Analysis of pesticides in soy milk combining solid-phase extraction and capillary electrophoresis-mass spectrometry

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Abbreviations: **ED** Experimental design; **NSM** Normal stacking mode; **TEA** Triethylamine

Abstract

In this work, the determination of a group of triazolopyrimidine sulfoanilide herbicides (cloransulam-methyl, metosulam, flumetsulam, florasulam and diclosulam) in soy milk by capillary electrophoresis-mass spectrometry (CE-MS) is presented. The main electrospray interface (ESI) parameters (nebulizer pressure, dry gas flow rate, dry gas temperature and composition of the sheath liquid) are optimized using a central composite design. To increase the sensitivity of the CE-MS method, an off-line sample preconcentration procedure based on solid-phase extraction (SPE) is combined with an on-line stacking procedure (i.e. normal stacking mode, NSM). Samples could be injected up to 100 seconds, providing limits of detection (LODs) down to 74 μg/L, i.e., in the low ppb level, with relative standard deviation values (RSD, %) between 3.8% and 6.4% for peak areas for the same day, and between 6.5% and 8.1% for three different days. The usefulness of the optimized SPE-NSM-CE-MS procedure is demonstrated through the sensitive quantification of the selected pesticides in soy milk samples.

1. Introduction.

In the last decade, capillary electrophoresis (CE) has gained much interest in analytical practice, especially in the analysis of agrochemicals [1]. In this sense, CE with MS detection is increasingly being used in pesticide determination, especially within very complex matrices [2-5]. CE-MS was first applied to the analysis of pesticides in 1989 by Lee et al. [6] and since then, several groups of pesticides have been determined by this technique [2,3,7-9]. Nowadays, among the different interfaces developed to couple CE with MS, electrospray ionization (ESI) [10] is the most frequently used. Although different types of ESI interfaces have been developed (e.g., sheath liquid, liquid junction, sheathless), the sheath-flow interface is the most commonly used due to its robustness and because, so far, it is the only ESI interface commercially available [11]. In spite of its robustness, the use of this interface requires a careful optimization of different parameters as, for instance, sheath-liquid composition, dry gas flow rate, dry gas temperature and nebulizer pressure. This optimization procedure is mainly carried out following a step-by-step approach requiring a high number of experiments and without taking into account possible interactions between the different factors or quadratic effects. A useful approach is to make use of experimental design (ED), in which each factor is varied in a programmed way, the obtained results can be easily interpreted and optimal conditions can be faster achieved. In this sense, few papers have made use of this tool to optimize ESI parameters [12-14]. Rudaz et al. [12], for example, have employed ED to optimize the separation of enantiomers using partial filling techniques and CE-MS. However, only the dry gas nebulization pressure was included in the ED optimization and no sensitivity problems were addressed. Another chemometrics application of ED to optimize ESI conditions was recently developed by

our group [3]. Thus, in that paper [3] ESI parameters were optimized by means of a suitable ED, allowing the CE-MS determination of a group of pesticides (namely, pyrimethanil, pyrifenox, cyprodinil, cyromazine, and pirimicarb) in fruit juices at ppb levels.

Cloransulam-methyl, metosulam, flumetsulam, florasulam and diclosulam [15] belong to the triazolopyrimidine sulfonanilide family of herbicides. They are used as pre-emergence and/or post-emergence herbicides mainly in soybeans or peanut crops in several countries. Its mode of action is through the inhibition of acetolactate synthase. As an example, cloransulam-methyl is frequently applied to the soil surface or incorporated in soybeans among others, as pre-emergence and post-emergence herbicide to control broadleaf weeds [16]. Together with diclosulam and flumetsulam, these three broadspectrum herbicides, are frequently used in the USA and registered by the USA Environmental Protection Agency (EPA) [17]. Florasulam is also registered by the European Union (EU) [18], while metosulam is registered and used in several countries around the world.

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In spite of the frequent combination of these herbicides for weed management, these compounds have mostly been analyzed individually by different techniques. Thus, ELISA has been applied to detect metosulam [19], HPLC to analyze cloransulam-methyl [20], MS to analyze florasulam [21] GC-MS for flumetsulam [22] and radiometric procedures to determine marked diclosulam [23]. To our knowledge, only one analytical procedure has been developed so far for the simultaneous analysis of these pesticides by using CE-UV [24]. This CE-UV procedure provides good results when applied to determine pesticides in different water samples, including tap and

stagnant water [24]. However, when this procedure was applied to soy milk (because of the frequent use of these herbicides in soy fields), a huge number of interferences was detected that precluded the determination of this group of pesticides by CE-UV (see below).

The aim of this work is, therefore, to develop a new and more selective analytical procedure able to determine in a single run a group of triazolopyrimidine sulfonanilide herbicides (diclosulam, cloransulam-methyl, flumetsulam, metosulam and florasulam) in complex matrices. In this sense, the use of CE-MS instead of CE-UV can be an interesting alternative based on the much higher selectivity and better sensitivity that MS can provide compared with UV detection. For this purpose, ESI parameters will be optimized using a suitable experimental design. SPE will be applied together with on-line preconcentration procedures (normal stacking mode, NSM) in order to improve the LODs. The usefulness of this optimized protocol that combines SPE, normal stacking and CE-MS, will be proved through the quantitative determination of these triazolopyrimidine pesticides in a complex matrix as soy milk at ppb levels.

2. Materials and methods.

2.1. Chemicals and samples.

All chemicals were of analytical reagent grade and used as received. Acetic acid, formic acid, ammonium hydroxide, ammonium acetate and ammonium carbonate were from Merck (Darmstadt, Germany). Triethylamine (TEA) was from Sigma Aldrich (Madrid, Spain). Methanol, isopropanol and acetonitrile (HPLC-grade) were from Scharlau (Barcelona, Spain). Distilled water was deionized by using a Milli-Q system (Millipore,

Bedford, MA, USA). Cloransulam-methyl (methyl 3-chloro-2-[[(5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl)sulfonyl]amino]benzoate), diclosulam (*N*-(2,6-dichlorophenyl)-5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-*c*]pyrimidine-2-sulfonamide), florasulam (*N*-(2,6-difluorophenyl)-8-fluoro-5-methoxy[1,2,4]triazolo[1,5-*c*]pyrimidine-2-sulfonamide), flumetsulam (*N*-(2,6-difluorophenyl)-5-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-2-sulfonamide) and metosulam (*N*-(2,6-dichloro-3-methylphenyl)-5,7-dimethoxy[1,2,4]triazolo[1,5-*a*]pyrimidine-2-sulfonamide) from Dr. Ehrenstofer GmbH (Cymit Quimica, Barcelona, Spain) were used without further purification. Standard solutions of each pesticide were prepared in acetonitrile and kept in the dark under refrigeration at 4ºC. Working mixtures of pertinent concentrations were daily made by appropriate combination and dilution with acetonitrile.

2.2. Capillary electrophoresis.

Two CE instruments have been used in this work. CE-UV analyses have been carried out using a PACE/5500 CE apparatus (Beckman, Fullerton, CA, USA) equipped with a DAD detector working at 205 nm. The instrument was controlled by a PC running the System Gold Software from Beckman. Bare fused silica capillaries with 50 μm i.d. were purchased from Composite Metal Services (Worcester, England). The effective length was 50 cm and the total length 57 cm. Injections were made at the anodic end using N_2 pressure of 0.5 psi (1 p.s.i. = 6894.76 Pa) for 60 seconds. CE-UV electrophoretic separation was carried out at 22ºC and +23 kV.

CE-ESI-MS analyses were carried out in a PACE/5010 CE apparatus (Beckman, Fullerton, CA, USA) equipped with a UV detector working at 214 nm and coupled

using an orthogonal electrospray interface (model 61607A from Agilent Technologies, Palo Alto, CA, USA) to the MS instrument. The detection length to the UV detector was 20 cm and the total length (corresponding to the MS detection length) was 87 cm. The CE instrument was controlled by a PC running the System Gold Software from Beckman. Injections were made at the anodic end using N_2 pressure of 0.5 psi (1 p.s.i. = 6894.76 Pa) for 100 seconds. CE-MS electrophoretic separation was carried out at 22ºC and at +25 kV.

Before their first use, fused-silica capillaries were washed with 0.1 M sodium hydroxide for 30 min and deionized water for 15 min. The separation electrolyte consisted of a 24 mM formic acid and 16 mM ammonium carbonate solution at a pH of 6.4. Capillary conditioning was done every morning rinsing with running buffer for 3 min and, between runs, by rinsing with running buffer for 1 min (all rinses were done using N_2 pressure at 20 psi). Buffer solutions were renewed every 5 runs to achieve a good reproducibility.

2.3. Mass spectrometry.

MS and CE-MS experiments were performed on a Bruker Daltonik Esquire 2000 ion-trap mass spectrometer (Bruker Daltonik, Bremen, Germany) equipped with an orthogonal electrospray interface. Electrical contact at the electrospray needle tip was established via a sheath liquid delivered by a 74900-00-05 Cole Palmer syringe pump (Vernon Hills, IL, USA). The mass spectrometer was operated in the negative ion mode. The spectrometer was scanned at 300-450 m/z range (target mass= 375 m/z, compound stability 35%, trap drive level 100%) at 13000 m/z/s during the separation and

detection. The instrument was controlled by a PC running the Esquire NT software from Bruker Daltonik. After ESI-MS optimization, selected parameters were as follows: nebulizer pressure of 3 psi, dry gas flow equal to 3 L/min, dry gas temperature at 50 ºC; and a sheath liquid made of methanol/water (82.5/17.5, v/v) with 2% TEA.

2.4. Software.

StatGraphics Plus Software Version 4.0 (Statistical Graphics, Rockville, USA) was used to generate the experimental design and data processing.

2.5. Solid phase extraction procedure.

Soy milk samples were bought in a local supermarket. The samples were spiked with the selected herbicides at several concentrations and left at room temperature for 2 hours. SPE of soy milk (performed using a Vac-Master manifold from IST Ltd, Hengoed, South Wales, UK) is a modification of a procedure described for water analysis elsewhere [24]. Briefly, 5 mL of soy milk were diluted with Milli-Q water to 20 mL and 4 mL of 1M hydrochloric acid were added. Afterwards, samples were centrifuged at 14000 rpm and 0ºC for 30 minutes. The supernatant was slowly passed through a C_{18} SPE cartridge (Sep-Pak Plus C_{18} Cartridge) from Waters (Milford, MA, USA) previously activated by flushing 5 mL acetonitrile followed by 2 mL 0.01 M hydrochloric acid. After loading the sample into the SPE cartridge, it was dried under vacuum of -10 mmHg (1 mmHg=133.322 Pa) for 15 minutes. The retained herbicides were eluted with 10 mL acetonitrile. The eluate was filtrated using a 0.22 μm filter (Millipore Corp., Bedford, MA, USA). The organic solvent was then evaporated to

dryness at 40ºC using a Rotavapor R-200 (from Büchi Labortechnik, Flawil, Switzerland). The residue was dissolved in 1 mL acetonitrile and directly injected in the CE instrument.

3. Results and discussion.

3.1. SPE-CE-UV of soy milk samples.

As demonstrated in our previous work [24], the combination of on-line stacking procedures (as normal stacking mode, NSM) and CE-UV is a suitable procedure to analyze this group of pesticides in different water samples in a sensitive way. As an example, Figure 1A shows the CE-UV separation of a standard dissolution of these pesticides concentrated by NSM. In this case, the analytes dissolved in acetonitrile could be injected up to 60 sec at 0.5 psi and the preconcentration obtained by NSM prior to CE-UV provided LODs (calculated as three times the signal/noise ratio) ranging from 133 μg/L for flumetsulam to 195 μg/L for cloransulam-methyl, i.e., in the sub-ppm range.

Since this group of pesticides is frequently used in soybean crops, they could obviously be present in soy milk, a beverage everyday more frequently taken. Soy milk is a very complex matrix to be directly injected into the CE system and, therefore, a suitable extraction and preconcentration procedure had to be developed. For this purpose, the use of SPE was investigated. First, the SPE method was studied by using standard solutions of the five pesticides in water. Under optimized conditions, the SPE protocol provided mean recovery values (n=3) around 100% for all pesticides except for

metosulam, for which a recovery around 55% was obtained. Since no further improvement could be achieved, these conditions were selected as optimum and applied to soy milk previously acidified and ultra-centrifuged as described under *Section 2.5*. The soy milk eluate obtained from SPE was evaporated to dryness, dissolved in 1 mL of acetonitrile and analyzed under CE-UV conditions identical to those used in Figure 1A. Figure 1B shows the result of the SPE-CE-UV analysis of a soy milk containing 200 µg/L of each pesticide. As it can be seen by comparing Figure 1A and 1B, there is no possibility to carry out a correct peak assignment due to the numerous interfering peaks from soy milk and, as a consequence, there is no possibility to quantify by CE-UV these pesticides in such complex matrix.

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Further experiments were carried out varying SPE conditions in order to improve the extraction selectivity without any success. Therefore, in order to feasibly detect and quantify these pesticides, a new CE-ESI-MS method was developed for their analysis in soy milk samples making use of the better selectivity and sensitivity provided by MS detection.

3.2. CE-MS preliminary studies.

As it has been previously indicated, no CE-MS method has been developed till now for the analysis of triazolopyrimidine sulfoanilide pesticides. Therefore, several preliminary studies were carried out testing different conditions in order to roughly evaluate the influence of the many factors that can play a role in CE-MS analysis.

First, a volatile separation buffer compatible with ESI-MS detection and able to provide a fast and efficient separation of the five pesticides is required. In this sense, the buffer used in Figure 1 composed of 24 mM formic acid, 16 mM ammonium carbonate at pH 6.4, fulfills all these requirements and, therefore, it was chosen for further CE-MS studies.

Thus, in order to evaluate the ionization process a dissolution containing 2 ppm of the five pesticides in acetonitrile was introduced in the MS instrument by direct infusion using as sheath liquid acetonitrile:water 1:1 (v/v). Since the selected pesticides are weak acids they could be detected more sensitively using the negative ion mode. A rough and quick optimization of the main ESI factors (sheath-flow rate, stability percentage of the skimmer, nebulizer pressure, dry gas flow rate, dry gas temperature) was carried out until obtaining an adequate value for these factors that provide a reasonable sensitivity, varying then the nature of the sheath liquid. The pre-optimized values were a sheath flow rate of 0.35 mL/h, 35 % stability in the skimmer, a nebulizer pressure of 2 psi, a dry gas flow rate of 5 L/min and dry gas temperature of 250ºC. With these pre-optimized values, several solvents and additives were tested as sheath liquids. In this sense, mixtures 1:1 of methanol:water, isopropanol:water and acetonitrile:water were tested together with different percentages of TEA, ammonium hydroxide, ammonium acetate or separation buffer. The highest signal intensities were achieved by using TEA. Once TEA was selected, mixtures of methanol:water, isopropanol:water and acetonitrile:water all of them 1:1 v/v and containing different percentages of TEA, were tested as sheath-liquids by directly infusing the five pesticides. Among the different sheath-liquids, acetonitrile:water gave the best results in terms of signal/noise ratio, however, the signal was not stable enough. Therefore, methanol:water containing TEA,

which provided similar signal/noise ratio as acetonitrile:water but better stability, was selected as the best sheath-liquid.

3.3. CE-MS optimization.

Once methanol, water and TEA were selected as sheath-liquid, a response surface design with five quantitative variables was carried out based on complete CE-MS separations using as running buffer the dissolution composed of 24 mM formic acid, 16 mM ammonium carbonate at pH 6.4. The five selected variables were: sheath liquid composition (percentage of methanol and TEA), nebulizer pressure, dry gas flow and dry gas temperature. Sheath flow rate and percentage of stability in the skimmer were set at constant values of 0.35 mL/h and 35%, respectively, since the variation of these parameters did not improve the signal intensity.

The sum of the CE-MS peak areas of the five pesticides was selected as response. A central composite design consisting of a full factorial design and a star design with three replicates of the central point was created using the Statgraphics Plus software. The factors and their levels are stated in Table 1. The limits of these parameters were imposed by different constraints as stability of the spray, instrumental limitations of the interface or siphoning effect inside the capillary. These 45 experiments were randomly carried out trying to nullify the effect of extraneous variables. The axial distance was set to 1.68. Data was introduced in Statgraphics Plus software for their ulterior analysis obtaining in that way the optimum. As it can be seen in Figure 2 –obtained from the statistical package-, the highest sum of peak areas could be obtained at low dry gas flows and low temperatures. Relatively high percentages of methanol and TEA in the

sheath liquid gave the best results. As it can also be seen, small variations in the percentage of TEA or methanol in the sheath liquid, lead to important variations in the response. These variations are lower for the rest of the variables and even neglectable for nebulizer pressure. This type of graphs –provided by the statistical package-, are very useful to know the individual effect of each factor and therefore, their experimental behavior. The optimum combination of factors according to the program was: sheath-liquid made of methanol:water (82.5:17.5, v/v) and 2% TEA, nebulizer pressure of 3 psi, dry gas flow equal to 3 L/min and dry gas temperature at 50ºC.

Once the experimental design provided the optimum conditions, these were experimentally checked, and it was found that indeed they provided the highest sum of areas values, which corroborated the usefulness of this approach. Moreover, several experiments were carried out varying slightly the different factors around the optimum in order to evaluate the obtained response. As an example, the dry gas temperature was increased till 100 ºC and decreased till 30 ºC, but 50 ºC gave again the highest area values, as expected. Similar results were obtained when the dry gas flow rate and the nebulizer pressure were slightly varied. Also, slight variations of the sheath liquid flow rate and the percentage of stability (initially set at 0.35 mL/h and 35 %, respectively) did not improve the intensity of the MS signal. Therefore, the same two values were used for all the future experiments.

3.4. CE-MS with normal stacking.

Although the use of on-line preconcentration strategies such as stacking [25-28] or sweeping [27,29,30] have provided significant sensitivity improvements in pesticide

analysis by CE, the use of on-line preconcentration techniques is limited in the case of CE-MS. For instance, many of these preconcentration techniques use surfactants, cyclodextrins, and other non-volatile compounds that are precluded in CE-MS. Also, stacking with matrix removal [25], another stacking procedure that provides very good results cannot be used in CE-MS since there is not outlet vial. Therefore, to improve the sensitivity of our procedure, normal stacking mode was evaluated. For this purpose, a low conductivity matrix is required since focusing takes place due to the abrupt change in the local electric field (and, as a consequence, in the electrophoretic velocity of the analytes) between the sample matrix and the BGE. In order to obtain adequate stacking conditions, we have tested several mixtures of the herbicides dissolved in acetonitrile and separation buffer. Relations 1:3, 1:1 and 3:1 as well as pure acetonitrile or separation buffer alone were tested. Among them, pure acetonitrile yielded the highest signal to noise ratios and consequently, the lowest LODs, in good agreement with that observed by other authors [31-33]. In our case, by using acetonitrile alone, the sample could be injected in the longer CE-MS capillary up to 100 seconds (that represents approximately 3.7% of the total volume of the capillary). Higher injections times induced higher electrical current instability while no significant improvement of the signal to noise ratio was attained.

LODs for the optimized NSM-CE-MS procedure ranged between 74 μg/L for metosulam and 150 μg/L for flumetsulam, which are in the low ppb range. Figure 3 shows the extracted ion electropherogram of the selected pesticides under the optimized NSM-CE-MS conditions, while Figure 4 shows the MS spectra of the five pesticides directly obtained from the electropherogram given in Figure 3. The MS patterns obtained for the five compounds are in good agreement with those expected from the

isotopic variability induced by sulfur and, mostly, chlorine atoms. The average mass values of the five pesticides were determined from a single CE-MS run and these experimental values are consistent with the theoretically expected, as can be seen in Table 2, corroborating the suitability of our approach.

Table 2 also shows a comparison of the LODs obtained by NSM-CE-UV and NSM-CE-MS. It has to be taken into account that the sample could be injected in CE-UV up to 60 sec at 0.5 psi (mainly due to peak overlap), which represents approximately 5.2% of the total volume of the capillary, while in CE-MS this percentage was 3.7% of the total volume of the capillary. Interestingly, the use of the extracted ion mode in MS detection allows a higher peak overlap than in UV detection. As can be seen in Table 2, LODs obtained by NSM-CE-MS are in general better than the ones obtained by using UV detection (up to 2.6 times better for cloransulam-methyl).

3.5. Method validation.

Once NSM-CE-MS conditions were optimized, a study about the intra-day and inter-day precision of this procedure was carried. To do this, three levels of concentrations (0.5, 1 and 2 mg/L) with three consecutives injections of each concentration (n=3) in three consecutives days (n=3) were used. Table 3 shows the results of the intra-day and inter-day precision studies. As it can be seen, good precision was achieved for migration times and peak areas in the same day and between days. Relative standard deviation (RSD, %) values for peak areas ranged from 5.2% for diclosulam to 6.4% for florasulam in the same day, and between 6.5% for cloransulam-methyl and 8.1% for diclosulam between days.

Table 3 also shows the calibration parameters obtained for the quantification of these pesticides, including calibration equations, determination coefficients (R^2) and limits of quantifications (LOQs) calculated as 10 times the signal to noise ratio. Calibration graphs were obtained at working concentrations ranging from 500 to 5000 μg/L by injecting by triplicate each concentration showing in all cases a good correlation with R^2 values higher than 0.992. These values allow an adequate quantification of the studied compounds by this new NSM-CE-MS procedure.

3.6. Application of SPE-NSM-CE-MS to soy milk.

Once the NSM-CE-MS procedure was validated, the same SPE extract from soy milk containing 200 μg/L of each pesticide that could not be analyzed by CE-UV due to multiple interfering peaks (see Figure 1B) was injected in the CE-MS instrument. Figure 5, shows the electropherograms of the SPE-NSM-CE-ESI-MS analysis of the mentioned soy milk sample. As it can be seen in Figure 5, the correct determination of the five pesticides in the soy milk sample free of interfering peaks can be now carried out by CE-MS as a result of the higher selectivity provided by the MS detector.

Using this validated quantitative analytical methodology, a study on the suitability of the SPE conditions used for these herbicides in soy milk can now be done. Thus, SPE recovery values were determined and they ranged between 75 and 95% for all the pesticides except for metosulam that was 40%. Although different SPE conditions (as introduction of several washing protocols, variation of the volume of both the sample and the eluate) were tried in order to improve the SPE extraction recoveries, no

significant improvement was attained for metosulam. A non-spiked commercial sample was submitted to the SPE protocol, injected into the CE-MS instrument and no signal for the selected pesticides was observed. Although these results show the great possibilities of this new SPE-NSM-CE-MS procedure to determine triazolopyrimidine sulfoanilide herbicides in real samples, more work needs to be done in order to improve the recoveries from the SPE protocol and to demonstrate its usefulness with different soy milk samples or other soy products.

4. Conclusions.

In this work, a new SPE-NSM-CE-ESI-MS procedure has been developed for the quantitative and simultaneous determination of pesticides in soy milk. To do this, i) multiple parameters that influence ESI conditions were optimized by means of a central composite design; ii) NSM was optimized as on-line preconcentration prior to CE-MS to improve sensitivity; iii) a new SPE protocol was also developed to extract the pesticides from soy milk. The SPE-NSM-CE-MS procedure allowed the simultaneous determination of a group of triazolopyrimidine sulfoanilide herbicides (namely, cloransulam-methyl, metosulam, flumetsulam, florasulam and diclosulam) at ppb levels in soy milk.

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Figure captions

Figure 1. NSM-CE-UV electropherogram of: A) a standard solution containing ca. 1000 µg/L of each pesticide and; B) a SPE extract from soy milk sample containing 200 µg/L of each pesticide. Injection: 60 s at 0.5 psi. Running electrolyte: 24 mM formic acid, 16 mM ammonium carbonate at pH 6.4; Total length: 57 cm (50 cm effective length); Voltage: +23 kV; Temperature: 22ºC. (1) Metosulam; (2) Cloransulam-methyl; (3) Diclosulam; (4) Florasulam; (5) Flumetsulam.

Figure 2. Effect of the individual factors on the sum of the CE-MS peak areas of the selected pesticides. Levels used: from 0 to 100 % for percentage of methanol in the make-up-flow; from 0 to 4 % of TEA in the make-up-flow; from 1 to 11 psi for nebulizar pressure; from 1 to 11 L/min for dry gas flow; from 50 to 300 ºC for dry gas temperature.

Figure 3. Extracted ion electropherograms of the selected pesticides under optimum CE-ESI-MS separation conditions. Carrier electrolyte: 24 mM formic acid, 16 mM ammonium carbonate pH 6.4. Total length of the capillary (effective length): 87 cm; Hydrodynamic injection at the anode for 100 s at 0.5 psi; Voltage: +25 kV; Temperature: 22 ºC; Nebulizer pressure: 3 psi; Dry gas flow: 3 L/min; Dry gas temperature: 50 ºC; Make-up flow: methanol-water 82.5:17.5 (v/v), 2 % (w/v) TEA, 0.35 mL/h; Stability: 35 %; Trap drive level: 100 %. Sample: 1 mg/L of each pesticide in acetonitrile. The extracted ions used (+/- 0.2 m/z) are indicated in each case.

Figure 4. Mass spectra directly obtained from the electropherograms given in Figure 3.

Figure 5. Extracted ion electropherograms of a soy milk sample containing 200 μg/L of each pesticide analyzed under SPE-NSM-CE-ESI-MS optimized conditions. Other conditions as in Figure 3.

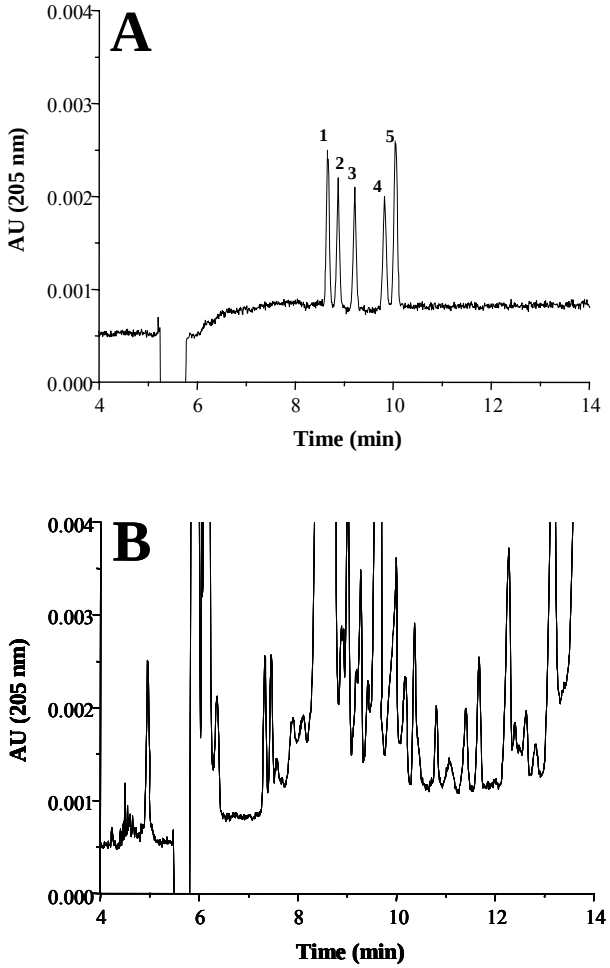
A
B
AU (205 nm)
Time (min)
1
2
3
4
5
0.000
0.001
0.002
0.003
0.004
4
6
8
10
12
14

Figure 1.

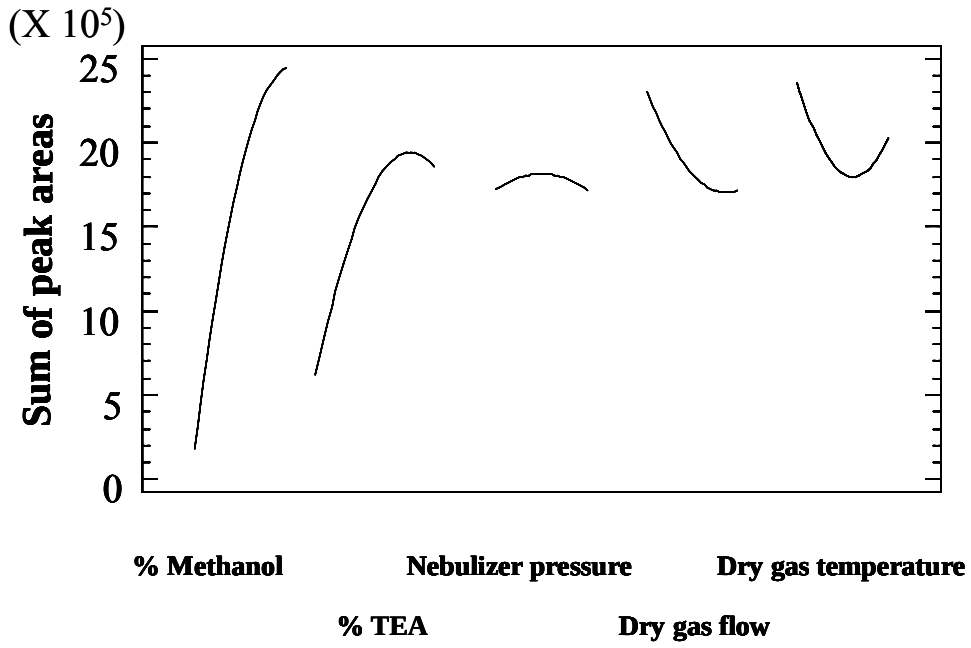
(X 10^5)
25
20
15
10
5
0
Sum of peak areas
% Methanol
Nebulizer pressure
Dry gas temperature
% TEA
Dry gas flow

Figure 2.

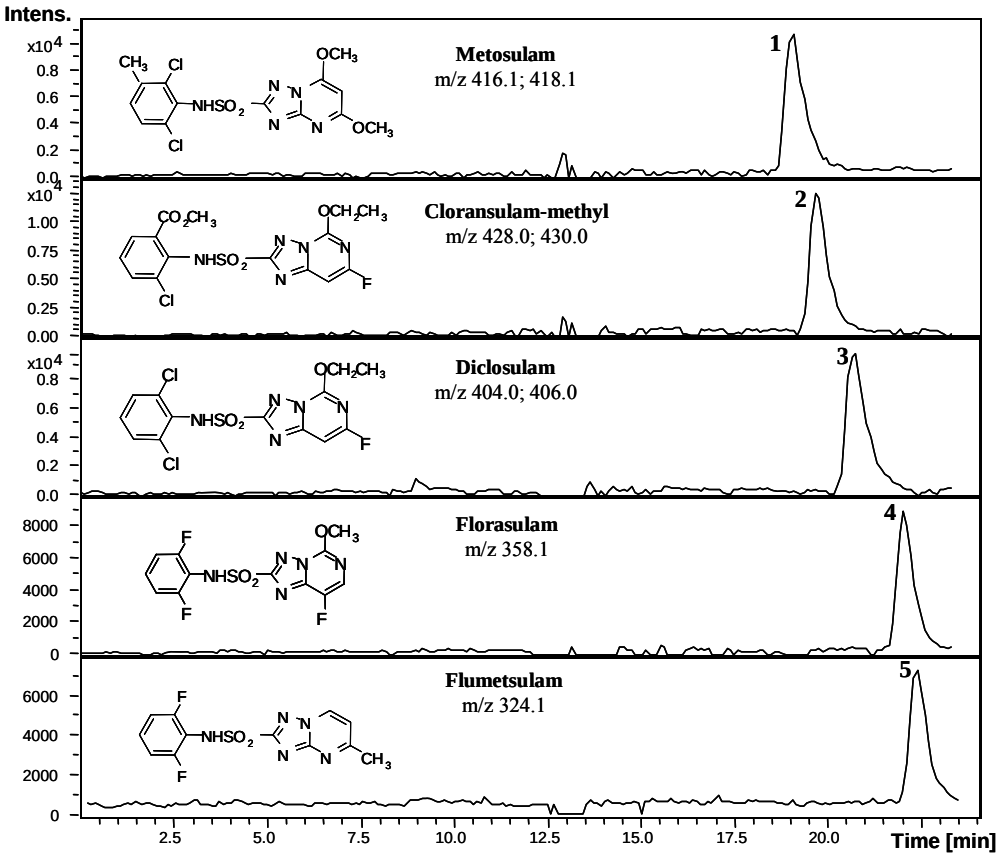
Intens.
x10^4
0.8
0.6
0.4
0.2
0.0
Metosulam
m/z 416.1; 418.1
1
x10^4
1.00
0.75
0.50
0.25
0.00
Cloransulam-methyl
m/z 428.0; 430.0
2
x10^4
0.8
0.6
0.4
0.2
0.0
Diclosulam
m/z 404.0; 406.0
3
8000
6000
4000
2000
0
Florasulam
m/z 358.1
4
6000
4000
2000
0
Flumetsulam
m/z 324.1
5
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
Time [min]

Figure 3.

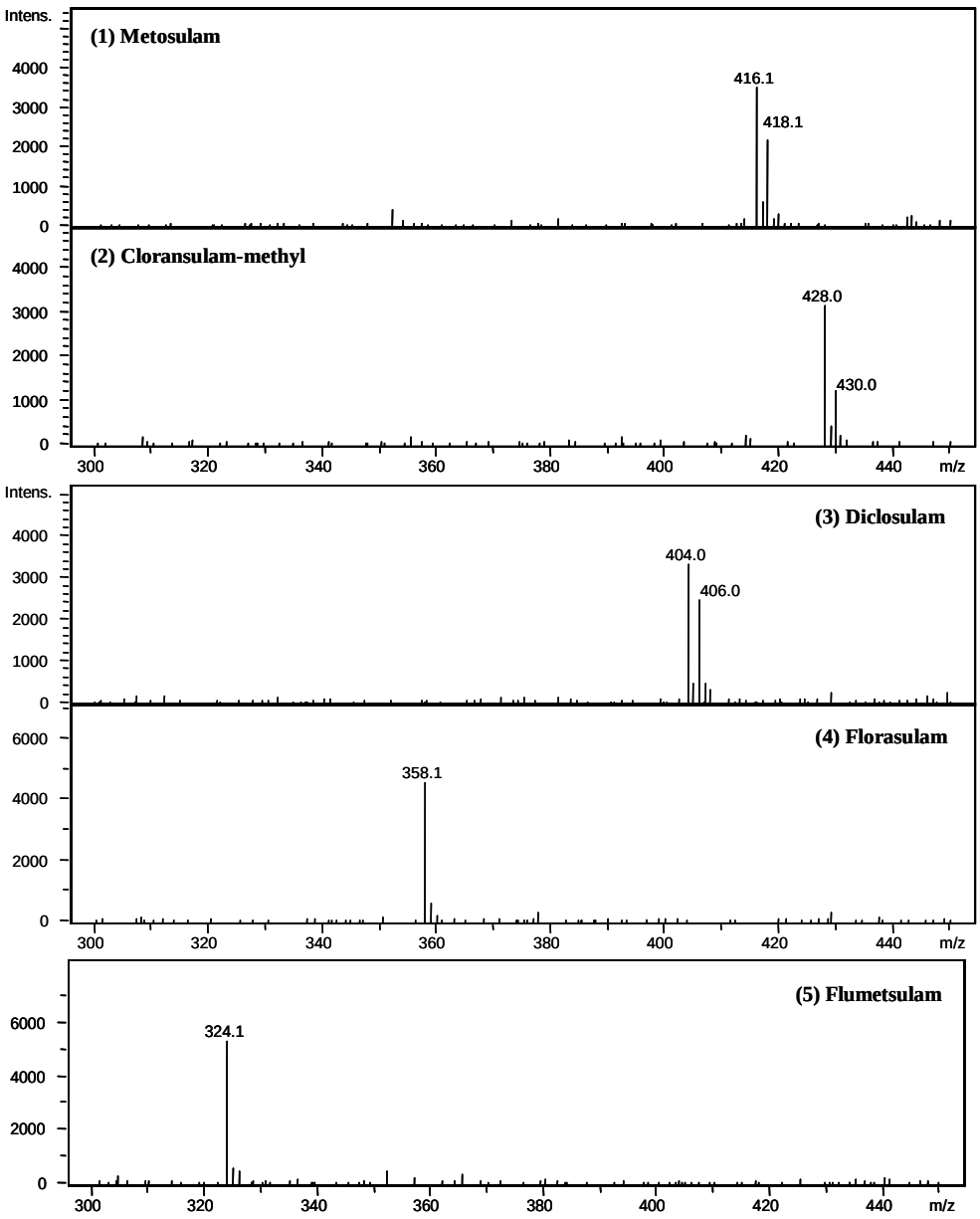
Intens.
(1) Metosulam
416.1
418.1
(2) Cloransulam-methyl
428.0
430.0
(3) Diclosulam
404.0
406.0
(4) Florasulam
358.1
(5) Flumetsulam
324.1
m/z

Figure 4.

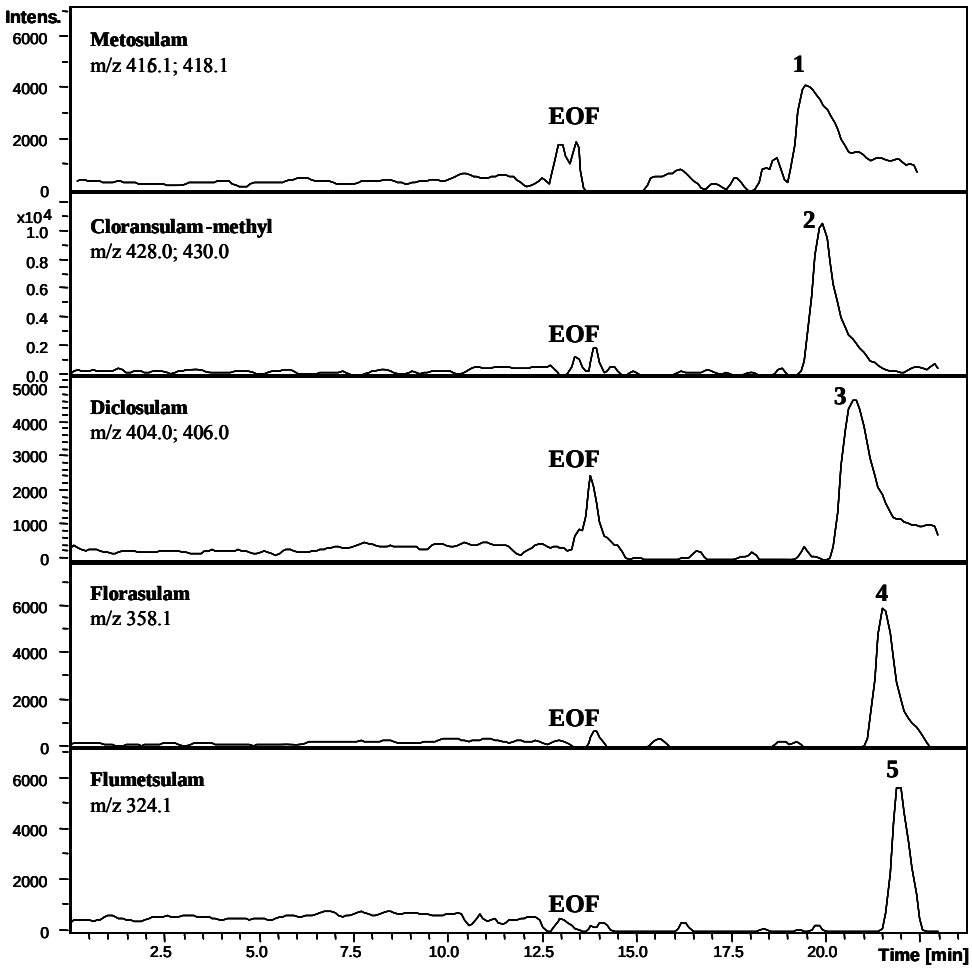
Intens.
Metosulam
m/z 416.1; 418.1
1
EOF
x10^4
Cloransulam-methyl
m/z 428.0; 430.0
2
EOF
Diclosulam
m/z 404.0; 406.0
3
EOF
Florasulam
m/z 358.1
4
EOF
Flumetsulam
m/z 324.1
5
EOF
Time [min]

Figure 5.

Table 1. Factors levels used in the central composite design.

	Central Composite Design				
	Full factorial design levels			Star design levels	
Factor	Low (-1)	Medium (0)	High (+1)	Low (-1)	High (+1)
Methanol in make-up flow (%)	20	50	80	0	100
TEA in make-up flow (%)	1	1.5	3	0	4
Nebulizer pressure (psi)	3	6	9	1	11
Dry gas flow (L/min)	3	6	9	1	11
Dry gas temperature (ºC)	100	175	250	50	300

Table 2. Theoretical and experimental molecular weight of the studied pesticides and comparison of the LODs obtained by using NSM-CE-UV and NSM-CE-MS procedures.

	$Mw_{theoret}$	$Mw_{exp.}$	NSM-CE-UV LOD[b)] (μg/L)	NSM-CE-MS LOD[b)] (μg/L)
Metosulam	418.3	418.1	143	74
Cloransulam-methyl	429.8	430.0	195	75
Diclosulam	406.2	406.0	191	80
Florasulam	359.3	359.1	185	105
Flumetsulam	325.3	325.1	133	150

a) $Mw_{exp.}$ Molecular weight determined by CE-ESI-MS in this work.

b) LODs calculated as 3 times the S/N ratio.

Table 3. Intra-day precision, day-to-day precision (both expressed as RSD %) obtained for the NSM-CE-ESI-MS procedure and calibration data for the studied herbicides.

Pesticide	Intra-day precision (RSD %) [a] (n=3)		Day-to-day precision (RSD %) [a] (n=3)		Calibration curve[b] (n=5)	R^2	LOD µg/L	LOQ µg/L
	t_m	Area	t_m	Area				
Metosulam	0.8	5.7	2.4	7.2	y=(279±11)x-(62±16)	0.997	74	247
Cloransulam-methyl	1.3	6.2	2.6	6.5	y=(398±26)x-(110±35)	0.996	75	250
Diclosulam	1.1	5.2	2.8	8.1	y=(257±24)x-(24±32)	0.992	80	267
Florasulam	1.4	6.4	2.9	7.2	y=(331±84)x-(84±11)	0.997	105	350
Flumetsulam	1.3	3.8	3.1	7.6	y=(228±20)x+(5±24)	0.997	150	500

a) Data given for 1 mg/L.

b) Slope and intercept $x10^3$.