

## Multiresidue Analysis of Pesticides in Vegetables using Liquid Chromatography with Atmospheric Pressure Ionisation Mass Spectrometry (LC-API-MS) and a Heated Nebuliser

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**Abstract:** A multi-residue analytical method using liquid chromatography/atmospheric pressure ionization-mass spectrometry (LC-API-MS) and a heated nebuliser with positive and negative chemical ionization (PCI/NCI) modes of operation was developed for the trace determination of selected pesticides in vegetables. The pesticide residues are extracted with 5% ethyl alcohol in ethyl acetate (v/v) and cleaned up with 100 mL acetonitrile. The extracts were then concentrated and injected into the LC-MS system. Both pseudo-molecular ion  $[M+H]^+$  / $[M-H]^-$  or the characteristic fragment ion of the pesticides were monitored under PCI or NCI using Selected Ion Monitoring (SIM) mode for identification and quantification. The heated nebuliser probe temperature and orifice voltages were optimized to achieve identification capabilities with the highest sensitivity. Detection limits were in the region of 1 – 3 pg and recoveries ranged from 70% to 110%.

**Abstrak:** Suatu kaedah analisis berbilang-baki yang menggunakan kromatografi cecair/spektrometri jisim- pengionan tekanan atmosfera dan sejenis penebula panas dengan cara pengionan kimia positif dan negatif adalah dimajukan untuk penentuan surih bagi beberapa racun perosak dalam sayur-sayuran. Baki racun perosak adalah diekstrakkan dengan 5% etil alkohol dalam etil asetat (v/v) dan dibersihkan dengan menggunakan sesuatu turus Florisil dan 100 ml asetonitril. Lepas itu, kesemua ekstrak adalah dipekatkan dan terus disuntik kepada alat LC-MS. Kedua-dua ion pseudo-molekul  $[M+H]^+$  / $[M-H]^-$  atau ion serpihan tertentu daripada racun perosak adalah dirakamkan dengan pengionan kimia positif atau negatif (PCI/NCI) menggunakan mod perakaman ion tunggal (SIM) untuk identifikasi dan kuantifikasi. Suhu dan voltan orifis bagi penebula panas itu adalah dioptimumkan untuk mencapai kebolehan identifikasi yang paling tinggi kepekaan. Had pengesanan dalam julat 1 - 3 pg dan pencapaian semula bernilai 70% ke 110% adalah dilaporkan.

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### Introduction

In modern day agricultural practices, the use of pesticides provides unquestionable benefits by increasing the production of crops. However, it has the drawback of pesticide residues which remain on the vegetables, constituting potential health risks to consumers. This leads on the one hand to the establishment of legal directives to control their levels through the maximum residue levels (MRLs) and on the other to a continuous search for pesticides which are less persistent and less toxic for human beings. Thus this search has led to the extraordinary increase in the number of pesticides registered or recommended for use and the analytical difficulties encountered for their control. In Malaysia, the total number of registered pesticides for the periods 1993 -1995 and 1995 -1997 are 1810 and 1815 respectively. This situation has put pressure on regulatory agencies to increase the scope of analysis and number of samples analysed in their monitoring programs. Procedures were developed to detect as many pesticides as possible, reliably and rapidly in the most cost effective manner.

To monitor agricultural products that contain pesticide residue levels higher than the MRLs, each country has its own government agencies which can determine pesticide residues through two different but

complementary approaches : (i) regulatory monitoring focused on raw agricultural commodities which measures the levels in individual lots for determining compliance with legal tolerances, and (ii) the total diet study. The methods of Storherr *et al* [1], Carson *et al* [2], Mills *et al* [3] and Luke *et al* [4-5] are used to determine organophosphorus pesticides (OPP) levels in the total diet study.

Analytical instruments are needed to determine, quantify and confirm pesticide residues in vegetables for both research and regulatory purposes. Currently, the pesticides are generally analysed by spectrophotometry, thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and gas chromatography (GC) or gas chromatography-mass spectrometry (GC-MS). Previous reports have noted that the spectrophotometric [6-8] and TLC [9-11] methods are inferior to the other methods in terms of detection sensitivity and specificity. HPLC [12-15] is a well-known separation technique that has good operability because it can be performed at room temperature. However, HPLC methods using retention time and fixed wavelength data via conventional detectors, such as ultraviolet absorption and fluorescence, might not be sufficiently specific and selective to perform certain identification of the target

pesticides in complex matrices. A fluorescence detector could not be used in a multiresidue procedure because it would not be able to detect non-fluorescence pesticides.

Similarly, GC [16-22] is also an easy analytical method to use for compounds that are volatile and do not thermally decompose at high temperature. In recent years, the separation capability of this method has been improved remarkably by the use of the capillary column, and simultaneous measurement of many different types and classes of pesticides has become possible when equipped with selective and sensitive detection methods such as electron-capture detection (ECD), nitrogen-phosphorus detection (NPD), and flame-photometric detection (FPD). However, GC is performed under high column temperature and has the disadvantage of decomposition of thermally unstable compounds [23] and a derivatisation reaction to convert the substance for measurement into a less polar compound is required in the analysis of a highly polar compound [24-25], which requires time consuming and complicated pre-treatment of the sample. GC-MS [24, 26-30] shows high reliability for both quantitative and qualitative analyses because of the characteristic peaks of compounds in the mass spectra.

Fillion *et al* [31] described a rapid and efficient multiresidue method for analysis of 189 pesticides in fruits and vegetables by GC-MS. However, it has the same problems encountered with GC analyses. For such compounds, liquid chromatography mass spectrometry (LC-MS), a comparatively new analytical method has been developed to overcome the above problems of GC, HPLC and GC-MS. It is recognized as an improved analytical method, which combines HPLC and MS in the measurement of thermally unstable, highly polar or non-volatile substances. Although the first coupling of LC and MS was reported about 20 years ago, its use for environmental monitoring is still in its infancy. This is so because of its complexity, lack of ruggedness, limited sensitivity and the technical difficulties in interfacing the solvent flow rate with the high vacuum of a mass spectrometer source. As a result, it limited the widespread use of LC-MS in method development during the early years. Subsequently the development of various types of ionisation interface such as moving belt [32,33], silicone membrane separator [34], direct liquid introduction (DLI)[35], thermospray (TSP)[36-38] and atmospheric pressure ionisation (API)[39-42] were utilised to introduce the effluent from the LC into the MS. Slobodnik *et al* [43] has published a review on the use of LC-MS technique for polar pesticides covering both the principles of ionisation and applications of LC-MS with TSP, particle beam (PB) and API interfaces.

The present trends in the development of pesticide residue analysis are towards multiresidue methods with adequate recovery characteristic (over 80% as a rule, but not less than 70%), good reproducibility and has

low detection limits. These methods also permit the simultaneous determination of pesticides of different structural types such as organophosphorus(OP), organochlorine(OC), N-methylcarbamate(NMC), and phenylureas. It is obvious that multiresidue methods using GC, HPLC and GC-MS with specific detectors can only cover a limited range of pesticides [44-49] and therefore multiple injections of each sample into different instruments and dual column confirmation of positive analytes are required. The development of LC-API-MS system offers new opportunities for the determination of thermolabile and polar compounds. API is a term covering different principles of ionization [50-55] such as IonSpray and the heated nebuliser.

The heated nebuliser is the most common interface currently used on an API source system. It takes full advantage of the large gas and solvent vapour throughput tolerated by the API source and provides a wide range of reversed phase solvent compositions at flow rates between 0.2 - 2.0 ml/min. Pneumatic nebulisation of the total HPLC effluent into the atmospheric pressure ionisation interface eliminates the vacuum system problems generally associated with LC-MS interfacing. This interface uses a nebuliser and make-up gas flow ( $N_2$ ) to ionise the HPLC effluent. The spray travels through a large diameter quartz tube which is heated sufficiently to dry the vapour. Ionisation of solvent molecules is initiated by a corona discharge needle. The solvent ions which are formed produce the analyte ions by atmospheric pressure chemical ionisation (APCI) of the analyte. These ions are focused and declustered through a dry nitrogen curtain gas and are then passed through the orifice into a high vacuum analyser region of the mass spectrometer where they are mass analysed. Fig. 1 shows the basic components of the heated nebuliser LC-MS interface and the atmospheric pressure chemical ionisation (APCI) source [43]

The APCI process is a soft ionisation method typically generating only protonated molecular ions  $(M+H)^+$  in PCI mode or deprotonated molecular ions  $(M-H)^-$  in NCI mode, thus producing the molecular mass information which is considered the most important criterion for identification of the analyte. However, if structural information is desired for identification purposes, the problem can be overcome by the application of an appropriate voltage difference on two regions - the sample cone and the first skimmer of the API source generally varies the frequency and energy of collision of the analyte and drying gas molecules and thus induces fragmentation of the primarily formed ions, this mode of operation is termed pre-analyser collision induced dissociation (CID). A typical example of enhancement of structural information by changing the pre-analyser CID voltage is shown in Fig. 6.

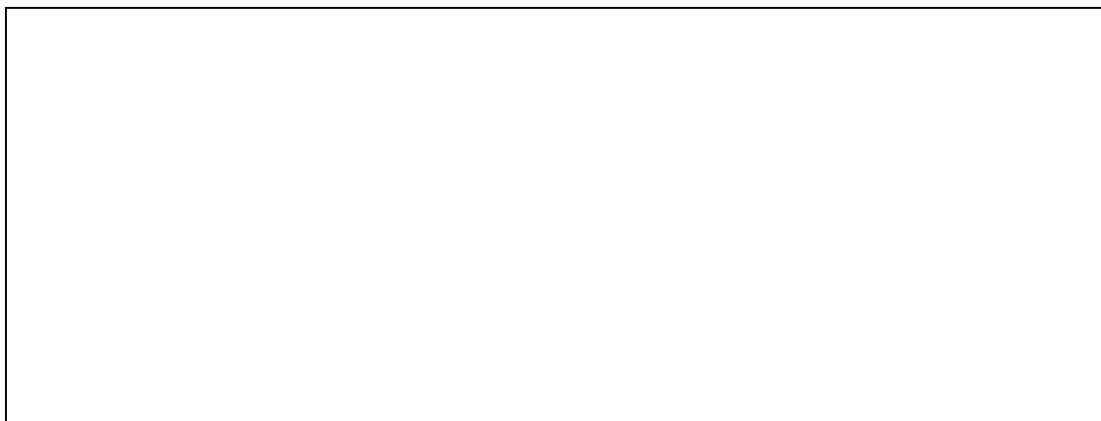


Fig. 1. The basic components of the heated nebuliser LC – MS interface and the atmospheric pressure chemical ionisation (APCI) source (43)

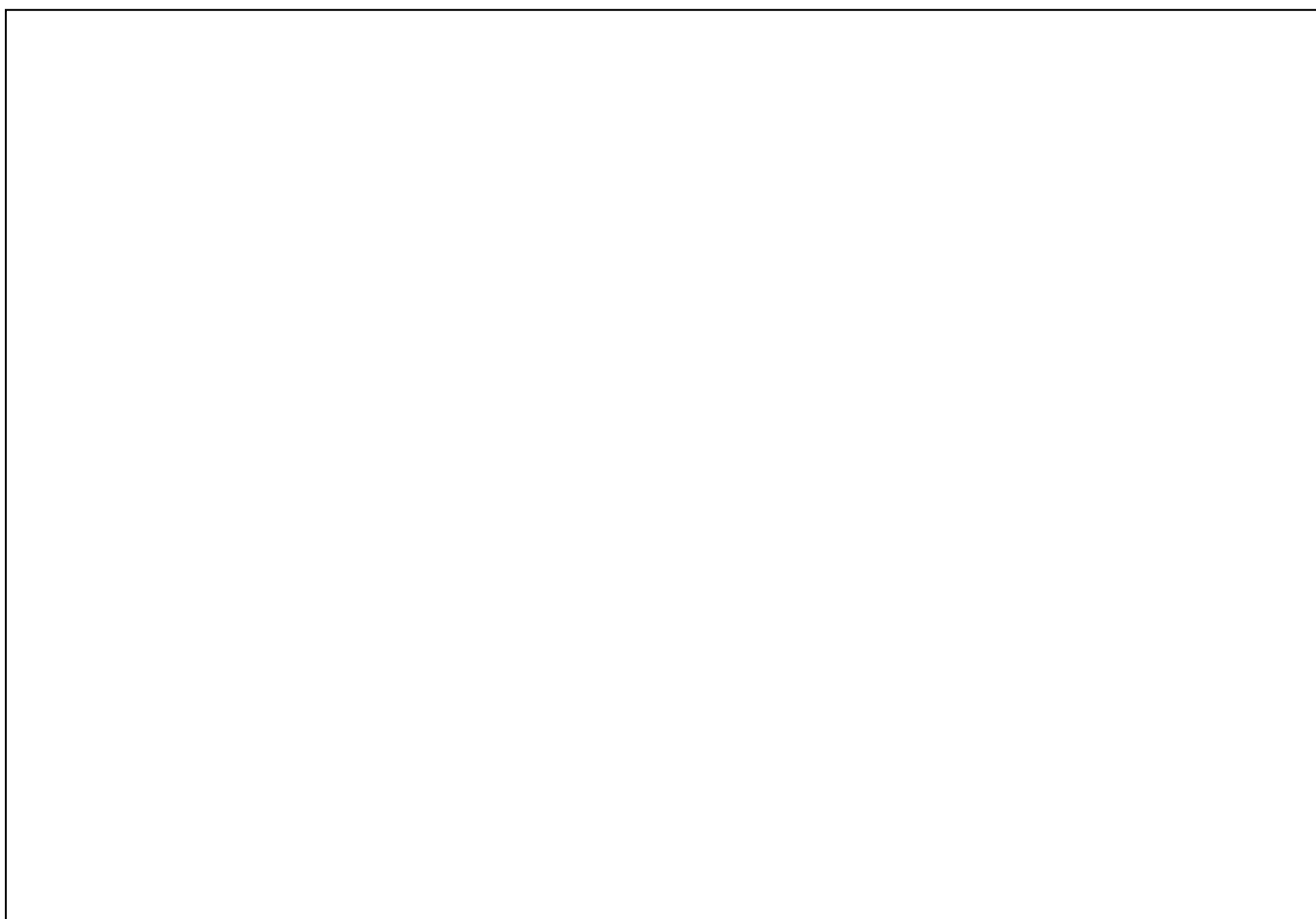


Fig. 2. The mass spectra of methamidophos at low orifice V = 20 volts (A) and high orifice V = 60 volts (B)

Giraud *et al* [56] reported that the selectivity of a single quadrupole MS can be considerably increased by monitoring two ions - the precursor ion derived from the intact molecule at low orifice voltage, the protonated molecular ion (M+H)<sup>+</sup> and its major fragment ion produced at high orifice voltage, thus allowing for the unambiguous pesticide identification and confirmation. Very few studies on the application of LC-MS with a heated nebuliser for pesticide analyses have been published. The potential of this technique for the analysis of some pesticides has been described by some workers [53,57,58]. Pleasance *et al* [57] used it for the determination of residues of carbamates in green peppers, Kawasaki *et al* applied this technique for the determination of organophosphorus pesticides [58] and carbamates [53] in human serum.

In this present study, we have further investigated the use of liquid chromatography coupled with atmospheric pressure ionization – mass spectrometry (LC-API-MS) and a heated nebuliser for the simultaneous determination of 11 different structural pesticides in a single run.

## Experimental

### Chemicals and Materials

All the solvents used were HPLC grade unless otherwise stated. Acetonitrile, methanol, petroleum ether, diethyl ether, ethyl acetate and ethanol were purchased from J. T. Baker, a division of Mallinckrodt Baker, Inc. Phillipsburg, NJ 08865 USA and anhydrous sodium sulphate was purchased from Fluka. Ultra-pure water and methanol were filtered through a 0.45 µm filter purchased from Millipore. All the pesticide standards of purity ranging 98.9% to 100% in methanol solution (1.0 mg/ml) were purchased from AccuStandard Inc., New Haven CT, USA except the N-methylcarbamates (carbaryl, carbofuran, methiocarb) and diuron which were purchased from PolyScience, Niles, IL, USA.

The use of high purity reagents and solvents help to minimise interference problems. The standard stock solution of the mixture was prepared by diluting aliquots of the individual pesticide standard solutions with methanol. The calibration standard solutions were subsequently prepared by diluting the standard stock solution with methanol.

### Optimising the Heated Nebuliser Parameters

The possible parameters affecting the performance of the heated nebuliser set-up are the eluent flow rate, heated nebuliser probe temperature, orifice and focusing ring voltages. No fixed relation between the pesticides and the parameters can be observed. To optimise the performance, the pesticides were injected individually by flow injection and then the above-

mentioned parameters were adjusted to maximize the signal-to-noise (S/N) ratio for each pesticide.

### Samples

In order to evaluate the pesticide recoveries, some vegetables – watercress (*kangkong*) were obtained from a pesticide-free farm under study. The known volume of each standard stock solution was added to the blank control samples to obtain spiked control samples. Recoveries of pesticides were determined by comparison of the amount of each analyte extracted from the spiked samples with that of the standard calibration solutions.

### Sample Preparation

The sample preparation method is adopted from that of the Queensland Health Scientific Services, Australia with some slight modifications [59]. The spiked samples were extracted and cleaned up as follows:

The vegetable was finely chopped and homogenised with a blender. 30 g of the homogenised sample was accurately weighed and placed in a 500ml conical flask. Then 200 µL of the standard stock solution was spiked into the sample to provide the spiked control sample. Table 1 shows the concentrations of 11 pesticides in the standard stock solution.

COMPOUND	CONCENTRATION / ppm
Methamidophos	10
Dimethoate	30
Carbofuran	15
Carbaryl	30
Diuron	30
Methiocarb	50
Chlorothalonil	30
Ethyl-parathion	50
Diazinon	10
Profenofos	50
Chlorpyrifos	50

Table 1. The concentrations of 11 pesticides in the mixed standard stock solution.

The mixture was thoroughly mixed and then 150 ml of 5% v/v ethanol in ethyl acetate was added. After ultrasonication for 10 minutes, the supernatant liquid was filtered into a flask containing about 60 g sodium sulphate. After adding the plant tissue to the funnel, the extraction and filtration steps were repeated once more with 100 ml of the 5% v/v ethanol in ethyl acetate. The combined extract was again filtered into a round bottomed flask and was evaporated to just dryness with a vacuum rotary evaporator (40°C water bath). The concentrated extract was dissolved in 1 ml of acetonitrile and further cleaned up with Florisil.

Florisil column chromatography clean-up was used for separation of analytes from coextractive matrices by elution with 100 ml acetonitrile. The chromatographic column (30cm x 1 cm I.D.) was slurry packed with 4 g Florisil activated at 450 °C overnight, made into a 3% v/w water/Florisil with distilled water and stirred for 1 hour before used. Approximately 0.5 cm anhydrous sodium sulfate was placed at the top of the column to absorb any water in the sample or the solvent. The column was pre-eluted with 50 ml of acetonitrile. Just prior to the exposure of the sodium sulfate layer to the air, the washings from the flask containing the distilled off solvent was placed into the column and allowed to sink below the sodium sulfate layer. The sample in the Florisil column was eluted with 100 ml acetonitrile and the eluate was collected in a round bottom flask. It was evaporated to just dryness by rotary evaporator and then dissolved in 2 ml of methanol before it was injected into LC-API-MS system. All samples were prepared in duplicates.

### Liquid Chromatography - Atmospheric Pressure Ionisation-Mass Spectrometry (LC-API-MS) and a Heated Nebuliser

The analyses were performed on a Perkin Elmer LC-200 pump fitted with a Rheodyne 8125 20- $\mu$ L loop injector. The LC column was connected to a single quadrupole Perkin Elmer/Sciex API 100 LC-MS system equipped with a heated nebuliser interface. The separations were performed on a Techsphere 5 ODS 25 cm x 4.6 mm ID column, purchased from HPLC Technology Ltd., Wellington House, Waterloo Street West, Macclesfield Cheshire, UK. The separation was carried out starting with methanol-water at 60:40 (v/v) with isocratic elution for 5 minutes and then followed by a linear gradient elution to a final methanol-water at 100:0 (v/v) in 35 minutes. The flow rate was set at 0.8 ml/min and 1 $\mu$ L of the sample/calibration solution was injected onto the LC-MS system.

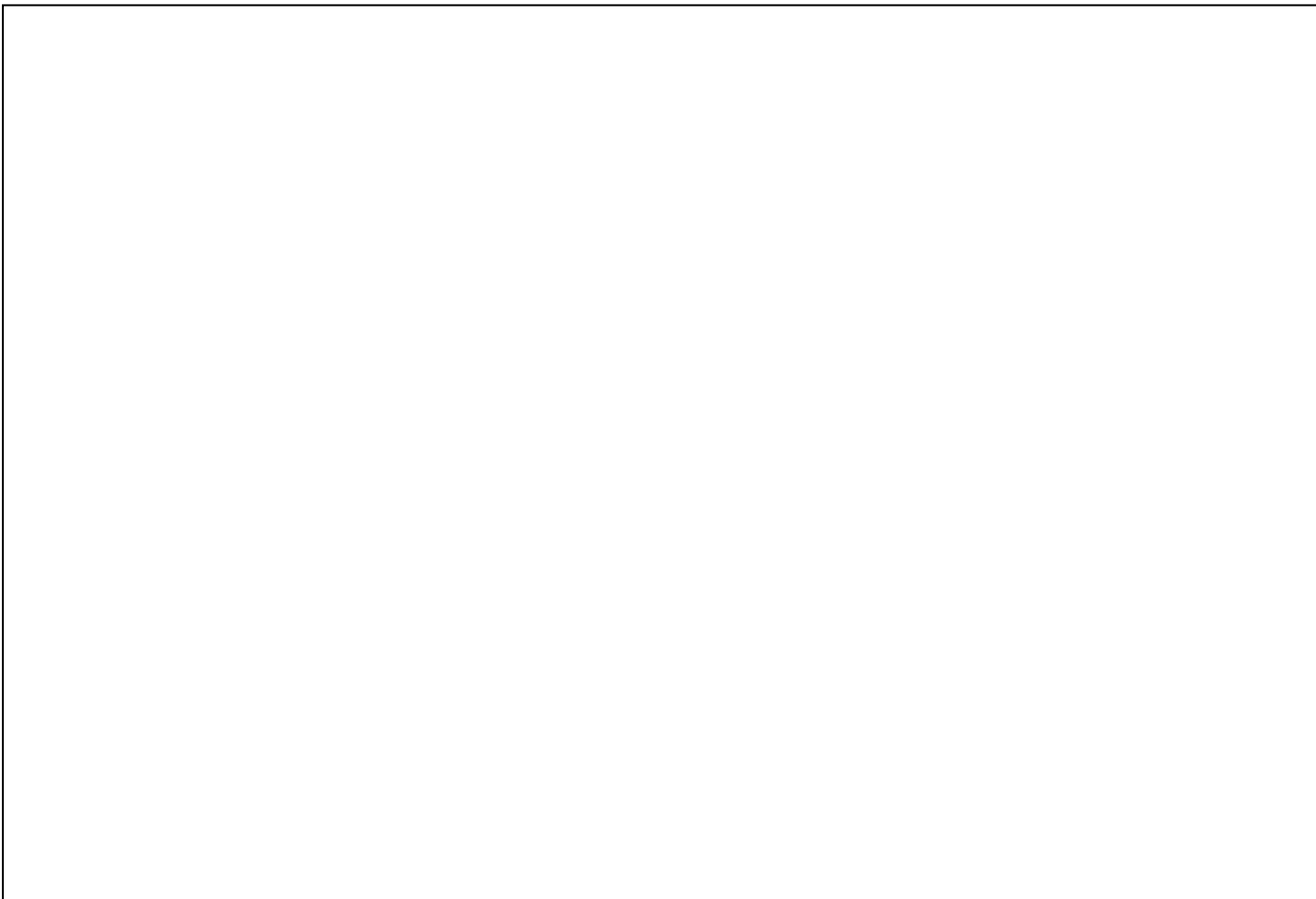


Fig. 3. The total ion chromatograms of the blank control sample (A) and spiked control sample (B) recorded under time-scheduled selected ion monitoring (SIM) conditions using an acquisition window for each pesticide. (1) methamidophos, (2) dimethoate, (3) carbofuran, (4) carbaryl, (5) iuron, (6) methiocarb, (7) chlorothalonil, (8) ethyl parathion, (9) diazinon, (10) profenofos, (11) chlorpyrifos

PERIOD	COMPOUND	MOL. WT	MONITORING ION M/Z	PCI/NCI MODE	ACQUISITION WINDOW (min)
1	Methamidophos	141.1	142.1[M+H] <sup>+</sup>	PCI	0 - 1.1
2	Dimethoate	229.0	230.1[M+H] <sup>+</sup>	PCI	1.1 - 4.4
3	Carbofuran	221.3	165.4[M+H-CONCH <sub>3</sub> ] <sup>+</sup>	PCI	4.4 - 6.7
4	Carbaryl	201.2	145.0[M+H-CONCH <sub>3</sub> ] <sup>+</sup>	PCI	6.7 - 10.0
5	Diuron	232.1	233.1[M+H] <sup>+</sup>	PCI	10.0 - 15.5
6	Methiocarb	225.0	169.3[M+H-CONCH <sub>3</sub> ] <sup>+</sup>	PCI	15.5 - 17.5
7	Chlorothalonil	266.0	245.0[M-Cl+O] <sup>-</sup>	NCI	17.5 - 22.0
8	Ethyl-Parathion	291.3	261.1[M-C <sub>2</sub> H <sub>5</sub> ] <sup>-</sup>	NCI	22.0 - 24.0
9	Diazinon	304.3	305.3[M+H] <sup>+</sup>	PCI	24.0 - 27.1
10	Profenofos	373.6	375.0[M+H] <sup>+</sup>	PCI	27.1 - 30.5
11	Chlorpyrifos	349.0	330.0[M-Cl+O] <sup>-</sup>	NCI	30.5 - 35.0

**Table 2. The periods and acquisition windows of the pesticides**

After each run the column was equilibrated for 5 minutes with the LC initial conditions. The chromatograms of the blank control sample and spiked control sample as shown in Fig.3 were recorded under time-scheduled selected ion monitoring (SIM) conditions using an acquisition window for each pesticide. Table 2 above shows the periods and the acquisition windows of the pesticides.

### Quantification

The instrument control and data processing utilities is based on the Macquan Software Version 1.5. The calibration graph of each pesticide was constructed using the calibration standard solutions. The above standard solutions were prepared by appropriate dilution of the stock solution. In the multiclass and multiresidue analysis of pesticides in vegetables, all pesticides were detected by the selected ion monitoring(SIM) mode on their respective [M+H]<sup>+</sup> or their characteristic fragment ions using the time-scheduled procedure and acquisition windows as shown in Table 2.

Compound	Recovery /%	% RSD	Corr. Coefficient	+ LOD /pg
Methamidophos	93.8	3.57	1.00	1.0
Dimethoate	82.0	5.11	1.00	1.0
Carbofuran	84.1	5.99	0.983	1.5
Carbaryl	106.8	4.09	0.993	1.5
Diuron	76.5	5.92	0.999	1.0
Methiocarb	88.9	5.10	0.995	2.5
Chlorothalonil	103.3	3.53	0.996	1.0
Ethyl parathion	92.3	3.15	0.977	1.5
Diazinon	77.4	2.34	0.999	1.0
Profenofos	78.6	3.30	0.997	1.5
Chlorpyrifos	74.4	2.14	0.999	1.5

**Table 5. The recoveries, % RSDs, correlation coefficients and LOD = limit of detection LODs of the 11 pesticides investigated.**

The recoveries, linearity of the method was examined on pesticide-free watercress (*Ipomoea aquatica*) samples. For each sample, the repeatability of each peak area measurement was evaluated from 5 consecutive injections. The relationship between peak area and concentration of the respective pesticide (correlation coefficient) was obtained for each pesticide. Recoveries of each pesticide were evaluated by the external calibration method. Table 5 shows the average recoveries, % relative standard deviation (RSD), correlation coefficients and limits of detection (LOD) of the 11 pesticides used in the study.

### Results and Discussion

Flow injection analysis - mass spectrometry (FIA-MS) experiments were performed in order to determine the optimum heated nebuliser parameters for absolute sensitivity and signal to noise (S/N) ratio, as well as to select the appropriate ion for identification and quantification of the pesticides in the SIM LC-API-MS experiments.

### Selection of Ionisation Mode

After analysing 0.1 µg of the above mentioned standard pesticides using FIA-API-MS with a heated nebuliser, the pesticides can be classified into 3groups:- Group 1- those which were detectable by only PCI mode, Group 2 – those which were detectable by only NCI mode and Group 3 – by both PCI/NCI modes. Table 3 shows the classification of pesticides by detectable ionization mode. Before optimisation, the mass spectrum of each compound was recorded in order to select the most abundant mass-to-charge ratio (m/z) for further studies. For all the compounds in Group1 & 3, the protonated (M+H)<sup>+</sup> pseudo-molecular ion were registered. Diuron is a phenylurea herbicide.

Group 1 ( PCI mode only )	Group 2 ( NCI mode only )	Group 3 ( PCI/NCI mode)
Carbofuran	Chlorothalonil	Methamidophos
Carbaryl	Ethyl-Parathion	Dimethoate
Methiocarb		Diazinon
Diuron		Chlorpyrifos
		Profenofos

**Table 3. Classification of pesticides by detectable Ionisation mode in LC-API-MS**

Fig. 4 shows the mass spectrum of diuron under PCI mode, the  $[M+H]^+$  pseudo-molecular ion of high intensity was registered as the base peak. The pesticides

in Groups 2 & 3 respond in NCI mode. Methamidophos yields the deprotonated pseudo-molecular ion  $[M-H]^-$ .

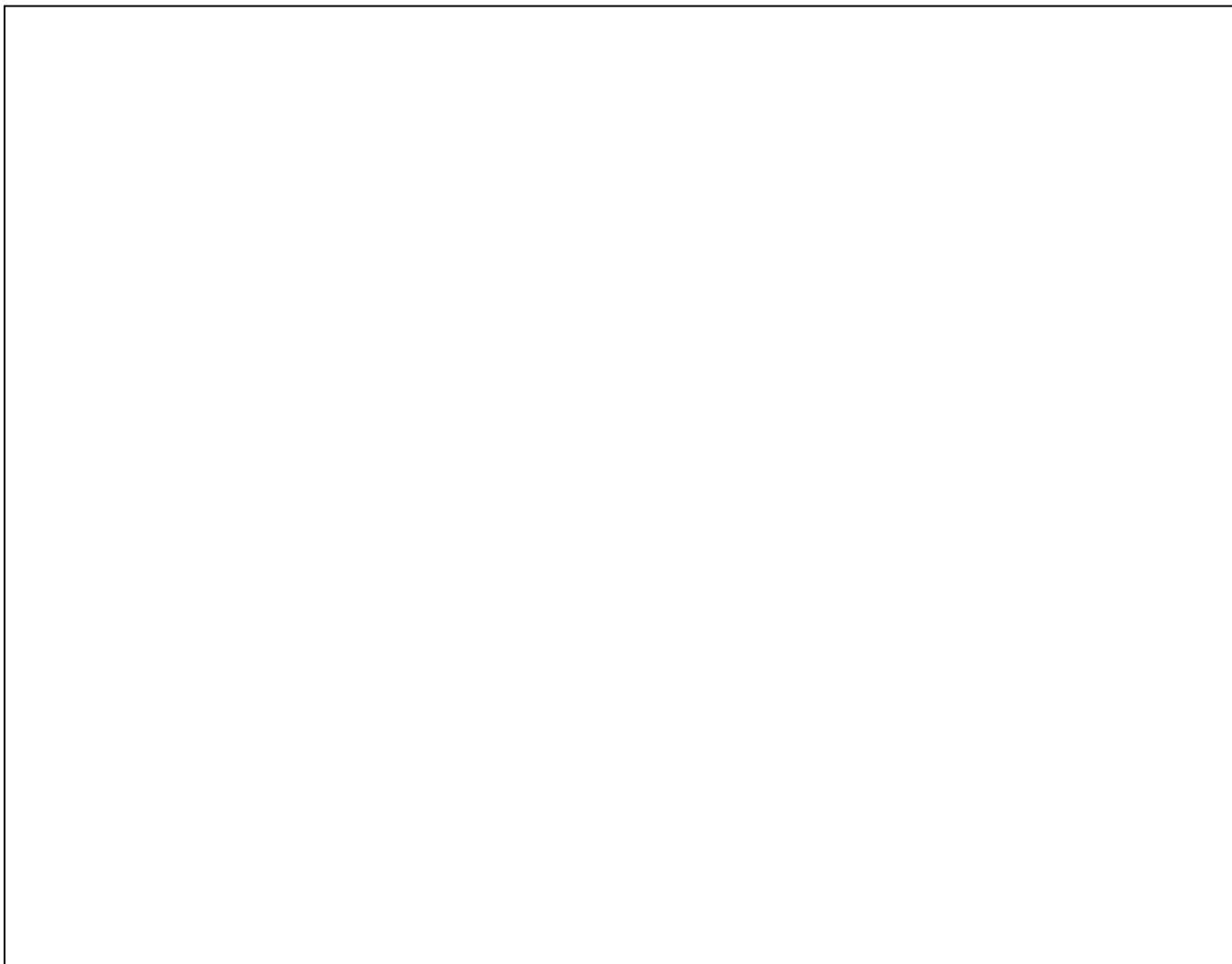


Fig. 4. The mass spectrum and the proposed fragmentation scheme of diuron under PCI mode.

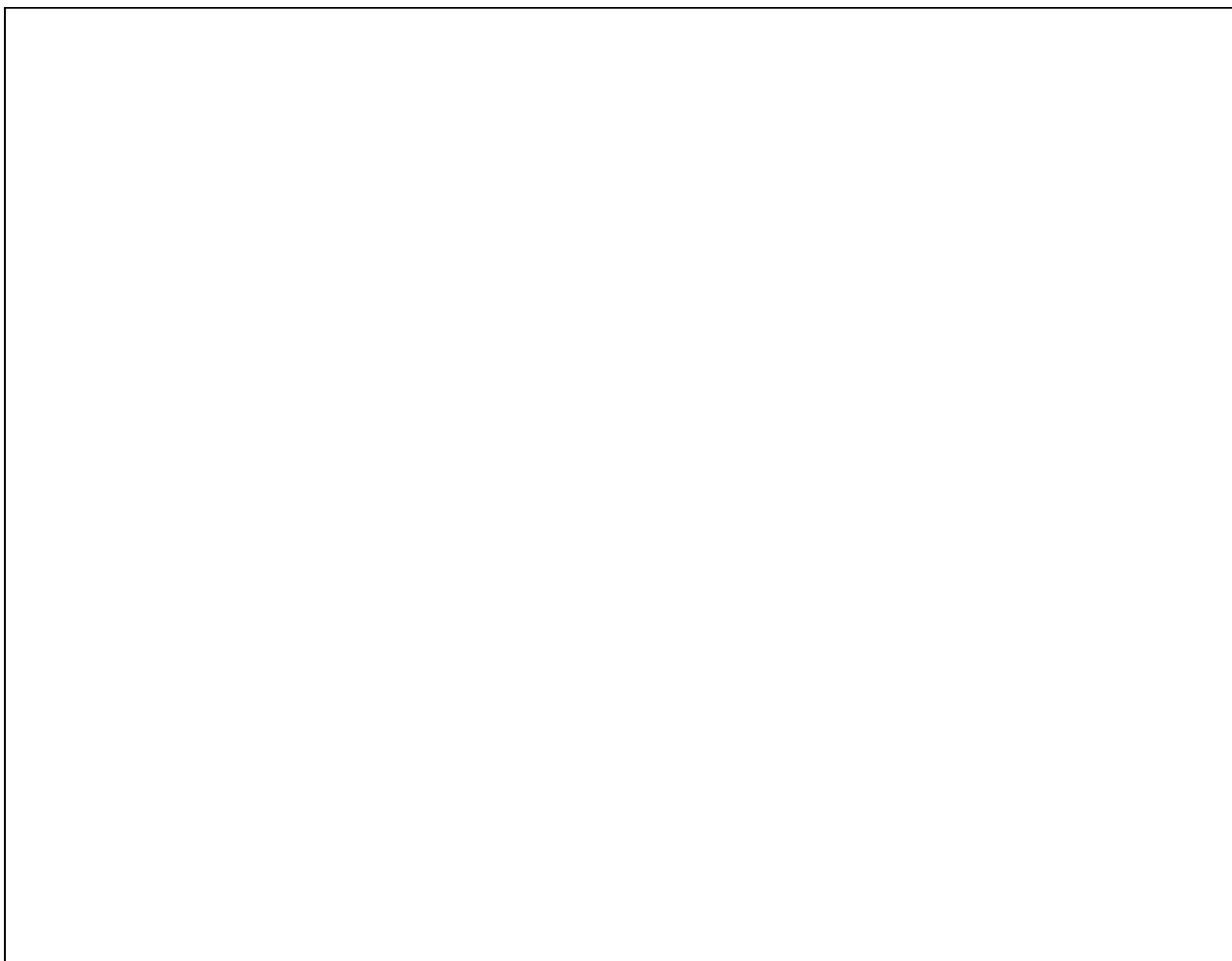


Fig. 5. The mass spectrum and the proposed fragmentation scheme of methamidophos under NCI mode

Fig. 5 shows the mass spectrum and the proposed fragmentation scheme of methamidophos under NCI mode, whereas for chlorothalonil and chlorpyrifos which are interhalogen compounds, the ions minus a chlorine atom is first obtained from the molecule and then replaced by an oxygen atom. Fig. 6 shows the mass spectrum and the proposed fragmentation scheme of chlorothalonil under NCI mode. Diazinon and dimethoate show demonoalkylation process in the NCI mode. Fig 7 shows the mass spectrum and proposed fragmentation scheme of diazinon under NCI mode.

#### Eluent Flow Rate

The method of calculating the signal-to-noise (S/N) ratio is to subtract the minimum value of the background signal from the maximum background signal. This difference is then compared to the signal from the suspected presence of a chemical

phenomenon. The signal is then divided by the difference of the background signal.

$$S/N = \frac{\text{signal}}{\text{Max}_{\text{background}} - \text{Min}_{\text{background}}}$$

In this study, different eluent flow rates (0.6 ml/min to 1.6 ml/min) were delivered to the heated nebuliser probe. Although the signal response remained unchanged, the use of the lower flow rates improved the base line stability, thereby increasing the signal-to-noise ratio. This is because the difference between the maximum and minimum background signal remains more stable at lower flow rates. At higher flow rates, the optimum temperature increases. The heat is used to vaporise the sample and solvent sprayed into the ion source chamber. If the temperature is set too low, the vaporisation is



Fig. 6. The mass spectrum and the proposed fragmentation scheme of chlorothalonil under NCI mode

incomplete, however setting the temperature too high induces thermal degradation of the sample. The optimum temperature is the lowest setting which ensures the complete vaporisation of the sample. For the orifice and focusing ring voltages, the higher the orifice and focusing ring voltages, the greater the energy imparted to the ions entering the analysing region of the mass spectrometer. The energy helps to decluster the ions and to reduce the chemical noise in the spectrum resulting in an increase in the signal-to-noise ratio or sensitivity. By increasing the voltages beyond optimum conditions, the increased energy can induce ion fragmentation which provides the potential for generation of alternative confirmatory ions but at the expense of molecular ion sensitivity. To minimise the fragmentation and thus maximise the sensitivity for the pseudo-molecular ion and characteristic fragment

ion on which quantification was based, the orifice and focusing ring voltages should be kept low to minimise further fragmentation in the pre-analyser zone. Table 4 shows the optimum parameters which were employed for the different pesticides – heated nebuliser probe temperature, focusing ring and orifice voltages used in this method.

The recoveries of 11 pesticides, their correlation coefficients and lowest detection limits are presented in Table 5. By this method, the polar pesticides could be quantitatively extracted. Matrix co-extractives were removed without loss of the more volatile or the polar pesticides. Extraction with 5% (v/v) ethanol in ethyl acetate resulted in a multiresidue extraction. After using the appropriate clean up steps, good repeatability and accuracy for the polar compounds

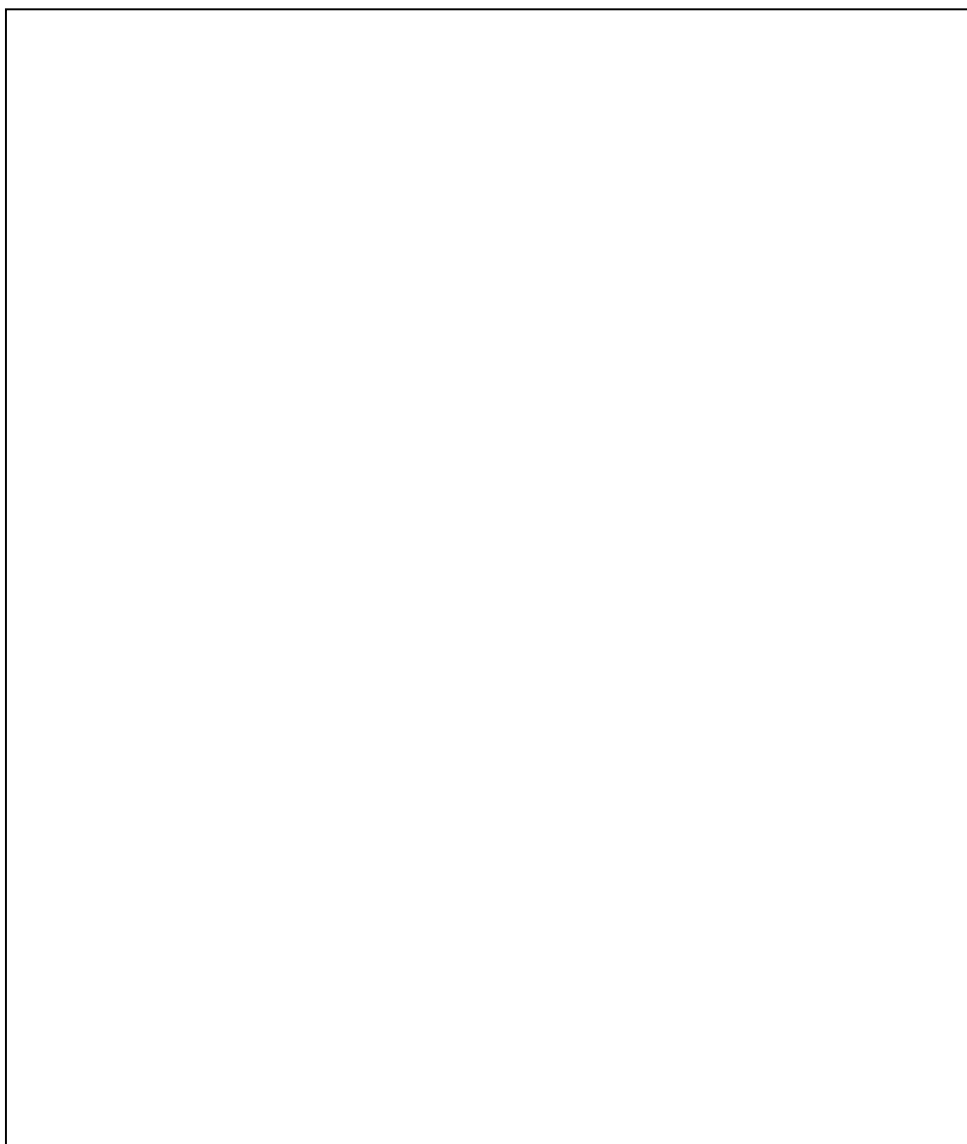


Fig. 7. The mass spectrum and the proposed fragmentation scheme of diazinon under NCI mode.

COMPOUND	HEATED NEBULISER PROBE TEMPERATURE / °C	FOCUSING RING VOLTAGE / V	ORIFICE VOLTAGE / V
Carbaryl	350	250	20
Carbofuran	350	300	25
Methiocarb	350	250	30
Chlorothalonil	350	-330	-35
Dimethoate	350	250	30
Diazinon	400	250	30
Diuron	350	300	25
Chlorpyrifos	400	-250	-27
Methamidophos	370	230	20
Profenofos	450	250	27
Ethyl Parathion	400	-350	-25

Table 4. The optimum parameters - the heated nebuliser probe temperature, focusing ring and orifice voltages.

were obtained. Table 5 shows the mean recoveries and its % relative standard deviations of 11 pesticides in the spiked samples. The use of 5% ethanol in ethyl acetate remedied the anhydrous sodium sulphate.

### Conclusion

In this study, an attempt was made to develop a multiresidue method that would combine the analyses of organophosphorus (OP), organochlorine (OC), N-methylcarbamates (NMC), phenylureas and several other pesticides into a single procedure. Different classes of pesticides are extracted and cleaned up at the same time. No fractional collection is required. The final extract was injected into the LC-API-MS system. The method has several advantages. No derivatization step is required as compared to the current official methods for the determination of polar, non-volatile and thermolabile compounds such as carbamates and phenylureas. Furthermore, the use of the mass spectrometer as the detector gives a higher degree of confirmation of the molecular identity as compared with methods based on fluorescence or ultraviolet detectors. It is also very time and labour consuming to analyse each pesticide or each group of pesticides with each official method.

This study also demonstrates that this method is sensitive, selective, rapid and is an excellent form of confirmation analysis for different groups of pesticides. It offers very low detection limits (1 – 3 pg) for all the 11 pesticides. The relationship between the peak area and concentration of each pesticide is linear (correlation coefficient,  $r^2 > 0.97$ ).

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