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Chemometric Strategies for the Extraction and Analysis Optimization of Herbicide Residues in Soil Samples

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1. Introduction

1.1 Herbicide benefits and concerns

The two cereal crops which are grown most abundantly in the EU are wheat and barley (Document No. SANCO/D3/SI2.396179, 2005). Herbicides play a very important role in effectively controlling annual grasses and broad-leaved weeds affecting these crops. Their use cannot be neglected due to the enormous benefits in agricultural outputs. Among them, acidic herbicides are widely used for control of broad-leaved weeds and other vegetation because they are relatively inexpensive and very potent even at low concentrations (Wells & Yu, 2000).

However, due to the herbicide widespread and possible toxicity in the environment, it is important to monitor their residues. Under realistic field situations there is a potential exposure to agricultural soils by these products indirectly through spray drift and run-off from crop vegetation surfaces, and directly through soil treatment practices (Document No. D/00/SuM/5277, 2000). They have harmful effects on the microflora of the soil when they are not degraded quickly enough (Santos-Delgado et al., 2000).

1.2 Herbicide characteristics

There are many compounds registered as herbicides intended for their use in cereal crops, which can be classified into several chemical classes in accordance with their chemical structures.

Substituted ureas are one of the oldest herbicide groups used in agriculture, being two of the most important, phenylureas, employed since early fifties, and sulphonylureas, developed more recently with a high herbicidal activity resulting in low applications doses (Tadeo et al., 2000). Among the basic herbicides, triazines are the most important selective herbicides (Pinto & Jardim, 2000). Triazinic herbicides have been widely used in the last years for crop protection in agriculture and weed removal in different lands. Among neutral herbicides, dinitroaniline herbicides are usually soil applied in a wide variety of agronomic crops and particularly, in winter and spring cereals. Thiocarbamates have been used as herbicides in maize and wheat for several decades (Tadeo et al., 2000).

Acidic herbicides consist of several families of compounds that are related by similarities in biological activity and chemical properties which influence the way they are extracted and analyzed. These families of compounds are derivatives of acidic functional groups including benzoic acid (dicamba), acetic acid [2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA)], propanoic acid [dichlorprop, diclofop, fenoxaprop p and 2-(4-chloro-2-methylphenoxy)propanoic acid (MCPP)], picolinic acid (clopyralid) and pyridinecarboxylic acid (fluroxypyr) among others (Wells & Yu, 2000). Acidic herbicides can be applied in the form of free acids, salts or esters (Analytical Methods for Pesticide Residues in Foodstuffs, Part I, 1996). Several studies have shown that in the environment, acidic herbicides formulated as esters undergo fast hydrolysis, on the order of 24–48 h, depending on pH and other conditions and in the presence of vegetable tissues and soil bacteria yielding the corresponding free acids (Budde, 2004; Marchese, 2001; Tadeo et al., 2000). Therefore, they are generally present as the corresponding acids and most frequently exist in ionized form at most environmental pH values (Wells & Yu, 2000).

1.3 Multiresidue determination and analysis

Since spray history or environmental background of most soil samples is unknown, method development efforts have concentrated on multiresidue methods (Regulation EC No 1107/2009, 2009; Document No. SANCO/825/00, 2004). They require universality of the isolation and clean-up procedure and, as far as possible, unification of the conditions of the chromatographic separation (Tekel & Kovacicová, 1993).

Acetone, acetonitrile and ethyl acetate, sometimes at acidic pH, are the most usual organic solvents employed in the extraction of a large number of herbicide residues belonging to different groups (Kremer et al., 2004; Jiménez et al., 2000; Mastovska & Lehotay, 2004; Papadopoulou-Mourkidou et al., 1997; Sánchez-Brunete & Tadeo, 1996). The neutral (dinitroanilines, phenylureas, thiocarbamates) and basic (triazines) multiresidue herbicide extractions from soils are usually carried out with organic solvents (Tadeo et al., 1996). The addition of water has been reported, in some cases, to increase desorption of herbicides from the matrix because it is both a solvent for the analyte and a solute that can compete for adsorption sites (Tadeo et al., 1996).

Acidic herbicides (phenoxyacids, benzoic acids, sulfonylureas) are reported in most cases to be extracted at low pH conditions that suppress the ionization of acids and make them neutral and more apt to be extracted with an organic solvent (Macutkiewicz et al., 2003; Marchese, 2001; Nolte & Kruger, 1999; Sánchez-Brunete & Tadeo, 1996; Wells & Yu, 2000). They are usually extracted from soils with solid-liquid extraction with organic solvent–water mixtures at an acid pH with a solvent of medium polarity or with an alkaline solution with sodium hydroxide 0.5 M (EPA Method 8151A, 1996). Afterwards, the extract is acidified and partitioned into an organic solvent immiscible with water or concentrated with solid-phase extraction (SPE) (Crespín et al., 2001; Menasseri & Koskinen, 2004; Patsias, 2002). Many procedures have been shown to effectively extract acid herbicides with organic solvents of medium polarity in mixtures with water and acetic acid (Ahmed & Bertrand, 1989; Crescenzi et al., 1999; Menasseri & Koskinen, 2004; Smith & Milward, 1981; Smith, 1995). In the same way, ammonium hydroxide has been reported to enhance basic herbicide recoveries (Smith & Milward, 1981).

No pH adjustment for non-ionic and basic analyte water removal by liquid partition has been reported (Papadopoulou-Mourkidou et al., 1997), meanwhile methods without

previous acidification (Ahmed & Bertrand, 1989) and with previous pH adjustment to pH 2 (Crespín et al., 2001; EPA Method 8151A, 1996; Sánchez-Brunete & Tadeo, 1996) have been found for acid analytes.

Herbicides formulated as esters have been reported to rapidly hydrolyze in contact with soil to their corresponding acids and phenols (Budde, 2004). The analyst, therefore, must either evaluate the herbicides in both the ester and hydrolyzed acid forms, or convert all components present to their free acids before analysis. For example, some analytical methods specify a strong base hydrolysis of any residual esters before conversion of the acids to methyl esters for GC (Wells & Yu, 2000). However, this approach is unsuitable for multi-class herbicide residue analysis because other analytes will be destroyed under such strong conditions. To avoid this loss, a unique multiresidue extraction and a simultaneous analysis for both esters and their corresponding free acids is intended.

The use of GC-MS is a very versatile and sensitive method for residue analysis due to the high sensitivity obtained and MS is a very valuable detection technique, because it provides information on the compound molecular structure and it is also highly sensitive and selective when used in the single ion monitoring (SIM) mode (Tadeo et al., 2000).

The acidic compounds, because of their polar nature, suffer from peak asymmetry and tailing in the GC stationary phases. Masking of these acidic hydrogens by derivatization to their corresponding esters is essential in order to yield products with enhanced volatility that can undergo analysis by GC (Catalina et al., 2000). The typical reactions of derivatization of phenoxyacids are trans-esterification, esterification, silylation, alkylation and extractive and pyrolytic alkylation (Rompa et al., 2004). The formation of methyl esters/ethers is particularly preferred, because they can be easily prepared and have reasonably short GC retention times (Macutkiewicz et al., 2003). The most useful reagent of pyrolytic alkylating reagents is TMSH ($(CH_3)_3SOH$ (Yamauchi et al, 1979) which provides an efficient methylation by pyrolysis of the previously formed salts of nucleophiles, e.g. carboxylic acids and phenols to their corresponding methyl esters and methyl ethers (Butte, 1983). It can be used in two ways, i.e. to methylate free acids by pyrolysis of the salt in the heated injection port of a GC, or to effect base-catalyzed trans-esterification of other esters to their methyl esters (Butte, 1983; Christie, 1993). As a result, methyl esters are the final product of both reactions. This reaction is very elegant and convenient, because it is just necessary to add the reagent to the sample solution with little or no work-up and reacts very rapidly (Butte, 1983; Halket & Zaikin, 2004). In addition, removal of excess reagent is not required, as in other derivatization reactions, because the only by-products of this reaction are dimethylsulphide (b.p. 37ºC), and methanol that elute with the solvent peak and do not disturb the chromatographic separation of analytes.

2. Introduction. Method development. Chemometric strategies

Since the extraction process for a number of analytes occurs, more or less, in a single run within multiresidue methods, the efficiency of the recovery of each individual component differs from each other, due to their different chemical structures. A detailed optimization of these multiresidue procedures would, therefore, help to adjust the applied conditions in a way to obtain the maximum recovery percentage for most of the constituents of the sample. These multiresidue methods, however, are in principle rather costly for implementation on a large scale, so they require the use of chemometric strategies applied to method development in order to ensure an efficient recovery.

A frame of integration between analytical procedures and chemometric methods has made the extraction of relevant underlying analytical information possible, largely applied in the environmental science where data interpretation is of great interest (Einax et al., 1997). Chemometrics is a chemical discipline that uses mathematics, statistics and formal logic to design or select optimal experimental procedures, to provide maximum relevant chemical information by analyzing chemical data; and to obtain knowledge about chemical systems (Massart et al., 1998). Some of these chemometric strategies are detailed in this chapter.

2.1 Pattern recognition: multivariate analysis

Pattern recognition is the scientific discipline whose goal is the classification of objects into a number of categories or classes. It "reveals" the organization of patterns into "sensible" clusters (groups), which will allow to discover similarities and differences among patterns and to derive useful conclusions about them.

Classification is synonymous with pattern recognition, and scientists have turned to it and PCA and cluster analysis to analyze the large data sets typically generated in environmental studies that employ computerized instrumentation. The set of measurements that describe each sample in the data set is called a pattern. The determination of the property of interest by assigning a sample to its respective category is called recognition, hence the term pattern recognition. Clustering and classification are the major subdivisions of pattern recognition techniques.

In a typical pattern recognition study, samples are classified according to a specific property using measurements that are indirectly related to that property. An empirical relationship or classification rule is developed from a set of samples for which the property of interest and the measurements are known. The classification rule is then used to predict this property in samples that are not part of the original training set (Lavine, 2000; McLachlan, 1992).

2.1.1 Cluster analysis

Cluster analysis (Kaufman & Rousseeuw, 1990; Massart, 1983) is the name given to a set of techniques whose basic objective is to discover sample groupings within data. For cluster analysis, each sample is treated as a point in an n-dimensional measurement space. The coordinate axes of this space are defined by the measurements used to characterize the samples. Cluster analysis assesses the similarity between samples by measuring the distances between the points in the measurement space. A basic assumption is that the distance between pairs of points in this measurement space is inversely related to the degree of similarity between the corresponding samples. Points representing samples from one class will cluster in a limited region of the measurement space distant from the points corresponding to the other class. Samples that are similar will lie close to one another, whereas dissimilar samples are distant from each other (Lavine, 2000). Samples within the same group are more similar to each other than samples in different groups.

Clustering methods are divided into three categories, hierarchical, object-functional, and graph theoretical. The hierarchical methods are the most popular. The results of a hierarchical clustering study are usually displayed as a dendogram, which is a treeshaped map of the intersample distances in the data set. The dendogram shows the merging of samples into clusters at various stages of the analysis and the similarities at which the clusters merge, with the clustering displayed hierarchically (Lavine, 2000).

Clustering has a lot of applications:

1. Data reduction: Many times, the amount of the available data, N, is very large and, as a consequence, its processing becomes very demanding. Cluster analysis can be used in order to group the data into a number of sensible clusters, m (<<N) and to process each cluster as a single entity.
2. Prediction based on groups: the resulting clusters are characterized based on the characteristics of the patterns by which they are formed. In the sequel, if an unknown pattern is given, it can be determined the cluster to which it is more likely to belong and it can be characterized based on the characterization of the respective cluster.
3. Hypothesis generation: cluster analysis is applied to a data set in order to infer some hypotheses concerning the nature of the data. Thus, clustering is used as a vehicle to suggest hypotheses. These hypotheses must then be verified using other data sets.
4. Hypothesis testing: In this context, cluster analysis is used for the verification of the validity of a specific hypothesis.

2.1.2 Principal component analysis

PCA (Brown, 1995; Joliffe, 1986, Wold et al., 1987) aims to reduce the dimensionality of a data set, while simultaneously retaining the information present in the data. It allows the transformation and visualization of complex data sets into a new perspective in which the more relevant information is made more obvious. PCA extracts maximal information from large data matrices containing numerous columns and rows because it calculates the correlations between the columns of the data matrix and classifies the variables according to the coefficients of correlations (Cserháti; 2010; Kaliszan, 1997; Mardia et al., 1979; Vandeginste et al., 1998).

The original measurement variables are transformed into new conceptually meaningful variables called principal components which account for most of the variation providing reduction of the dimensionality of the dataset. By plotting the data in a coordinate system defined by the two or three largest principal components, it is possible to identify key relationships in the data, that is, find similarities and differences among objects in a data set.

The first component is the linear combination of variables that contribute most to the total variance. The second principal component is orthogonal to the first and accounts for most of the residual variance. Each principal component describes a different source of information because each defines a different direction of scatter or variance in the data (the scatter of the data points in the measurement space is a direct measure of the data's variance). Hence, the orthogonality constraint imposed by the mathematics of PCA ensures that each variance-based axis will be independent (Lavine, 2000).

One measure of the amount of information conveyed by each principal component is the variance of the data explained by the principal component. The variance explained by each principal component is expressed in terms of its eigenvalue. For this reason, principal components are usually arranged in order of decreasing eigenvalues or waning information content. The most informative principal component is the first and the least informative is the last. By examining the eigen vector for those variables that load heavily to the component axis, it is possible to give the principal axis a physical interpretation. The closer the values are to 1 or -1, the more they contribute to that component, i.e. the axis aligned to the variable is also closely aligned to the component axis. If the value is closer to 0, the axis for the variable is at a right angle to the component axis and does not influence it greatly.

Due to its versatility and its easy-to-use multivariate mathematical–statistical procedure, PCA is frequently used in many fields of up-to date research, such as environmental protection studies (Cserháti; 2010; Hildebrandt et al., 2008).

2.2 Optimization experimental designs. Orthogonal Arrays

The optimization of any process can be tried either by the trial and error method, the one-at-a-time design or achieved by experimental design methods. The one-at-a-time design is a classical **Univariate method** which consists of investigating the response for each factor while all other factors are held at a constant level. Therefore, the variation of response can be attributed to the variation of the factor. They are time-consuming methods which do not take interactive effects between factors into account because the real optimum cannot be achieved. In this case, the use of factorial designs, which are based in blocking, is very useful because the response is measured for all possible combination of the chosen factor levels.

Blocking is one of the fundamental principles of good experimental design because it reduces the variability from the most important sources and hence increases the precision of experimental measurements. Essentially, experimental units are grouped into homogeneous clusters in an attempt to improve the comparison of treatments by randomly allocating the treatments within each cluster or "block" (Hanrahan et al., 2008).

Screening techniques such as **Factorial Designs** allow the analyst to select which factors are significant and at what levels. Such techniques are vital in determining initial factor significance for subsequent optimization. The most general (two-level design) is a full factorial design and described as 2k designs, where the base 2 stands for the number of factor levels and k is the number of factors each with a high and a low value (Bruns et al., 2006; Otto, 1999). One obvious disadvantage of factorial designs is the large number of experiments required when several variables are examined. However, this number can be considerably reduced by the use of **Fractional Factorial Designs**, such as **Orthogonal Array** designs **(OA)** (Lan et al., 1994; Lan et al., 1995), orthogonal meaning balanced (Wan et al., 1994). The theory and methodology of OA, as a chemometric method for the optimization of the analytical procedure, have been described in detail elsewhere (Lan et al., 1994; Lan et al., 1995). They imply the use of a strategically designed experiment which deliberately introduces changes in order to identify factors affecting the procedure, and estimate the factor levels yielding the optimum response with minimal experimental investment (Oles, 1993; Wan et al., 1994). They assign factors to a series of experiment combinations whose results can then be analyzed by using a common mathematical procedure. The main effects of the factors and preselected interactions are independently extracted.

Although the optimization by factorial designs is regarded as a simultaneous method, the optimum is actually located step by step as in sequential approaches. Therefore, previous knowledge of the variables, past experience and intuition are very helpful in arranging the variables and levels of the experiment because OA only cover a predefined region (Wan et al., 1994).

Taguchi Parameter Design, which uses OA, introduces, in addition, the concept of the signal-to-noise ratio to evaluate the variation of the response around the mean value due to experimental noise, which makes the optimum response robust against uncontrollable external variability, named noise factors (Barrado et al., 1998; Bendell et al., 1989; Ross, 1988; Taguchi, 1991). It allows separating the effect of each factor on the output variable in terms of mean response (regular analysis) and signal-to-noise ratio analysis. It has the following aims: to identify factors affecting the procedure, to estimate the factor values leading an optimum response and to decrease the process variability without controlling or eliminating causes of variation, which yields a process robust against noise factors.

The steps for implementing the experimental design are the following:

1. To select the output variable to be optimised,

2. To identify factors and their interactions affecting the output variable and to choose the levels to be tested,
3. To select the adequate orthogonal array,
4. To assign factors and interactions to the columns of the array,
5. To perform the experiments,
6. To carry out an statistical analysis of the data and determine the optimum factor levels, and
7. To conduct a confirmatory experiment.

Different OA have been applied in analytical method development allowing the identification of the principal and interaction effects of the extraction conditions on the recovery of pollutants (Mostert et al., 2010), and more specifically to pesticides from various environmental samples, such as vegetables (Pena et al., 2006; Quan et al., 2004; Wan et al., 2010), soils (Delgado-Moreno et al., 2009; Fuentes et al., 2007; Sun et al., 2003) or water samples (Bagheri et al., 2000; Chee et al., 1995; Lin & Fuh, 2010; Pasti el al, 2007; Wells et al., 1994; Wan et al., 1994). OA have also been applied to the optimization of derivatization procedures to analyse pesticides by GC (Stalikas & Pilidis; 2000).

3. Experimental procedures

3.1 Principle of the experimental method

The application of some chemometric strategies in order to develop a multiresidue extraction and analysis method for nearly 40 herbicides, belonging to very different chemical families, in agricultural soils of barley crops is shown.

The influence of some variables in recovery was studied by a set of previous experiments analyzed by PCA and Clustering techniques. Then, the most important factors affecting the multiresidue herbicide extraction were optimized by an OA.

The acidic and phenol herbicide methylation by TMSH in order to analyse their methyl esters/ethers by GC, was also optimised by an OA.

3.2 Reagents, equipment and analysis

Reagents

- The herbicides studied in this work are summarised in Table 1 together with some important physicochemical properties (The FOOTPRINT Pesticide Properties DataBase, 2006). All herbicide standards were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Individual stock standard solutions (1000 mg/l) were prepared in acetone and stored in the dark at -20ºC. They were kept for 1 hour at ambient temperature previously to their use. Working standard mixtures in acetone, containing 10 mg/l of each pesticide were prepared by dilution.
- Calibration standards were prepared by dilution in acetone acidified with 1% acetic acid. The internal standard was prepared by dissolving Alachlor (a sunflower herbicide) in acetone to make stock solutions of 1000 mg/l and diluted in acetone acidified with 1% acetic acid to 1 mg/l before the addition of 20 µl to samples.
- Organic solvents intended for extraction, were at least HPLC grade and were provided by Labscan (Dublin, Ireland) together with the glacial acetic acid and the ammonium hydroxide (28% in water).
- Trimethylsulfonium hydroxide (TMSH) purum 0.25 M in methanol, was purchased from Fluka (Buchs, Switzerland) and stored at 4ºC.

- Bulk quantities of Na_2SO_4, obtained from Merck (Darmstadt, Germany), were heated to 500°C for more than 5 hours to remove phthalates and any residual water prior to its use in the laboratory.
- The same soil was used for all the tests: 46% sand, 37% silt, 17% clay; 0.69% organic matter, 8.5 pH (H2O) and 9.2 meq/100 g ion exchange capacity. Soil samples were allowed to dry at room temperature in the dark, sieved and frozen at -20ºC till extraction.

Equipment and analysis

- An Agilent Technologies 6890N Network GC System Chromatograph (Waldbronn, Germany) equipped with an Agilent Technologies 7683 Series Splitless Injector and an Agilent Technologies 5973 Quadrupole Mass Selective Detector operated in the SIM mode was used. Injector temperature was set at 250ºC and the transfer line temperature at 280ºC. Splitless injection volume was 1 µl.
- A J & W Scientific, DB-17, (30 m × 0.25 mm I.D.), 0.25 µm film thickness column, was employed with helium (99.999% purity) as carrier gas at a constant flow of 1 ml/min.
- The oven temperature for neutral and basic analytes, was maintained at 60ºC for 1 min and then programmed at 6ºC/min to 165ºC, then at 12ºC/min to 215ºC, then at 2ºC/min to 230ºC and finally at 8ºC/min to 280ºC, held for 10 min.
- The oven temperature for acids analysed as their methyl esters/ethers, was maintained at 60ºC for 1 min and then programmed at 22ºC/min to 290ºC, held for 4.55 min.
- Acidic herbicides were compared with procedural standards, i.e. mixtures of acid standards of known concentration derivatized in the same way as samples.

3.3 Working procedure

3.3.1 PCA and cluster analysis. Previous experiments for soil extraction OA

Table 2 shows the previous tests designed to characterize the influence of the variables that would be further optimized with the OA after their analysis by PCA and Cluster techniques. These experiments were designed taking into account the Kovacs series of extraction solvents (Kovacs, 1996), together with the use of water and different modifiers (acetic acid and ammonium hydroxide) in order to increase recoveries of ionic herbicides as already detailed in section 2.3. Acetone was chosen as the unique organic solvent in these previous experiments because it has been widely used in herbicide extraction (Sánchez-Brunete & Tadeo, 1996), and its medium polarity and water miscibility provided a general overview. All quantities were made equivalent in order to compare recoveries. The same sample:solvent ratio was used in all these previous tests (1:3.2). A fixed water volume of 7.5 ml, enough to adequately wet 15 g of the spiked soil, was added in all the experiments where water addition was tested.

After shaking 15 g of blank soil samples, spiked at 0.05 mg/l, with the corresponding extraction mixture for 1 hour, and centrifugation at 2500 rpm for 5 min, an extract volume equivalent to 8 g of soil was recovered and concentrated until near dryness in a turbo vap at 35°C. Then, the concentrated extract was filled up with acetone:1% acetic acid until an equivalent concentration of 8 g/ml, filtered through a 0.45 µm PTFE filter and added the internal standard previously to the GC–MS analysis. In case water was present in the extraction mixture, the supernatant was previously partitioned after the centrifugation with 30 ml of dichloromethane and enough Na_2SO_4 to bind the water. No pH adjustment and pH

No.	Compound	MF	Structural group	MW	log Kw	pKa
1	Dicamba	$C_8H_6Cl_2O_3$	Benzoic acid	221.0	0.55	1.87
2	2,4-D	$C_8H_6Cl_2O_3$	Aryloxyalkanoic acid	225.7	-0.83	2.87
3	MCPP	$C_{10}H_{11}ClO_3$	Aryloxyalkanoic acid	214.6	0.64	3.11
4	Dichlorprop p	$C_9H_8Cl_2O_3$	Aryloxyalkanoic acid	235.1	-0.56	3.67
5	MCPA	$C_9H_9ClO_3$	Aryloxyalkanoic acid	200.6	2.80	3.73
6	Amidosulfuron	$C_9H_{15}N_5O_7S_2$	Sulfonylurea	369.4	1.63	3.58
7	Tribenuron methyl	$C_{15}H_{17}N_5O_6S$	Sulfonylurea	395.4	0.78	4.70
8	Fenoxaprop p	$C_{16}H_{12}ClNO_5$	Aryloxyphenoxypropionic acid	333.8	1.83	4.60
9	Diclofop	$C_{15}H_{12}Cl_2O_4$	Aryloxyphenoxypropionic acid	326.2		3.60
10	Flamprop	$C_{16}H_{13}ClFNO_3$	Aryaminopropionic acid	221.0	2.90	3.70
11	Bromoxynil	$C_7H_3Br_2NO$	Hydroxybenzonitrile	276.9	1.04	3.86
12	Ioxynil	$C_7H_3I_2NO$	Hydroxybenzonitrile	370.9	2.20	4.10
13	Cyanazine	$C_9H_{13}ClN_6$	Chlorotriazine	240.7	2.10	0.63
14	Terbuthylazine	$C_9H_{16}ClN_5$	Chlorotriazine	229.7	3.21	2.00
15	Terbutryn	$C_{10}H_{19}N_5S$	methylthiotriazine	241.4	3.65	4.30
16	Metribuzin	$C_8H_{14}N_4OS$	Triazinone	214.3	1.65	
17	Carfentrazone ethyl	$C_{13}H_{10}Cl_2F_3N_3$	Triaolinone	412.2	3.36	
18	Metoxuron	$C_{10}H_{13}ClN_2O_2$	Phenylurea	228.7	1.60	
19	Isoproturon	$C_{12}H_{18}N_2O$	Phenylurea	206.3	2.50	
20	Chlortoluron	$C_{10}H_{13}ClN_2O$	Phenylurea	212.7	2.50	
21	Methabenzthiazuron	$C_{10}H_{11}N_3OS$	Urea	221.3	2.64	
22	Linuron	$C_9H_{10}Cl_2N_2O_2$	Phenylurea	249.1	3.00	
23	Tralkoxydim	$C_{20}H_{27}NO_3$	Cyclohexadione oxime	329.4	2.10	
24	Flamprop isopropyl	$C_{19}H_{19}ClFNO_3$	Aryaminopropionate	363.8	3.69	
25	Mefenpyr diethyl	$C_{16}H_{18}Cl_2N_2O_4$	Herbicide safener	373.2	3.83	
26	MCPA tioethyl	$C_{11}H_{13}ClO_2S$	Phenoxyacid	244.7	4.05	
27	Bifenox	$C_{14}H_9Cl_2NO_5$	Diphenyl ether	342.1	4.48	
28	Fenoxaprop p ethyl	$C_{18}H_{16}ClNO_5$	Aryloxyphenopropionate	361.8	4.58	
29	Diclofop methyl	$C_{16}H_{14}Cl_2O_4$	Aryloxyphenopropionate	341.2	4.60	
30	Prosulfocarb	$C_{14}H_{21}NOS$	Thiocarbamate	251.4	4.65	
31	Triallate	$C_{10}H_{16}Cl_3NOS$	Thiocarbamate	304.7	4.66	
32	Diflufenican	$C_{19}H_{11}F_5N_2O_2$	Carboxamide	394.3	4.90	
33	Pendimethalin	$C_{13}H_{19}N_3O_4$	Dinitroaniline	281.3	5.18	
34	Trifluralin	$C_{13}H_{16}F_3N_3O_4$	Dinitroaniline	335.3	5.27	
35	MCPP isoctylic	$C_{18}H_{27}ClO_3$	Phenoxypropionate	327.6		
36	Bromoxynil octanoate	$C_{15}H_{17}Br_2NO_2$	Hydroxybenzonitrile	403.0	5.40	
37	Ioxynil octanoate	$C_{15}H_{17}I_2NO_3$	Hydroxybenzonitrile	497.1	6.12	

Table 1. Chemical characteristics of the herbicides of study: Molecular Formula (MF), Structural group, Molecular Weight (MW), Octanol-water coefficient (log Kw), and Acid dissociation constant (pKa 25ºC).

Previous Tests	% HAc or NH_3	Solvent (ml)	Water (ml)	% Rec. Total	%RSD (n=5)	% Rec. Acids	%RSD (n=5)	% Rec. Basic-Neutrals	%RSD (n=5)
Ac NaOH 0.5N DCM pH 2		47.50	47.50	97.55a	0.49	95.09a	0.90	98.72a	0.64
Ac:H_2O DCM		40.0	7.50	81.12d	0.86	49.62c	3.69	96.24ab	1.41
Ac:H_2O DCM pH2		40.0	7.50	86.55c	1.16	97.34a	1.88	81.37d	0.90
Ac:HAc	1.0	47.5		87.37c	2.51	60.81b	4.65	100.12a	1.90
Ac:H_2O:HAc DCM	1.0	40.0	7.50	92.70b	0.77	92.42a	0.86	92.84b	0.75
Ac:H_2O:HAc DCM pH2	1.0	40.0	7.50	89.72bc	0.61	97.08a	0.57	86.19c	1.21
Ac:NH_3	0.1	47.5		69.77e	0.60	20.79d	8.33	93.29b	1.04
Ac:H_2O:NH_3 DCM	0.1	40.0	7.50	78.58d	1.09	47.19c	2.13	93.64b	0.85

Table 2. Previous tests recoveries (% Rec.) and relative standard deviations (RSD) at 50 μg kg^{-1} spiking level soil samples. Means followed by different letters in the same column are significantly different at $p < 0.01$ level according to Tukey honest test for equal number of replicates. Acetone (Ac), Acetic Acid (HAc), DCM (dichloromethane).

adjustment to pH 2 were developed previously to the dichloromethane partition to study whether polar herbicides were lost in the aqueous phase with the different solvent combinations tested.

3.3.2 Optimization of operational variables. Herbicide soil extraction OA

The average recoveries were used as the output variable to optimize. Different OA were developed for acidic analytes and for basic and neutral herbicides due to dual methyl ester formation from the TMSH derivatization of acids and their ester forms prior to their GC analysis, in order to know which form the methyl ester came from.

After carefully studying the results obtained from the previous experiments analyzed by PCA, the following variable values were selected for the multiresidue extraction OA: solvent type and ratio, pH (percentage of acetic acid) and shaking time as showed in Table 3. Acetone, ethyl acetate and acetonitrile were selected as organic solvents because they are among the most used extractants in neutral and basic multiresidue herbicide procedures in soils. Ammonium hydroxide was not suitable for acids and showed no effect in basic recoveries, therefore it was not further used. The same water volume used in the previous experiments was taken for the OA due to its utility in mixtures with acetic acid and acetone. Due to different solvent volumes, water percentages changed from 14.3% to 33.3%, acetic acid percentages changed from 0.3% to 1.7%, and organic solvent percentages changed from 65.3% to 85.3%, covering the values found in the references (Ahmed & Bertrand, 1989; Crescenzi et al., 1999; Smith & Milward, 1981; Sutherland et al., 2003; Thorstensen & Christiansen, 2001).

Three levels for each control factor instead of two were chosen to detect any quadratic or non-linear relation between the factors and the output variable, and to obtain information over wider ranges of the variables. Four control factors at three levels contain eight degrees of freedom, and can be fitted to the $L_9(3^4)$ OA. The nine different trials resulting from this design were duplicated to calculate the residual error, and randomized to minimize the effects of uncontrolled factors that may introduce a bias on the measurements (Table 6).

Notation	Factor	Level 1	Level 2	Level 3
S	solvent + water	acetone	ethyl acetate	acetonitrile
A	% acetic acid	0.5	1	2
V	volume (ml)	15 (1:1.5)	30 (1:2.5)	45 (1:3.5)
T	shaking time (seg.)	15	30	60

Table 3. Factors and levels for the herbicide soil extraction $L_9(3^4)$ OA optimization.

A 15 g amount of blank soil spiked at 0.05 mg/l was added 7.5 ml water, shacked the corresponding time with the appropriate solvent mixture, centrifuged and partitioned with dichloromethane. A fixed extract volume equivalent to 8 g of soil was evaporated to dryness in every experiment, dissolved in 1 ml of acetone:1% acetic acid and split in two aliquots. One of them was directly analyzed by GC-MS and the second one was derivatized before the acidic analyte analysis with an optimized procedure described afterwards, which consists on adding 100 µl of TMSH derivatization reagent to 500 µl of final extract directly in the vial. The effect of the presence of substance/s in the matrix in the chromatographic determination, was corrected with the use of calibration lines prepared in 900 µl of blank soil extracts obtained in the same way as samples in each trial, i.e. matrix-matched standard calibration (Analytical Methods for Pesticide Residues in Foodstuffs, 1996).

3.3.3 Optimization of operational variables. Acidic herbicide analysis OA

Acidic herbicides were divided in two groups, those only present in their acidic form and those also esterified. These esters were called "original" to differentiate them from the methyl esters produced after derivatization. Due to dual methyl ester formation, different OA were developed for the acidic herbicides (named "Acid matrix") and for the original esters (named "Ester matrix") in order to know which form the methyl ester came from and the way factors affected both esterification and trans-esterification reactions.

The total peak area value, defined as the total sum of peak areas, was used as variable to optimize because the formation of peaks as high as possible was the goal, therefore no calibration was necessary. Two output variables were chosen to be optimized due to dual methyl ester formation and the separately OA for acidic and original ester herbicides. TMEPA (total methyl ester peak area) was calculated in both matrices to study methyl ester formation meanwhile, TOEPA (total original ester peak area) was only evaluated in the "Ester matrix" to know the amount of remaining non-trans-esterified original esters.

Notation	Factor	Level 1	Level 2	Level 3
S	solvent	acetone	ethyl acetate	acetonitrile
T	time of incubation (min)	5	30	45
C	temperature of incubation (ºC)	20	40	70
P	pH	–	1 % acetic acid	1 % phosphoric acid

Table 4. Factors and levels for the acidic herbicide analysis $L_9(3^4)$ OA optimization.

Organic solvents alone, slightly and strongly acidified (added 1% acetic acid and 1% phosphoric acid, respectively) were selected as reaction media because they are usually employed for acidic herbicide extraction as already detailed in section 2.3. Subsequently, final extract derivatization reactions were affected by pH values, which have been reported to play an important role in the process (Catalina et al., 2000).

The direct injection of analytes and TMSH mixtures into the hot injection port of the GC has been reported (Zapf & Stan, 1999). For some weak acids deprotonation and thermally decomposition of the resulting salts after derivatization have been reported to occur simultaneously in a heated GC injector (Rompa et al., 2004), meanwhile other authors recommend pre-heating in an oven in a closed sample vial previously to injection (Halket & Zaikin, 2004). In order to evaluate the usefulness of pre-heating, standard mixtures were incubated for 5-30-45 min at three different temperatures: 40 ºC (recommended maximum heating temperature recommended in the TMSH label), 70 ºC, both maintained in an oven, and 20 ºC, kept constant in an incubation chamber to simulate the absence of pre-heating.
Consequently, the following variables were selected: temperature and time of incubation, solvent and pH (composition of reaction mixture) (Table 4).
All experiments were carried out with standards diluted in the tested solvent at a concentration of 250 μg/l in order to avoid the possibility of finding matrix derivatized interferences.
Previously, the optimum quantity of TMSH was studied and 100 μl of a solution of TMSH 0.25 M in methanol added to 500 μl standard solutions were shown enough to provide a high excess of derivatizing reagent and to ensure the complete derivatization of all compounds present in the sample.
Four control factors at three levels contain eight degrees of freedom, and can be fitted to the $L_9(3^4)$ OA. The nine different trials resulting from this design were randomized and duplicated in order to calculate the residual error, so a total number of 18 standard solutions were derivatized and analysed by GC-MS to determine the corresponding total peak area values as described above (Table 8).

3.4 Results and discussions

3.4.1 PCA and cluster analysis results. Previous experiments for soil extraction OA

PCA was applied to the average herbicide recovery values of 5 replicates obtained from the previous experiments (Table 2) in order to provide a global overview and clarify the relationships among the several variables related to the extraction procedure and their effects on extractability. Both average recoveries for basic and neutral herbicides with acetone extraction and for acidic herbicides with alkaline extraction were taken together as the specific method results.
Statistical analyses were performed using the Minitab v.13.0 program package, with the Ward linkage method, and using none rotation option.
From the PCA it was found that 93.80% of the variation of the dataset could be explained using four factors. From the loading on the four factors of the PCA (Table 5) some conclusions can be drawn. The factor pattern of component 1 showed contributions from a set of procedures intended for neutral and basic herbicides while those more specific for acid herbicides formed the component 2. Component 3 and 4 consisted on both the specific methods and the acetone-water-acetic acid combination for all the herbicides of study.
The PCA showed groupings of the herbicides based on their chemical nature (Fig. 1). Acidics are grouped separately from the basics and neutrals, which did not show a different trend between them implying that both types of analytes could be extracted with the same procedures. However, acids are very different in nature and needed specific extraction methods. Loadings for both methods with pH adjustment before the partitioning step, lay near the acidic analytes (dotted lines) while loadings for acetone in combination with water and ammonium hydroxide (striped lines) are orientated to the basic and neutral grouping.

	Components			
Variables	1	2	3	4
Specific		-37.89	-82.52	-35.91
Ac:H_2O DCM	-92.51			
Ac:H_2O DCM pH2		-90.25		
Ac:HAc	-92.58			
Ac:H_2O:HAc DCM		-50.72	51.77	-64.08
Ac:H_2O:HAc DCM pH2		-90.82		
Ac:NH_3	-94.85			
Ac:H_2O:NH_3 DCM	-88.78			
%Variance	45.80	26.20	12.40	9.40

Table 5. Loading of variables on the four first components resulting from the PCA of extraction procedures with different solvent combinations with water and modifiers. Component loading less than |0.35| are omitted.

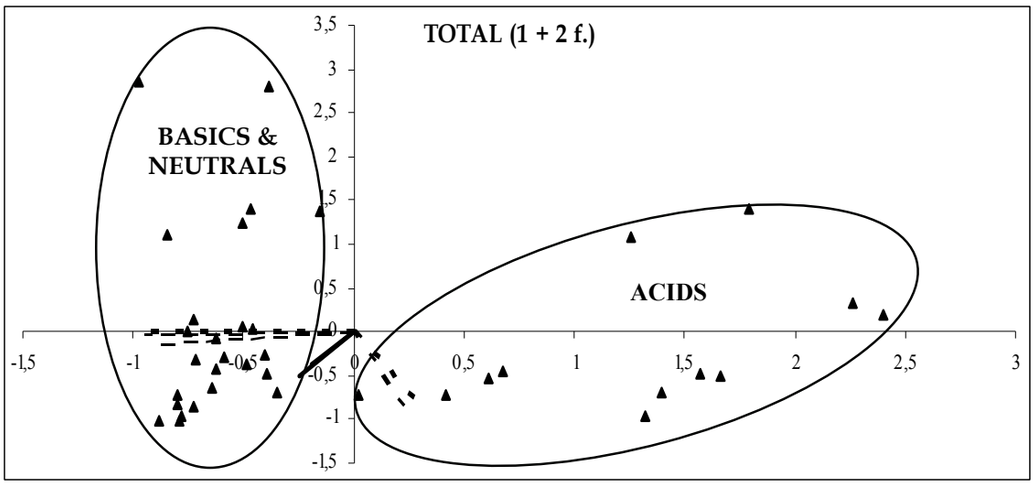

Fig. 1. Score plot for first two factors for all the herbicides. Loadings for the 8 different methods tested have been represented as lines.

Loadings for the specific methods and the acetone-water-acetic acid combination (black lines) are directed in the same way, their direction of maximum dispersion laying between the acid and basic and neutral groupings, what it could indicate the suitability of this combination of solvents and modifiers as multiresidue methods.

This result was also found in the cluster analysis (Fig. 2), where procedures were grouped in similarity in this way: neutral and basic herbicides, liquid-liquid partitioning with dichloromethane at pH 2 for acids and, specific methods and acetone-water-acetic acid for all of the studied herbicides.

The acetone-water-acetic acid combination was significantly the more efficient in extracting the whole range of different herbicides apart from the specific methods. The best acidic average recoveries were found for those combinations using water-acetic acid and those using a partitioning step with prior pH adjustment to pH 2. However, these both last methods were exactly the less effective in extracting basic and neutral analytes, although they were significantly recovered by the rest of the tested extraction methods. Basic recovery

showed no enhancement with the use of ammonium hydroxide as expected (Smith & Milward, 1981).

Basic herbicides behaved in the same way as neutral analytes; therefore their recoveries were averaged together. The significance of differences among the procedure recoveries were examined by applying analysis of variance (ANOVA). Values represent means for the average recovery replicates for all the spiked blank soil samples extracted with the different procedures tested. Means followed by different letters in the same column are significantly different at $p < 0.01$ level according to Tukey honest test for equal number of replicates (Table 2).

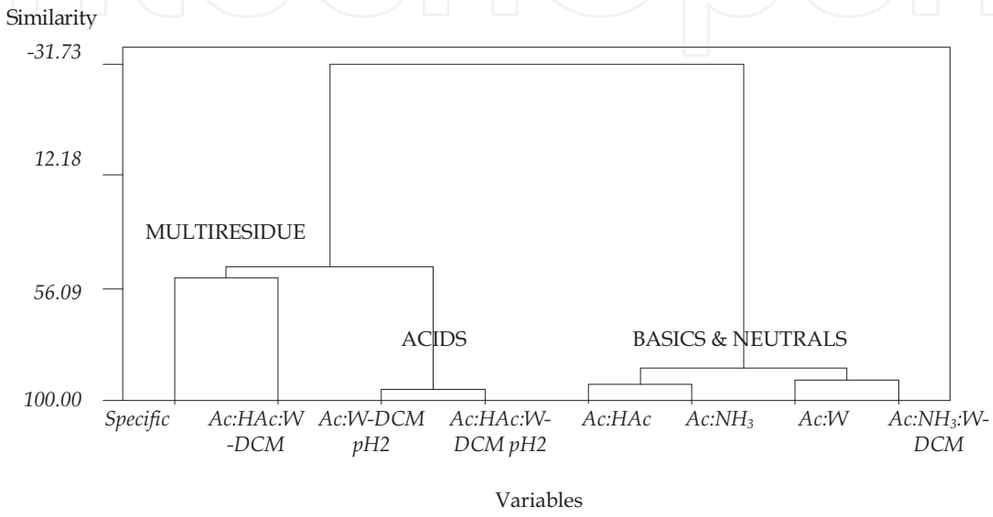

Fig. 2. Cluster variables for the eight extraction solvent combinations tested in the previous experiments.

The addition of water alone did not significantly recover more residues than the organic solvent as previously reported (Tadeo et al., 1996). However, the addition of acetic acid to the water and acetone combination enhanced significantly the acidic recovery with no detrimental in the basic and neutral extraction, and no pH adjustment prior to the dichloromethane partition was needed.

After carefully studying the results obtained from the previous experiments by PCA and Cluster analysis, the following variables were selected for the subsequent OA design: solvent type and ratio, pH (percentage of acetic acid) and shaking time.

3.4.2 Herbicide soil extraction OA results

Table 6 shows the average recovery data obtained by duplicate for each of the 9 experiments. For the regular analysis, an ANOVA table with pooled errors was calculated from these experimental data in order to identify individual sources of variation and to calculate the contribution of each factor to the response variation (Table 7).

ANOVAs of the recovery data obtained for both matrices revealed that factor S, the type of solvent, contributed by the highest percentage to the variability of the recoveries (49.1% for acids and 67.2% for basics and neutrals). Maximum recovery for all the analytes was obtained for level S1, acetone (Fig. 3). In contrast to acetone and acetonitrile, ethyl acetate was practically immiscible with water which could be easily removed by using only

anhydrous Na_2SO_4 as a drying agent. However, the dichloromethane partition was also carried out in order to develop all experiments in the same way and to benefit from the polar interference removal provided by the partitioning. In addition to the variability in recoveries due to the immiscibility of ethyl acetate with water and acetic acid, pesticides with a thioether group (ureas) have been reported to degrade in the ethyl acetate (Mastovska & Lehotay, 2004), what explains the lower recoveries observed when using this solvent. Acidic herbicides were very influenced by the acetic acid percentage (46.1%), meanwhile the contribution for basic and neutral was low (4.1%). Maximum recovery of the acid herbicides was obtained for level A3 (2% acetic acid), meanwhile level A1 (0.5% acetic acid) provided the maximum recovery for basics and neutrals (Fig. 3).

	Control Factors and Levels				Acids		Basics & Neutrals	
Trial	*S*	*A*	*V*	*T*	*1*	*2*	*1*	*2*
1	1	1	1	1	80.13	77.64	100.54	100.84
2	1	2	2	2	93.34	92.12	93.65	94.40
3	1	3	3	3	94.18	94.30	83.12	83.34
4	2	1	2	3	69.67	69.51	76.73	76.59
5	2	2	3	1	74.86	74.35	67.68	68.83
6	2	3	1	2	81.77	82.54	78.15	79.28
7	3	1	3	2	79.73	80.23	84.77	85.91
8	3	2	1	3	88.13	88.21	93.67	93.84
9	3	3	2	1	93.83	92.97	86.46	87.08

Table 6. Experimental average recoveries obtained for each duplicated trial in the herbicide soil extraction $L_9(3^4)$ OA optimization.

Variation source		S. Solvent + Water	A. % Acetic acid	V. Volume	T. Shaking time	Residual	Total
Degrees of freedom		2	2	2			8
Basics & Neutrals	Sum of squares	1055.34	65.30	441.03	3.99		1567.96
	Variance ratio (F)[a]	923.58	57.15	385.96			
	Pool	No	No	No	Yes	Yes	
	Pooled sum of squares	1054.20	64.16	439.89		9.71	
	Contribution (%)[b]	67.23	4.09	28.05		0.62	100.00
Acids	Sum of squares	313.14	293.88	10.01	10.88		1260.59
	Variance ratio (F)[a]	87.42	82.05				
	Pool	No	No	Yes	Yes	Yes	
	Pooled sum of squares	619.11	580.59			60.89	1260.59
	Contribution (%)[b]	49.11	46.06			4.83	100.00

[a]Critical variance ratio for a 95% confidence level is 19.00.
[b]Contribution is defined as 100 x (pooled sum of squares/total sum of squares).

Table 7. Pooled ANOVA for the regular analysis of the mean average recoveries obtained for acidic, basic and neutral herbicide soil extraction $L_9(3^4)$ OA optimization.

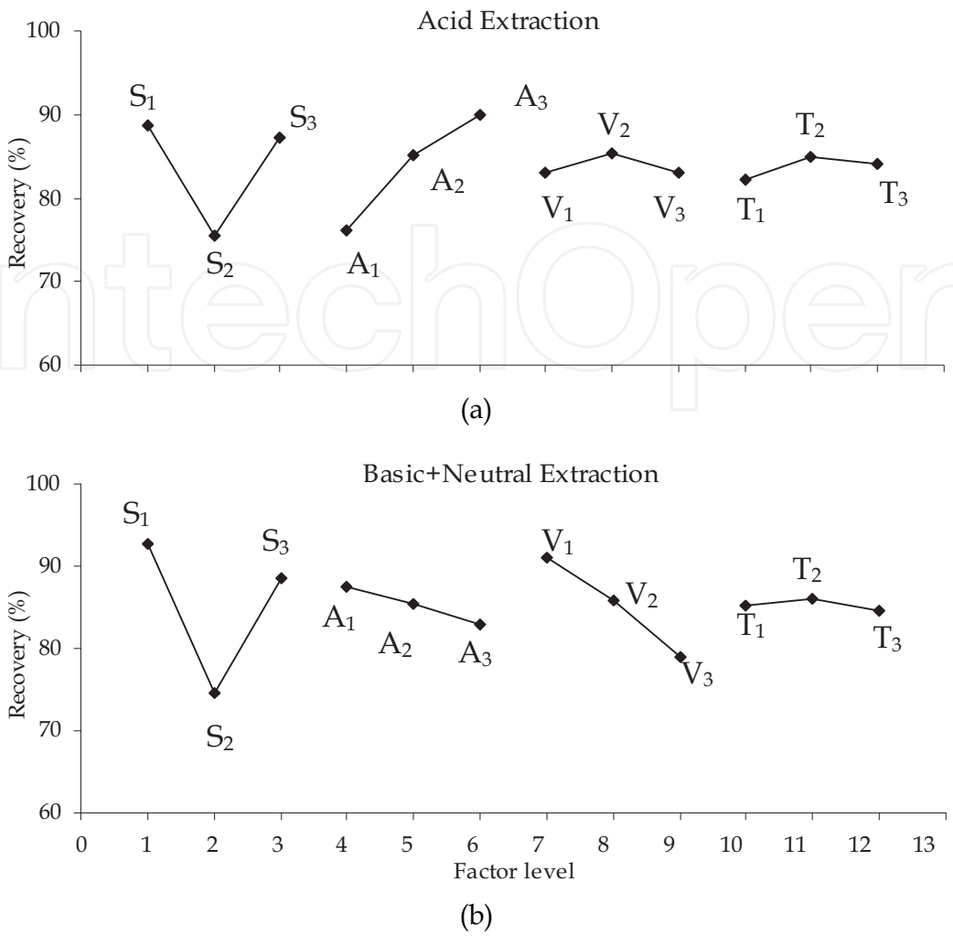

Fig. 3. Effect of the interaction of control factors on the mean response obtained for acidic (a), and basic and neutral (b) herbicide soil extraction $L_9(3^4)$ OA optimization.

Solvent volume was significant for basics and neutrals (28.1%) due to their volatility; however, this factor was pooled for acids. The maximum recovery was obtained for level V1, 15 ml, for basics and neutrals. An equivalent volume of 8 g of soil was taken to near dryness in all the tests, therefore more extract volume was concentrated when using 30 and 45 ml of solvent, leading to a higher loss of more volatile analytes, even after adding 20% of ethylene glycol in acetone as a holder solution when evaporating. However, solvent volume had not a statistically significant effect (at 95% confidence level) on the acid recoveries. Acids are not lost during evaporation in the same way as basics and neutrals, because they are taken to near dryness in their non-volatile acidic form and converted to the more volatile methyl esters/ethers just before analysis. As a compromise between both types of analytes, level V2, 30 ml, was selected. The main advantage of acetone over ethyl acetate and acetonitrile was its greater volatility, having the smallest boling point (56.2ºC for acetone, 77.1ºC for ethyl acetate and 81.6ºC for acetonitrile) and therefore, minimizing volatile losses due to evaporation.

The time of extraction was negligible indicating that there were no significant differences (at 95% confidence level) among the levels tested, its contribution being pooled for all the analytes. Level T2, 30 min, was chosen, because it gave a slightly higher response than 15 min.

The contribution of the residual error to the recovery variability (4.8% for acids and 0.6% for basics and neutrals) indicates the experimental design took into account all the variables affecting the response, the levels tested were fit for the purpose and the variance of the experimental data was explained by the effect of factors and interactions

Fig. 3 shows the effects of control factor levels on the output variable. Factor T variation (shaking time) had a slight influence on recoveries, and a change in their level produced very small variation in the multiresidue herbicide extraction. However, the significant influence of the solvent type (S) for all analytes, the acetic acid percentage (A) for acids and the solvent volume (V) for basics and neutrals can be observed by the statistically different recoveries obtained when changing these variables.

3.4.3 Acidic herbicide analysis OA results

Table 8 shows the output variables, TMEPA and TOEPA, obtained by duplicate for each of the 9 experiments. For the regular analysis, an ANOVA table with pooled errors was calculated from these experimental data in order to identify individual sources of variation and to calculate the contribution of each factor to the response variation (Table 9).

ANOVAs of the TMEPA and TOEPA for both matrices revealed that factor P (pH) contributed by the highest percentage to the variability of the signal (93.78 % for methyl ester formation, 78.56 % for methyl ester conversion and 97.04 % for original ester permanence).

Although very small, contribution made by the other variables for methyl ester trans-esterification was the only one that could not be neglected. In both the cases of methyl ester formation and permanence of original esters, the rest of factors were negligible indicating that there were no significant differences (at 95% confidence level) among the levels tested.

The pH of the solution (P) during both esterification and trans-esterification processes has been shown to play an important role. The presence of the anionic form of the acids was essential for the formation of the trimethylsulfonium salts as well as for the previous saponification in trans-esterification. Both esterification and trans-esterification reactions were enhanced in a strong basic environment provided by the addition of TMSH that yielded a solution pH value of 9. However, the presence of 1 % acids neutralized this strong

Control Factors and Levels					*TMEPA* (x 10^5)				*TOEPA* (x 10^5)	
					Acids		*Esters*		*Esters*	
Trial	*S*	*T*	*C*	*P*	*1*	*2*	*1*	*2*	*1*	*2*
1	1	1	1	1	69.3	76.0	95.6	140.1	63.7	67.5
2	1	2	2	2	106.8	115.5	37.1	32.6	419.7	412.6
3	1	3	3	3	13.8	12.4	9.0	7.4	503.4	450.2
4	2	1	2	3	11.4	12.3	9.3	7.4	480.8	448.1
5	2	2	3	1	89.3	83.3	101.0	92.2	79.4	70.9
6	2	3	1	2	91.2	117.6	52.2	45.6	491.1	494.3
7	3	1	3	2	108.9	105.4	42.7	48.9	543.5	480.9
8	3	2	1	3	14.3	13.0	5.9	6.0	510.8	543.6
9	3	3	2	1	110.6	104.8	208.1	207.0	66.5	68.7

Table 8. Experimental average recoveries obtained for each duplicated trial in the acidic herbicide analysis $L_9(3^4)$ OA optimization.

Variation source		S. Solvent	T. Time of incubation	C. T[a] of incubation	P. pH	Residual	Total
Degrees of freedom		2	2	2	2		8
TMEPA (Acid Matrix)	Sum of squares (x 10^4)	3.81	3.77	5.41	301.80		319.30
	Variance ratio (F)[a]				129.20		
	Pool	Yes	Yes	Yes	No	Yes	
	Pooled sum of squares				294.40	19.90	319.30
	Contribution (%)[b]				93.78	6.22	100.00
TMEPA (Ester Matrix)	Sum of squares (x 10^4)	47.94	56.20	36.40	566.51		718.05
	Variance ratio (F)[a]	19.61	22.99	14.89	231.75		
	Pool	No	No	No	No	Yes	
	Pooled sum of squares	45.50	53.75	33.95	564.07	20.78	718.05
	Contribution (%)[b]	6.34	7.49	4.73	78.56	2.89	100.00
TOEPA (Ester Matrix)	Sum of squares (x 10^4)	69.00	1.58	70.98	6778.50		6960.01
	Variance ratio (F)[a]				280.03		
	Pool	Yes	Yes	Yes	No	Yes	
	Pooled sum of squares				6754.25	205.75	
	Contribution (%)[b]				97.04	2.96	100.00

Table 9. Pooled ANOVA for the regular analysis of total methyl ester peak area (*TMEPA*) in the Acid Matrix and *TMEPA* and total original ester peak area (*TOEPA*) in the Ester Matrix obtained for acidic herbicide analysis $L_9(3^4)$ OA optimization.

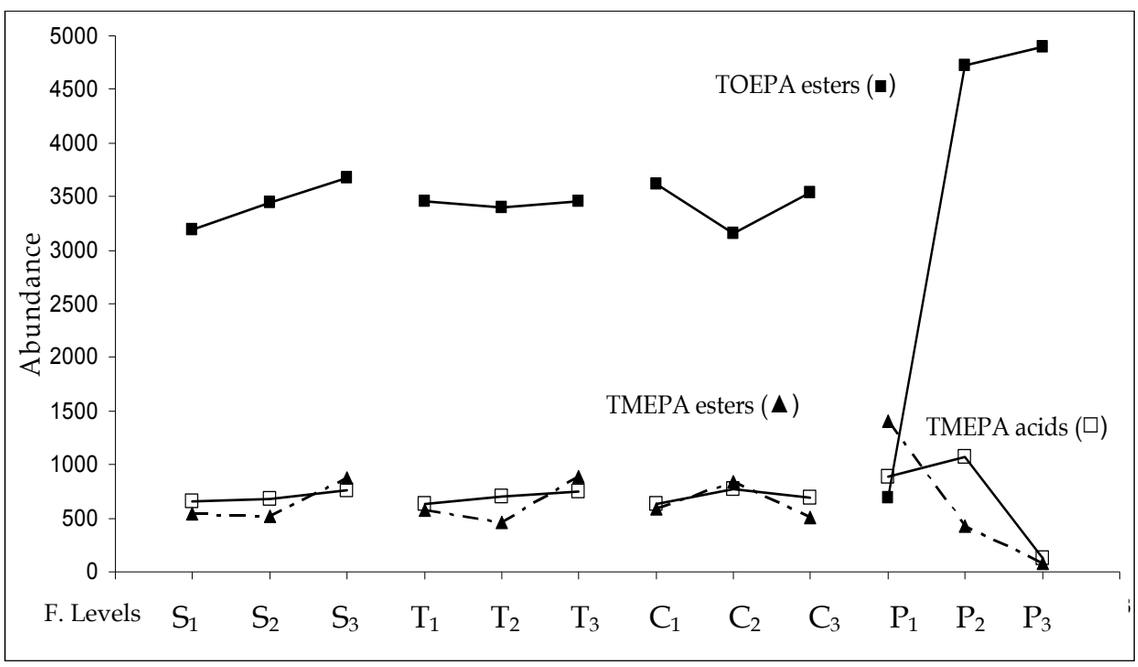

Fig. 4. Comparison of the effect of the interaction of control factors (f. levels) on the mean response for Original remaining Esters (■) and Methyl Esters in the Acid matrix (□) and in the Ester matrix (▲) obtained for acidic herbicide analysis $L_9(3^4)$ OA optimization.

basic TMSH media, and as a result, anionic forms of acids were not promoted and methylation yields decreased. A solution containing 1% of acetic acid had a pH value of 6 after adding TMSH meanwhile the strongest phosphoric acid decreased TMSH solution pH value till 2. Data in Table 8 clearly showed the effect of pH. All experiments developed at the same pH conditions had near TMEPA and TOEPA values regardless to the solvent, incubation time and temperature used.

Maximum methylation of acidic herbicides was obtained for P2, (pH) 1 % acetic acid (pH value of 6). The other three factors had not a statistically significant effect (at 95% confidence level) on the signal ratio; however, level S3 (solvent), acetonitrile; T3 (incubation time), 45 min; and C2 (incubation temperature), 40ºC, gave a slightly higher ratio. A slightly acidic environment gave the highest methyl ester formation but results were very close to those obtained in a basic medium. The very low methyl ester peak areas obtained with 1% of phosphoric acid, suggest that TMSH reaction was more influenced by very acidic pH values and the reaction worked properly from a neutral to a basic pH.

The contribution of the residual error to the TMEPA and TOEPA variability (6.22 %, 2.89 % and 2.96 % respectively) indicates the goodness of the experimental design used.

Fig. 4 shows the effects of control factor levels on the output variable. It can be observed that control factors different than pH (P) had a slight influence on the TMEPA and TOEPA value, and a change in their level produced very small variation in the conversion or permanence efficiency.

Fig. 4 also shows the effect of control factors on trans-esterification. TMEPA esters and TOEPA esters representations were obviously found to be opposite, the highest the methyl ester conversion, the smallest the permanence of remaining original esters. Both esterification and trans-esterification methyl ester formation were affected in the same way by pH being very diminished at strongly acidic pH values, although it seemed that trans-esterification needed a stronger basic media and did not work properly at a pH value of 6 (1% acetic acid) as esterification.

5. Conclusions

Herbicides play a very important role in agriculture but the toxicity and widespread of their residues pose a potential risk for the environment. In addition, their determination in soils is of primary importance because their dispersion in the environment depends on their behaviour in soils. The integration between analytical procedures and chemometric strategies has proved very valuable in the always difficult herbicide multiresidue extraction and analysis optimization development. The optimized methods have been applied to environment soils where herbicide residue data interpretation is of great interest.

The statistical analysis of the OA data revealed that all the factors were significant being the most important, the type and ratio of solvent for basic and neutral herbicides and the acetic acid percentage for acid herbicides. The final optimized method consisted of shaking previously wet soil samples for 30 min with 30 ml of acetone acidified with 1% acetic acid.

As a result, any organic solvent acidified with 1 % acetic acid was suitable for methylation with TMSH and as, pre-heating was shown not to improve derivatization yield, it was just necessary to add the derivatizing reagent to the sample vial and methylation was completely carried on in the injector port of the GC system.

6. Acknowledgement

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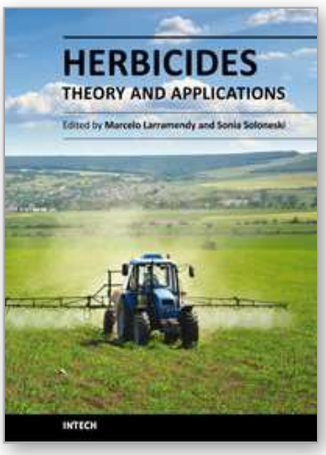

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The content selected in Herbicides, Theory and Applications is intended to provide researchers, producers and consumers of herbicides an overview of the latest scientific achievements. Although we are dealing with many diverse and different topics, we have tried to compile this "raw material" into three major sections in search of clarity and order - Weed Control and Crop Management, Analytical Techniques of Herbicide Detection and Herbicide Toxicity and Further Applications. The editors hope that this book will continue to meet the expectations and needs of all interested in the methodology of use of herbicides, weed control as well as problems related to its use, abuse and misuse.

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