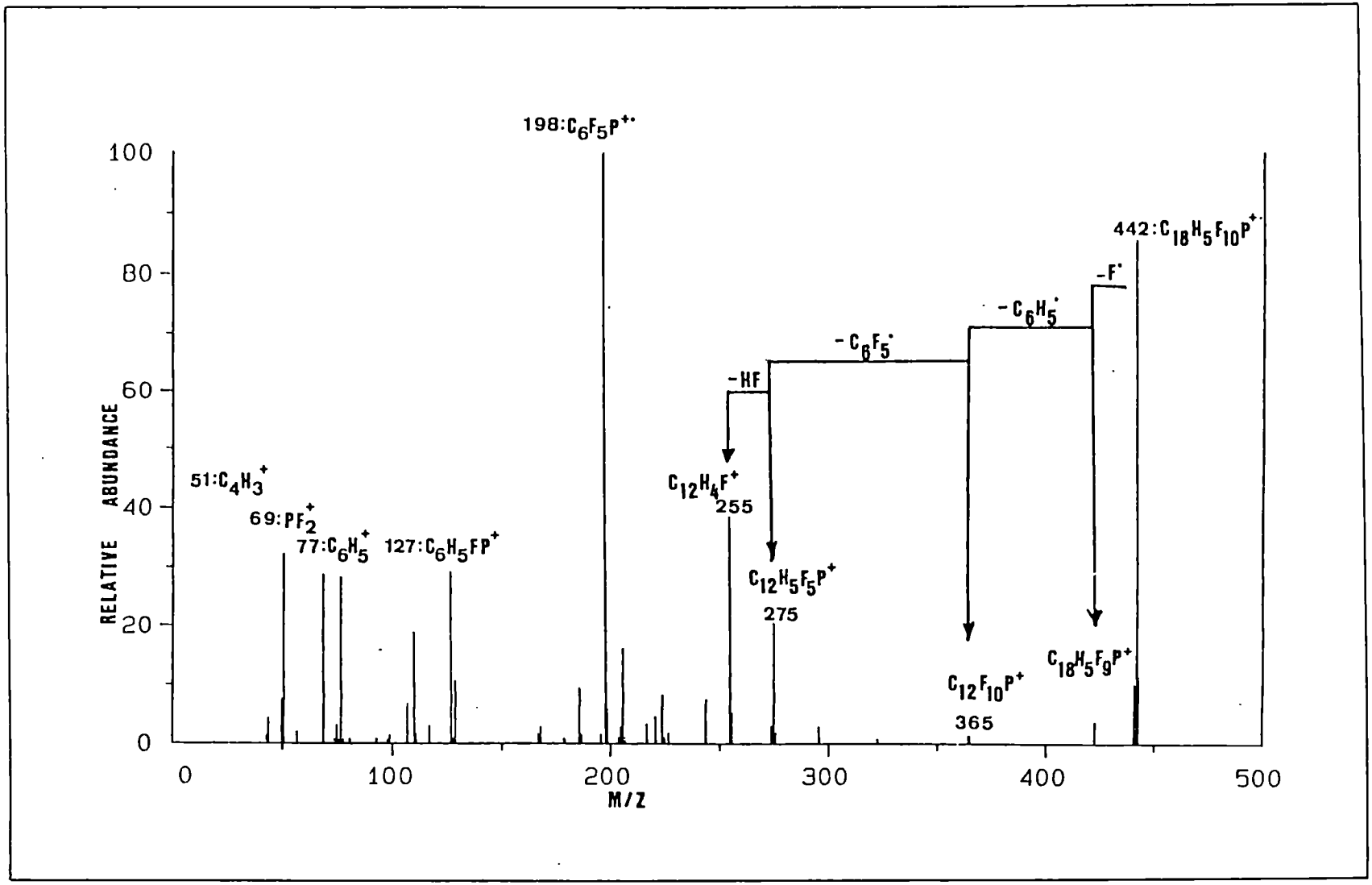

Figure A.3.2 EI mass spectrometry of methyl stearate

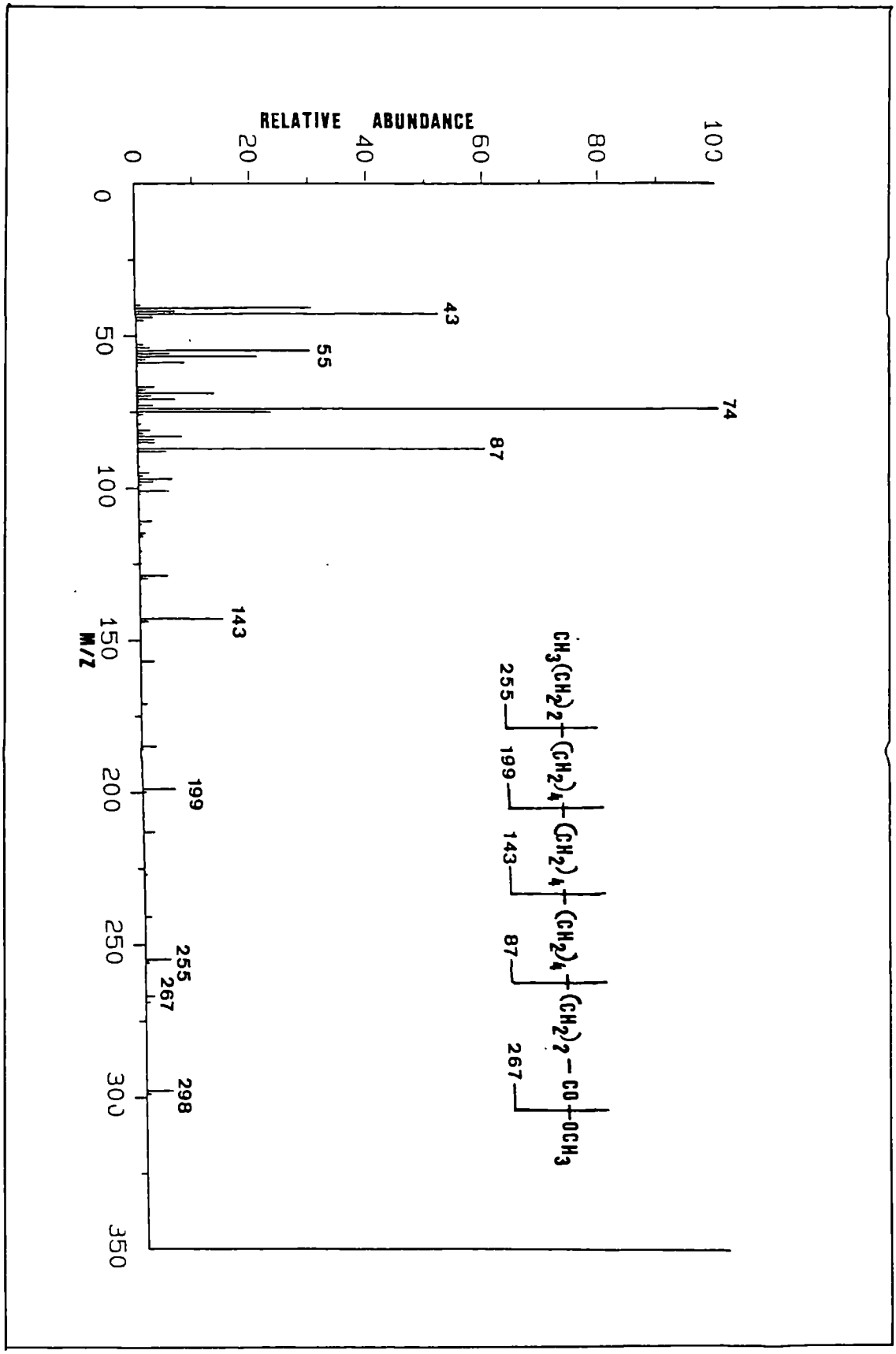

Figure A.3.3 EI mass spectrometry of DFTPP