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Development of a method for the simultaneous determination of phosphoric and amino acid group containing pesticides by gas chromatography with mass-selective detection Optimization of the derivatization procedure using an experimental design approach

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Abstract

An integrated gas chromatographic–mass-selective detection method for the analysis of the phosphoric and amino acid group containing pesticides is presented. The analytes are derivatized using a single-step procedure for the simultaneous esterification and acetylation of the active groups of analytes (–OH, –COOH, $-NH_2$) by means of acetic acid and trimethyl orthoacetate. An experimental design approach based on the central composite design is used to investigate the dependence of the derivatization variables with the total yield of derivatization of pesticides. The variables selected for study were: the amount of reagents, the temperature and the reaction time. When considering the total pesticide derivatization yield, the amount of acetic acid, the reaction temperature and the reaction time are found to be statistically significant. The electron impact ionization mass spectra of the resulting derivatives are acquired and properly interpreted. Under the chromatographic conditions employed, acceptable peak separation is attained. When the selective ion monitoring mode is used for quantitation purposes, low detection limits in the range 0.05 to 14 μg/l are achieved. Recoveries of spiked water samples range from 96 to 103% and the mean RSD of the method do not exceed 3.5%.

Keywords: Experimental design; Central composite design; Chemometrics; Derivatization, GC; Method development; Optimization; Pesticides; Organophosphorus compounds

1. Introduction

Glyphosate [*N*-(phosphonomethyl)glycine] (GLYP), glufosinate (DL-homoalanine-4-yl-methyl phosphonic acid) (GLUF) and bialaphos (L-2-amino-4-hydroxymethyl phosphonoyl) butyryl-L-alanine (BIAL) are widely used as non-selective, post-emergence contact herbicides [1,2]. Ampropylfos, (±)-1-aminopropylphosphonic acid (AMPP), is a fungicide with protective and curative action marketed under the tradename ''Appa'' [3]. These chemicals are of relatively low toxicity and have therefore been used in agriculture. GLYP is registered for a number of preplant and postharvest uses. GLUF is a synthetic herbicide related to the natural product BIAL, a

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tripeptide-type herbicide, which is produced by the *Streptomyces viridochromogenes L.* Both chemicals contain phosphinothricin as the active ingredient, a potent inhibitor of the enzyme glutamine synthetase [4,5].

Because of their high polarity and the poor detectability, there are limited methodologies for determining these compounds in the sub-ppb levels in aqueous samples. In addition to their polar nature, their high water solubility makes their extraction difficult and limits the use of common derivatization techniques for gas chromatographic analysis. This is the reason that information on the occurrence of this category of pesticides in the environment is not yet available [6]. Methodology on GLYP and its main metabolite aminomethylphosphonic acid (AMPA) is well documented using gas chromatography (GC) [7–15] and liquid chromatography (LC) techniques [16–22]. Fewer methods related to the analysis of GLUF and its major degradation product 3-methylphosphinico propionic acid are published [13,15,20,23–25]. As far as AMPP and BIAL are concerned no or very little information with regard to analytical methodology can be found in the literature [13,15]. Especially for BIAL, certain difficulties related to poor analytical reproducibility and sensitivity have prevented many researchers from dealing systematically with it.

In the present work we aim to develop a highly sensitive and relatively rapid method for the simultaneous determination of the above mentioned pesticides in environmental matrices. The method relies on the efficient conversion of the analytes to methyl ester and amino acetyl derivatives [23,26] using acetic acid and trimethyl orthoacetate (TMOA) and their detection by GC as intact molecules with a mass-selective detector operated in the selective ion monitoring (SIM) mode.

Before proceeding with the elaboration of the mass spectral data of the derivatives and the evaluation of the analytical figures of the method, we thoroughly optimized the derivatization procedure in order to obtain the best reaction yield for the total of pesticides. It is well-known that an experimental design that investigates the effect of one factor while keeping all the other factors constant (single-factor-at-a-time method) is unable to detect the presence of factor interactions. To overcome this problem several methods are called on such as the steepest ascent, the simplex methods, the factorial designs etc., [27–31]. Steepest ascent and simplex optimization can be used for moving towards the optimum in as few experimental runs as possible. Mainly, simplex optimization has been used in many areas of analytical chemistry with relative success [32–36]. Although two-level factorial designs are excellent tools providing a means whereby the factors involved in an experiment can be simultaneously estimated, they can not be applied for optimization of response surfaces (searching for maximum response). To determine the optimum conditions for a reaction, one has to use optimization designs, which can assume non-linear models. These so-called response surface models require at least three levels for each factor. The central composite design (CCD) is based on a full factorial two-level design which is augmented by the center and star points and can achieve a saving in the number of the experimental runs required. Further details about the mentioned methods of optimization can be found in the relevant Refs., [37–40].

Based on the above, the present work constitutes an integrated analytical effort towards the development of a method for the analysis of an important category of agrochemicals. It incorporates both the optimization of the derivatization reaction and the subsequent employment of GC–mass-selective detection for analyte quantitation after proper interpretation of mass spectra. The main variables for the derivatization of the studied pesticides to be optimized were: the concentrations of the reactants, the temperature and the reaction time. A full second-order polynomial model was chosen to approximate the region of the multifactor response surface. The method of the experimental design chosen in order to estimate the parameters of the model was an approach using CCD which allowed all four operating variables to be investigated individually as squared terms and to consider two-component interaction effects. The results of the chemometric design were evaluated using multiple linear regression analysis.

2. Experimental

2.1. Chemicals and glassware

AMPP and *n*-docosane (internal standard) were purchased from Aldrich. GLUF and its metabolite

3-methylphosphinico propionic acid (MPPA) were supplied by Riedel-de Haën. GLYP and acetic acid were purchased from Fluka; AMPA and TMOA were obtained from Sigma. BIAL (purity 87.4%) was kindly donated by the Pharmaceutical Research Center, Meiji Seika Kaisha (Tokyo, Japan). All solvents were of purity suitable for GC trace analysis. Derivatization reactions were performed in 4-ml PTFE-lined screw-capped glass vials.

2.2. Equipment – gas chromatography

GC analyses were carried out with a chromatographic system consisting of a Shimadzu GC-17A gas chromatograph equipped with a QP5000 mass-selective detector and a Class 5000 ChemStation (Shimadzu, Kyoto, Japan) for data acquisition process. Electron impact (EI) mass spectra confirmed the structures of the derivatives. EI ionization was employed at 70 eV with an electron multiplier set at 1200 V in either full scan operation mode for peak identification or SIM mode for quantitation purposes. The mass-selective detector was manually tuned using perfluorotributylamine with the masses m/z 69, 219, 502. The chromatograph was installed with a split/splitless injection system operated in the splitless mode with the liner purged 0.75 min after the injection. The chromatographic column used was an OV-5 fused-silica capillary (30 m×0.25 mm I.D., 0.25 μm film thickness) (Marietta, OH, USA).

The GC operating parameters were as follows: detector temperature, 280°C, injector temperature, 250°C, oven temperature, 60°C (hold 2 min), 5°C/min to 180°C, 15°C/min to 280°C, (hold 5 min). Helium was used as carrier gas regulated at 1.0 ml/min.

2.3. Preparation of samples

Lake water was collected and filtered through paper filter. Both drinking water and lake water samples were derivatized after preconcentrating to dryness an aliquot of 50 ml by rotary evaporation, at 55°C.

2.4. Derivatization reaction

The derivatization procedure encompasses the simultaneous esterification of hydroxyl and carboxylic groups and the acetylation of amino groups of pesticides. To the dry residue received after preconcentration of sample aliquot, were added the appropriate amounts of glacial acetic acid and TMOA. In the course of the optimization procedure the amounts of acetic acid and trimethyl orthoacetate as well as the reaction temperature and reaction time varied. Before heating, the reaction mixture was sonicated for 5 min, to enhancing reaction rate. After cooling to room temperature, the excess reagents were removed under a gentle stream of nitrogen. To ensure complete removal of the acidic residues, the evaporation was continued for an additional 5 min after apparent dryness. Next, the residue was dissolved in 200 μl of ethyl acetate and after sonication for complete dissolution, the derivatives were injected into the gas chromatograph.

The regression was performed by using the STATISTICA software package (StatSoft, USA).

3. Results and discussion

3.1. Optimization of the derivatization reaction

As reported before, the derivatization of the functional groups to the methyl esters and acetyl derivatives, is completed in a single step. A 5-min sonication of the reaction mixture before the incubation appreciably affects the responses probably due to reversible adsorption of the herbicides from the glass walls to the reaction mixture [26].

The derivatization procedure was optimized using an experimental design approach. Initial preliminary experiments using the classical single-factor-at-a-time method served to detect the variables and their respective working ranges that have influence on the yield of the derivatization reaction. Four variables were included in the preliminary experimental design: the amount of the reagents (acetic acid, TMOA), the reaction temperature and the reaction time. The chromatographic responses revealed different behavior of the reaction conditions to the yields of the individual pesticides. Based on this information, a full second-order CCD was proposed in order to study the reaction variables. Since it is advisable to keep the reagent consumption at the bare minimum and the reaction time low, the variable values were narrowed down and each variable

Table 1
Factor levels of the central composite design used for the study of the derivatization yield

Coded levels	Acetic acid (ml)	TMOA (ml)	Temperature (°C)	Reaction time (min)
$-\alpha$	0.20	0.5	60	30
−1	0.40	1.0	70	60
0	0.60	1.5	80	90
+1	0.80	2.0	90	120
$+\alpha$	1.0	2.5	100	150

was assigned the limits given in Table 1. Each of the variables had levels set at five coded levels: $-\alpha$, −1, 0, +1, $+\alpha$ as dictates the CCD. The chemometric design required 27 experiments (24 experimental points and three center points) which were conducted at random. The values representing the overall derivatization yields were calculated using normalization of data of the individual pesticides followed by summation according to the equation:

$$\text{Mean normalized yield} = \sum \frac{(\text{pesticide peak height in the injection/peak height of internal standard})}{(\text{pesticide peak height/peak height internal standard})_{\max}} \cdot 100$$

The normalization against the maximum peak height of the respective pesticide and the internal standard is recommended in order to convert the signals obtained to the mean derivatization yields

Table 2
The four-factor central composite design and the percentage total normalized yield

Exp. No.	Acetic acid (x_1) (ml)	TMOA (x_2) (ml)	Temperature (x_3) (°C)	Reaction time (x_4) (min)	Total normalized yield (%)
1	+1	+1	+1	+1	87.1
2	+1	+1	+1	−1	79.0
3	+1	+1	−1	−1	76.8
4	+1	−1	−1	−1	76.8
5	+1	−1	+1	+1	82.3
6	−1	+1	+1	+1	72.5
7	−1	−1	+1	+1	79.5
8	−1	−1	−1	+1	72.5
9	−1	+1	−1	−1	63.7
10	−1	+1	−1	+1	71.7
11	−1	+1	+1	−1	73.7
12	+1	−1	−1	+1	79.7
13	+1	+1	−1	+1	78.3
14	−1	−1	+1	−1	72.0
15	+1	−1	+1	−1	79.2
16	−1	−1	−1	−1	66.0
17	0	0	0	0	91.9
18	0	0	0	0	93.2
19	0	0	0	0	91.8
20	−2	0	0	0	43.0
21	+2	0	0	0	65.0
22	0	+2	0	0	63.8
23	0	−2	0	0	76.3
24	0	0	+2	0	68.7
25	0	0	−2	0	55.2
26	0	0	0	+2	93.0
27	0	0	0	−2	78.0

and to compensate for variations in the injection volume thereby making the results not ''conditional''.

The results from the optimization procedure with regard to the derivatization yield based on the total of six pesticides are given in Table 2. To fully understand the way in which the reaction variables affect the derivatization yield, the variables must be considered along with non linear effects and interaction terms. Hence, the results were subjected to multiple linear regression using the following full second-order polynomial model:

$$Y = b_0 + \sum_{j=1}^{4} b_j x_j + \sum_{j=1}^{4} b_{jj} x_j^2 + \sum_{0<j<k\leq 4} b_{jk} x_j x_k$$

where Y is the normalized reaction yield, x_j are the coded variables of the derivatization, b_0 is the intercept term, b_j are the slopes with respect to each of the variables, b_{jj} are the curvature terms and b_{jk} are the interaction terms.

A summary of the statistical treatment of data, based on the total of pesticides is shown in Table 3. It is seen that at 95% confidence level ($P<0.05$) certain terms are significant. The intercept of the model corresponds to the estimated normalized yield at the center point of the experimental domain where all the parameters studied assume the coded level 0 (0.6 ml acetic acid, 1.5 ml TMOA, 80°C temperature, 90 min time). The values of b_1, b_2, b_3 and b_4 represent the main effects and describe the variation of yield corresponding to the increase of one coded unit of each variable. The main effects of acetic acid, temperature and derivatization time have a positive sign since yield has to increase with increasing the values of these variables. TMOA is negative indicating that the maximum is obtained below the center point value. With the derivatization time being an exception, the rest of the second-order parameters, which describe curvature effects, are statistically significant. The interaction parameters responsible for curvature and twisting effects are non-significant. Slightly stronger significance is observed for all variables (data not shown) when the insignificant interactions are excluded from the model.

Response surfaces are plots of the derivatization yield versus each of the factors and visualize their influence on the derivatization yield. Allowing for that the factors involved in the optimization are four it is possible to obtain two typical three-dimensional response–surface plots for the total of pesticides, as shown in Fig. 1. In this way, we are able to assess graphically the maxima on the response surfaces and therefore the parameter settings, which produce the highest yields of the derivatized pesticides. The parabolic plot of Fig. 1a shows clear optima for acetic acid and TMOA at approximately 0.7 ml and 1.4 ml, respectively. The relationship between temperature and derivatization time shows a maximum ridge (Fig. 1b) in the experimental domain. It is observed that better derivatization yield is achieved as the temperature increases up to 80°C and declines beyond this value possibly due to secondary non-predicted reactions which occur during the derivatization or destruction of the target derivatives. The yield ameliorates with the reaction time although this improvement is not highly pronounced. In order to save time in the experiment an optimum reaction time of 120 min was chosen. The model developed

Table 3
Multiple linear regression results for total pesticides derivatization yield[a]

Parameter	Variable[b]	Regression coefficient	P Value[c]
b_0		92.0	*0.0000*
b_1	x_1	4.65	*0.0026*
b_2	x_2	−1.26	0.3261
b_3	x_3	2.78	*0.0427*
b_4	x_4	2.77	*0.0439*
b_{11}	x_1^2	−8.15	*0.0000(4)*
b_{22}	x_2^2	−4.14	*0.0074*
b_{33}	x_3^2	−6.16	*0.0005*
b_{44}	x_4^2	−0.275	0.8363
b_{12}	x_1x_2	0.725	0.6387
b_{13}	x_1x_3	−0.487	0.7516
b_{14}	x_1x_4	−0.325	0.8327
b_{23}	x_2x_3	0.237	0.8772
b_{24}	x_2x_4	0.225	0.9975
b_{34}	x_3x_4	0.087	0.9546
R		0.931	
R^2		0.867	
Adjusted R^2		0.818	

[a] The R, R^2 and adjusted R^2 are calculated after excluding from the model the insignificant interaction terms and quadratic term of derivatization time.

[b] x_1: Amount of acetic acid, x_2: amount of TMOA, x_3, temperature and x_4: derivatization time.

[c] The significance of data in italics is 95% ($P<0.05$).

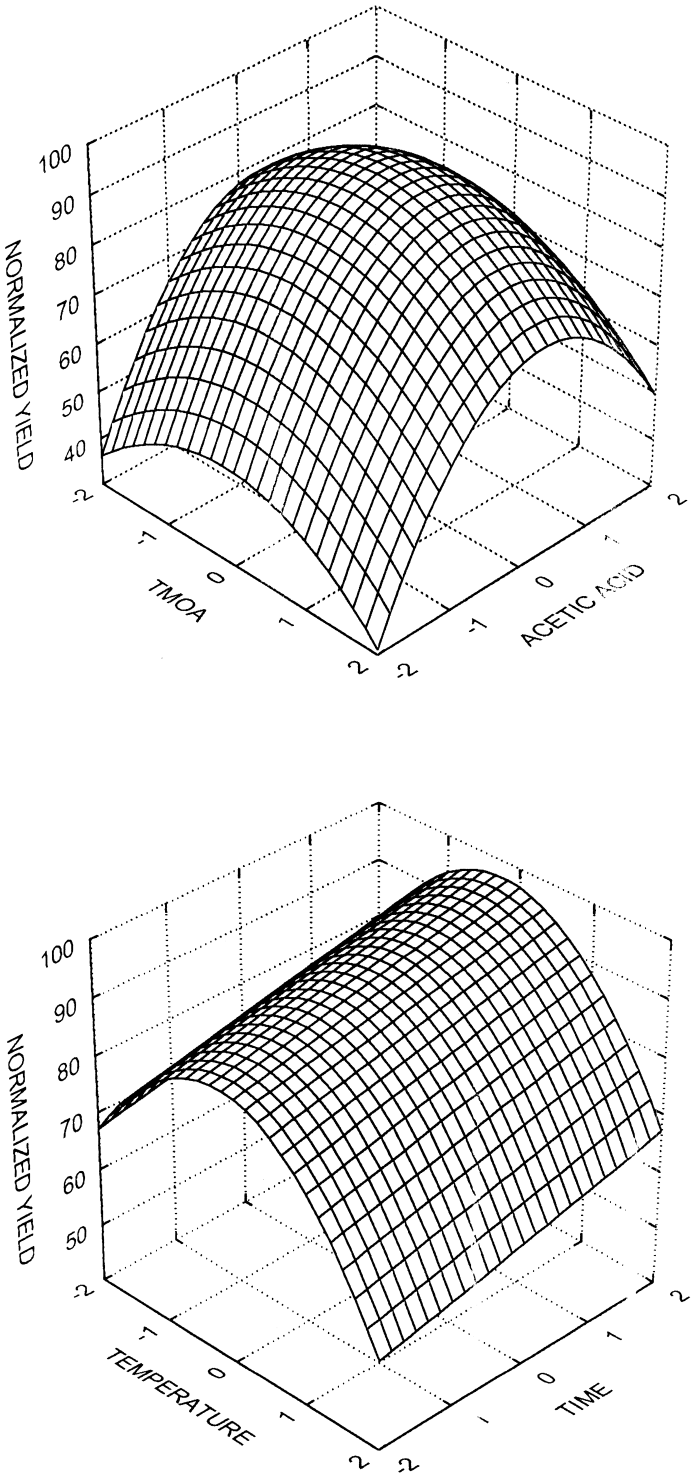

Fig. 1. Three-dimensional response–surface plots of (a) TMOA vs. acetic acid and (b) temperature vs. derivatization time.

for the yield of reaction which derives from considering only the significant terms, seemed adequate with a satisfactory R^2 value (0.867).

The calculated values for the dependent variable (derivatization yield) obtained from the model reproduce the experimental results with errors of less than 5% in the major part of the experimental domain. Higher errors are observed mainly on the upper and lower boundaries of the studied variables.

It should be emphasized that daily injection of a standard solution revealed that with no exception, the derivatives are stable at room temperature for at least 2 weeks. If strictly followed, the derivatization procedure was proved to be highly reproducible.

3.2. Gas chromatography

Careful selection of the oven temperature profile reconciles sufficient peak separation and relatively rapid chromatographic elution. Based on this assumption, improved performance showed an oven temperature program that included a low temperature ramp for the separation of the majority of the methyl ester/acetyl derivatives followed by a higher temperature ramp for the elution of BIAL. Under the chromatographic conditions selected and detailed in the experimental section, acceptable separation is attained and, except for BIAL, the derivatives exhibit relatively rapid chromatographic elution using the OV-5 fused-silica capillary column. Pesticides and metabolite derivatives elute from the non-polar capillary column in the order of increasing molecular mass, an exception being GLYP and GLUF derivatives which have very similar molecular masses and their elution order seems not to be governed by common interaction properties. No interfering peaks were identified in the obtained gas chromatograms. Fig. 2 illustrates a full scan chromatogram of a lake water fortified with a mixture of the pesticides and their metabolites. A peak of relatively high intensity appearing at a retention time of 38.8 min is attributed to the derivatization mixture itself as demonstrates the injection of a blank chromatogram run under the same experimental conditions. Finally, the derivatives are thermally stable to the point of withstanding temperatures of 280°C at which they are chromatographed as intact molecules.

3.3. Mass-selective detection

Developing a SIM method for the mass-selective detector requires prior interpretation of the mass spectra of the derivatized analytes and confirmation of the expected structures of the derivatives. Fig. 3 gives the structures of the derivatized pesticides. Reasonable electron impact mass spectrometry (MS) fragmentation patterns with a multitude of fragment ions give assurance that the pesticides studied can

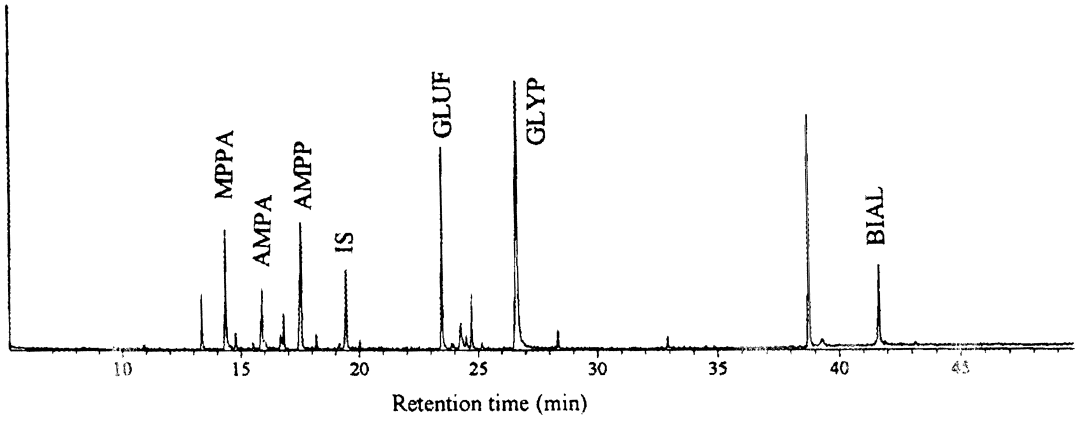

Fig. 2. Full scan gas chromatogram of lake water fortified with the analytes studied. Chromatographic conditions are detailed in the text. MPPA: 3-methylphosphinico propionic acid (prederivatized analyte concentration: 140 ng/ml), AMPA: aminomethylphosphonic acid (20 ng/ml), AMPP: ampropylfos (125 ng/ml), GLUF: glufosinate (368 ng/ml), GLYP: glyphosate (181 ng/ml), BIAL: bialaphos (1.11 μg/ml), I.S.: internal standard.

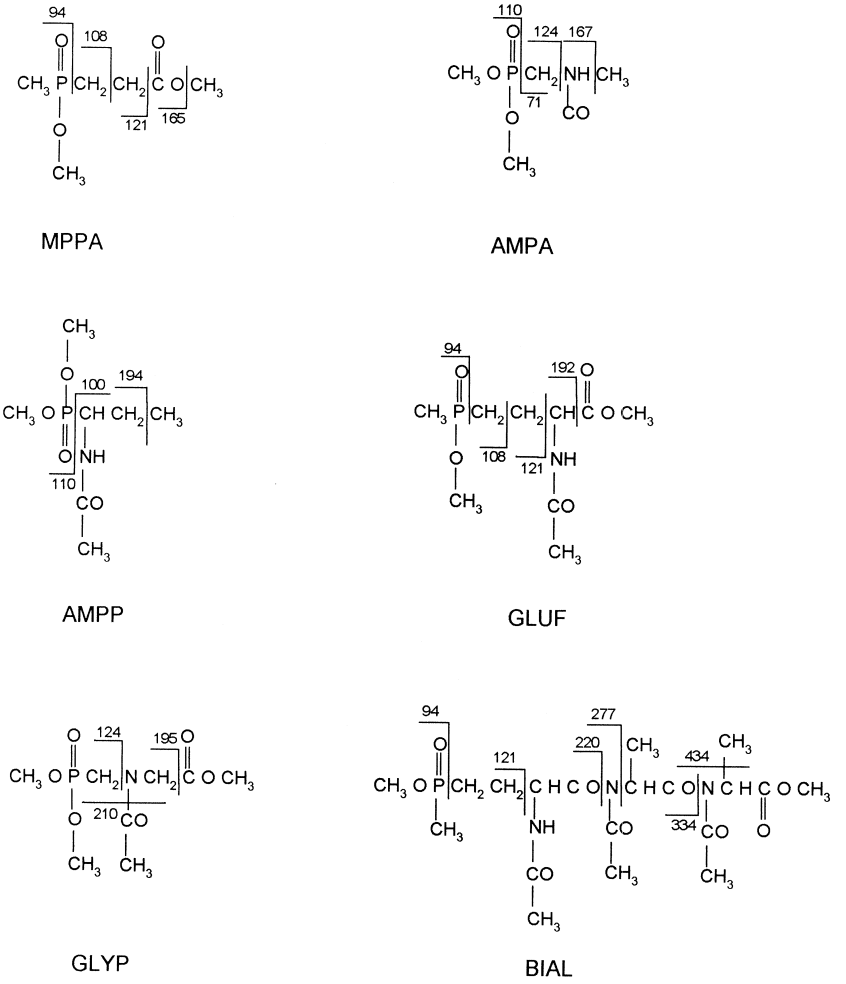

Fig. 3. Structures of the derivatized: 3-methylphosphinico propionic acid (MPPA), aminomethylphosphonic acid (AMPA), ampropylfos (AMPP), glufosinate (GLUF), glyphosate (GLYP) and bialaphos (BIAL).

conveniently and effectively be converted into the respective derivatives.

Fig. 4 illustrates that molecular or quasi-molecular ions (M^+) when present, are sensibly the minor ions in the EI spectra of the derivatized analytes. Close examination of the mass spectra reveals that each derivative produces an easily interpretable mass spectrum with specific and prominent fragment ion peaks at M^+-15 (CH_3), M^+-31 (CH_3O), M^+-43 ($COCH_3$), M^+-58 ($NHCOCH_3$). The combination of the molecular ion confirmation and electron impact fragmentation pattern suggests that amino-, hydroxy- and carboxylic- moieties in the molecules of compounds studied have unequivocally reacted during the derivatization procedure. GLUF and BIAL show pretty alike fragmentation pattern because of great similarities of the molecular structures. Ions at m/z 94, 108, 121 and 192 feature the methyl ester/acetyl derivative of GLUF. Further to these fragment ions, the fragmentation pattern of BIAL shows two low intensity ion peaks, at m/z 434 and 334. It is speculated that the former arises from the detachment of the $COCH_3$ group (m/z 43) from the molecule and the latter originates from the cleavage of the CO–N bond being adjacent to the carboxylic group of the molecule. A peak at m/z 263

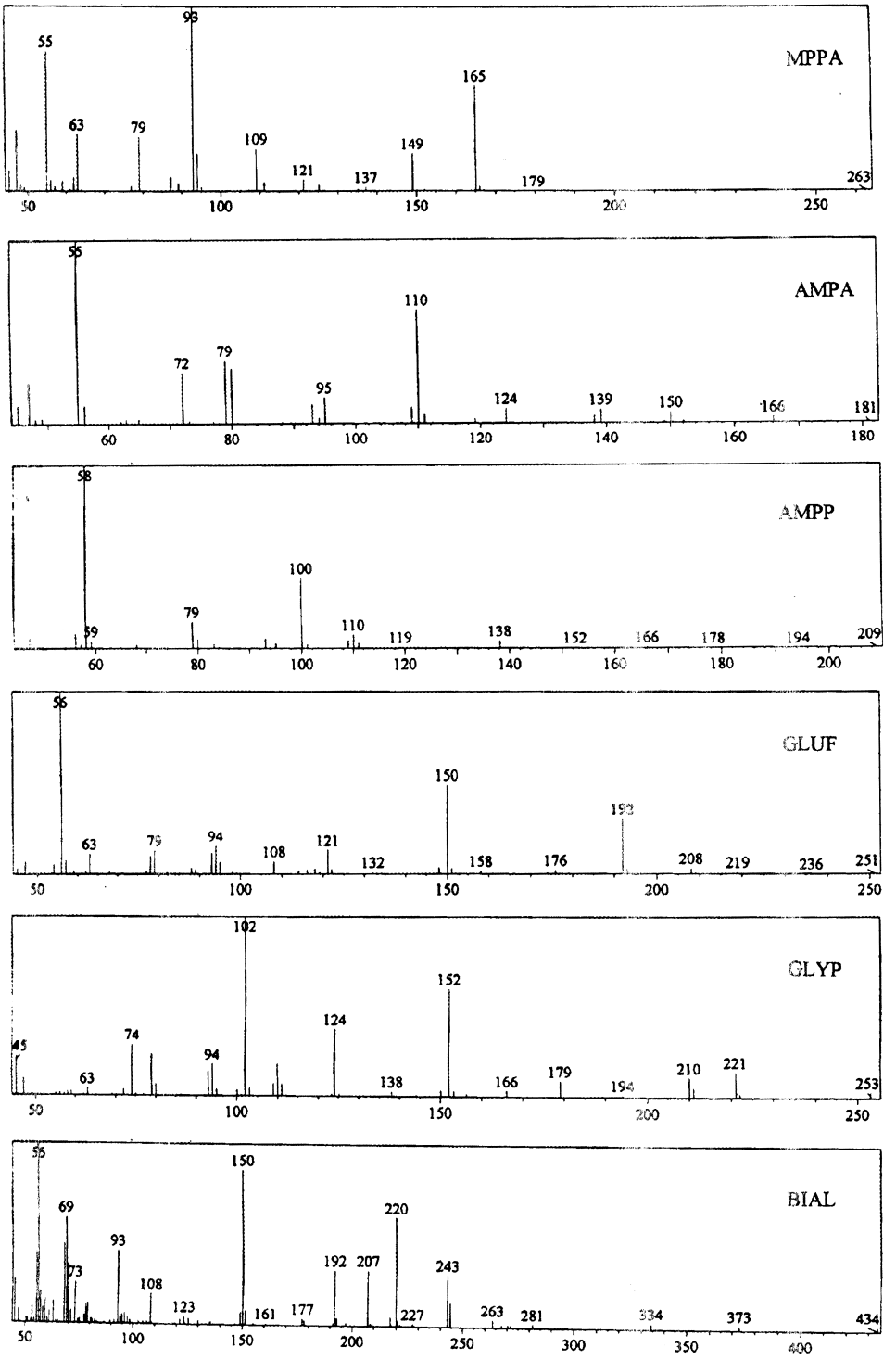

Fig. 4. Mass spectra of the derivatized analytes. MPPA: 3-methylphosphinico propionic acid, AMPA: aminomethylphosphonic acid, AMPP: ampropylfos, GLUF: glufosinate, GLYP: glyphosate, BIAL: bialaphos.

is attributed to the cleavage of the central N–CH and the loss of a CH_3 group while another peak at m/z 220 is ascribed to the cleavage of the CO–N bond located in the center of the molecule. Further peaks, m/z 177, 161 and 150 can be considered as secondary fragments emanating from the breaking of CH–CO bond and the subsequent loss of certain groups.

Important and possible diagnostic ions are compiled in Table 4 and consistently correspond to the fragmentation pathways of each derivatized compound.

3.4. GC–MS analysis – applications – recoveries

On a routine basis, the analysis of the pesticides studied can conveniently be carried out using the SIM mode. Selected ions are monitored in order to achieve sub-ppb levels for most of the derivatized compounds. Up to two fragments were monitored simultaneously for the quantitation. The selection of ions for the SIM method was based on either their abundance (e.g., base peaks) or the prominence of fragment ions related to parts of molecules of the formed derivatives. The minimum detectable amounts of the analytes to give signal three-times the noise are 0.05, 0.21, 0.29, 0.32, 0.65 and 14 μg/l for AMPA, AMPP, MPPA, GLYP, GLUF and BIAL, respectively. In order to demonstrate the applicability of the method to environmental samples, lake and drinking water were subjected to a simple pretreatment described in the experimental part. It was proved that the method could be applied to surface waters, without facing any interference problem. The tested drinking and lake water samples were not found to contain any traces of the pesticides concerned. In order to simulate real-life situation, six surface water samples were spiked with known amounts of the pesticides in the range 0.09 to 35 μg/l. The recoveries were as high as 96–103% while the RSD of the method ($n=5$) does not exceed 3.5%.

4. Conclusions

A GC–MS method is described for the simultaneous determination of phosphoric and amino acid group containing pesticides. The CCD experimental design approach in relation to multiple linear regression analysis performs a detailed investigation of the influential parameters for the derivatization reaction. The proposed method scrutinizes the optimum conditions for the simultaneous derivatization of six relevant analytes taking into account the total yield of derivatization through data normalization. The model resulting from the use of the multiple linear regression analysis describes well the experimental data, in a major part of the experimental domain.

Mass fragmentation pathways which are dominated by the loss of prominent fragment ions (CH_3, CH_3O, $COCH_3$, $NHCOCH_3$) afford unambiguous identification for the detection and quantitation of the pesticide derivatives. The method provides enhanced sensitivity and simple sample pretreatment. Finally, the detection limits are sufficiently low, for regular monitoring analyses.

References

[1] C.D.S. Tomlin (Ed.), The Pesticide Manual, 11th ed., BCPC, 1997.
[2] S.O. Duke, J. Lydon, Weed Technol. 1 (1987) 459.
[3] L. Cerenius, S. Rufelt, K. Söderhäll, Pestic. Sci. 36 (1992) 189.

Table 4
Diagnostic ions in the EI mass spectra of the derivatized compounds

Compound	M^+	M^+-15	M^+-31	M^+-43	M^+-58	Others
MPPA	179	165	149	137	121	55, 93 (BP)
AMPA	181	166	151	139	124	55, 79, 110 (BP)
AMPP	209	194	178	166	152	58 (BP), 100
GLUF	251	236	219	208	192	56 (BP), 121, 150,
GLYP	253	–	221	210	194	102 (BP), 124, 152
BIAL	–	–	–	434	–	56 (BP), 93, 150, 192, 220, 243

[4] A. Wild, R. Manderschied, Z. Naturforsch. C39 (1984) 500.
[5] R. Manderschied, A. Wild, J. Plant Physiol. 123 (1986) 135.
[6] M. Fielding, D. Barcelo, A. Helweg, S. Galassi, L. Torstensson, P. van Zoonen, R. Wolter, G. Angeletti, in: Pesticides in Ground and Drinking Water, Water Pollution Research Report, 27, Commission of the European Community, Brussels, 1992, pp. 1–67.
[7] R.A. Guinivan, N.P. Thompson, W.B. Wheeler, J. Assoc. Off. Anal. Chem. 65 (1982) 35.
[8] R.A. Guinivan, N.P. Thompson, W.B. Wheeler, J. Agric. Food Chem. 30 (1982) 977.
[9] C.L. Deyrup, S.-M. Chang, R.A. Weintraub, H.A. Moye, J. Agric. Food Chem. 33 (1985) 944.
[10] D.N. Roy, S.K. Konar, J. Agric. Food Chem. 37 (1989) 441.
[11] S.K. Konar, D.N. Roy, Anal. Chim. Acta 229 (1990) 277.
[12] P.L. Eberbach, L.A. Douglas, J. Agric. Food Chem. 39 (1991) 1776.
[13] N. Tsunoda, J. Chromatogr. 637 (1993) 167.
[14] P.L. Alferness, Y. Iwata, J. Agric. Food Chem. 42 (1994) 2751.
[15] H. Kataoka, S. Ryu, N. Sakiyama, M. Makita, J. Chromatogr. A 726 (1996) 253.
[16] H.A. Moye, A.J. Boning Jr., Anal. Lett. 12 (1979) 25.
[17] L.N. Lundgren, J. Agric. Food Chem. 34 (1986) 535.
[18] Y.Y. Wingfield, M. Lanouette, Anal. Chim. Acta 233 (1990) 311.
[19] M.P. Abdullah, J. Daud, K.S. Hong, C.H. Yew, J. Chromatogr. A 697 (1995) 363.
[20] J.V. Sancho, F. Hernández, F.J. López, E.A. Hogendoorn, E. Dijkman, P. van Zoonen, J. Chromatogr. A 737 (1996) 75.
[21] R.J. Vreeken, P. Speksnijder, I. Bobeldijk-Pastorova, Th.H.M. Noij, J. Chromatogr. A 794 (1998) 187.
[22] E.A. Hogendoorn, F.M. Ossendrijver, E. Dijkman, R.A. Baumann, J. Chromatogr. A 833 (1999) 67.
[23] A.E. Smith, J. Agric. Food Chem. 37 (1989) 267.
[24] J.V. Sancho, F.J. López, F. Hernández, E.A. Hogendoorn, P. van Zoonen, J. Chromatogr. A 678 (1994) 59.
[25] M.J. Faber, G.R. Stephenson, D.G. Thompson, J. Agric. Food Chem. 45 (1997) 3672.
[26] M. Tsuji, Y. Akiyama, M. Yano, Anal. Sci. 13 (1997) 283.
[27] C.K. Bayne, I.B. Rubin, Practical Experimental Designs and Optimization Methods for Chemists, VCH, Weinheim, 1986.
[28] S.M. Deming, S.L. Morgan, Experimental Design: A Chemometric Approach, Elsevier, Amsterdam, 1987.
[29] R.G. Brereton, Chemometrics: Applications of Mathematics and Statistics to Laboratory Systems, Ellis Horwood, Chichester, 1990.
[30] D.C. Montgomery, Design and Analysis of Experiments, 3rd ed., Wiley, New York, 1991.
[31] A.G. González, Anal. Chim. Acta 360 (1998) 227.
[32] D. Betteridge, A.F. Taylor, A.P. Wade, Anal. Proc. 21 (1984) 373.
[33] D. Betteridge, A.P. Wade, A.G. Howard, Talanta 32 (1985) 723.
[34] K.S. Kurtz, S.R. Crouch, Anal. Chim. Acta 254 (1991) 201.
[35] C.D. Stalikas, M.I. Karayannis, S.M. Tzouwara-Karayanni, Talanta 41 (1994) 1561.
[36] C.D. Stalikas, G.A. Pilidis, M.I. Karayannis, J. Anal. At. Spectrom. 11 (1996) 595.
[37] R.S. Strange, J. Chem. Educ. 67 (1990) 113.
[38] J.A. Palasota, S.N. Deming, J. Chem. Educ. 69 (1992) 560.
[39] E. Morgan (Ed.), Chemometrics: Experimental Design, Wiley, Chichester, 1991.
[40] A.G. González, D. González-Arjona, Anal. Chim. Acta 298 (1994) 65.