Simple and rapid analytical methodology based on liquid chromatography - tandem mass spectrometry for monitoring pesticide residues in soils from Argentina

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ABSTRACT

Rapid analytical methodology has been developed for multi-residue determination of pesticides in soils using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) with triple quadrupole analyzer. Soil samples were collected from Argentina in 12 representative agricultural areas, and 18 pesticides were selected on the basis of their use. Special attention was paid to minimize sample preparation, making easier the method application to routine analysis. Several extraction procedures were tested, performing a careful study on matrix effects. The method finally proposed (extraction with acetonitrile and subsequent 2-fold dilution with water without any clean-up step) was fully validated at 0.05 and 0.5 mg/kg on the basis on European SANCO 12571/2013 and 825/00 guidelines. The method applicability and robustness was demonstrated by analysis of quality control (QC) samples, consisting on eleven soils spiked at 0.5 mg/kg. These soil samples were collected from different experimental plots, and were very diverse in their physico-chemical characteristics. The methodology developed is of easy application, there is low consumption of solvents and reagents, and no clean-up is required despite the complexity of the soil matrix. In the near future, the method developed will be used to monitor the presence of pesticides in large agricultural areas of Argentina.

Key words: Pesticide residue analysis; soil; QuEChERS; LC-MS/MS triple quadrupole; matrix effects

1. INTRODUCTION

Argentina is ranked the tenth agricultural nation in the world considering the area under cultivation, based on figures produced by the Food and Agricultural Organization of the United Nations [1]. With 31 million hectares given over to agriculture, Argentina accounts for 2.2% of the world's total area under cultivation [2]. The Argentine agrochemical market has strongly expanded over recent years, with an increase in the consumption from 73 to 236 million kg per year over the last 10 years. This represents a total turnover of US$ 2381.16 million in 2012, with the following distribution: 64% herbicides, 16% insecticides, and 20% fungicides, acaricides and seeds cure [3].

Transgenic crops account for three quarters of the Argentina's total cultivated land. In addition, 78.5% of agricultural lands are direct seeding [4], where the only way of controlling weeds, during cultivation and fallow periods, is by using agrochemicals.

Nowadays, the analysis of pesticide residues in environmental waters and soils has become indispensable in agricultural areas. Information provided by both, soil and water, is required to have a realistic knowledge on the impact of agricultural activities on the environment. Soil is a complex-matrix sample, where the presence of many different co-extracted components negatively affects pesticides residue determinations. Particularly, soil organic matter has notable impact on analyses and therefore it is common the inclusion of clean-up steps in the analytical procedure for its removal, even using powerful analytical techniques such as liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS) [5–7].

Many different techniques have been used along the time for soil sample preparation, including extraction and/or clean-up, such as Soxhlet extraction, pressurized-liquid extraction (PLE) , ultrasound-assisted extraction (UAE), dispersive liquid-liquid microextraction, microwave assisted extraction, liquid–liquid extraction, accelerated solvent extraction, solid-phase extraction (SPE), or solid-phase microextraction [8–11]. However, the classical solid-liquid extraction with organic solvents, commonly followed by appropriate clean-up procedures, has been one of the most

widely used in pesticide residue analysis (PRA) in soils, employing mechanical agitation and/or ultrasounds [12,13].

The increasing need to reduce solvent amounts and manual labor in analytical laboratories has led to the commercial introduction of alternative extraction approaches. Among these, the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) procedure is one of the most popular [14]. Originally developed for fruits and vegetables, QuEChERS is being applied to many other matrices, including soil samples [15–17]. The original procedure consisted on extraction with acetonitrile, separation of water by addition of anhydrous $MgSO_4$ and NaCl, and subsequent clean-up using dispersive solid-phase extraction (d-SPE) [14]. Some modifications have been included in the QuEChERS procedure due to the possible negative influence on the recovery arising from the retention properties of the soil matrix. Thus, UAE, PLE [18–20], as well as modifications in the clean-up step [21–23] have been applied to deal with the matrix interferences commonly found in soil analysis.

In relation to the analytical techniques applied for PRA in soil, there has been a clear evolution along the time from the first analysis, commonly performed by GC-MS and/or LC-UV, to the most recent ones, based on GC-MS/MS and/or LC-MS/MS, which nowadays are the techniques of choice in this field [19,24–28]. The wide majority of pesticides currently applied are of medium-high polarity and of low volatility; therefore LC-MS/MS is the preferred technique for most of them [29]. Despite the excellent characteristics of this technique (robustness, and high sensitivity and selectivity), matrix effects are commonly a problem in LC-MS/MS methods and may notably affect the ionization of the analytes leading to important analytical errors if not satisfactorily corrected. This is an important issue in soil analysis, as the matrix sample can largely vary from one soil to the other, making matrix effects correction or minimization troublesome.

The goal of this study was to develop a rapid and simple LC–MS/MS multiresidue method for the determination of pesticides in soils commonly found in Argentina. We pursued the minimization of sample manipulation and solvent consumption (i.e. extract the sample and directly inject into the LC-MS/MS) in order to make the method easier to apply and to implement in Argentinian laboratories. To this aim, soil extraction with different organic solvents was tested and the results compared with the QuEChERS in order to select the most appropriate in terms of extraction efficiency, less matrix effects, minimum sample treatment and better robustness. The method developed was validated and evaluated for several soil samples collected from different agricultural areas of Argentina.

2. EXPERIMENTAL

2.1 Reagents and chemicals

Pesticide reference standards were from Dr. Ehrenstorfer (Augsburg, Germany). HPLC-grade methanol (MeOH), HPLC-grade acetonitrile (ACN), and acetone for residue analysis from Scharlab (Barcelona, Spain). HPLC-grade water was obtained by purifying demineralized water in a Milli-Q Gradient A10 (Millipore, Bedford, MA, USA). Formic acid (HCOOH, content >98%) and ammonium acetate (NH_4Ac, reagent grade) were supplied by Scharlab.

QuEChERS (CEN) standard method EN 15622 reagents were purchased from Scharlab.

Stock standard solutions were prepared dissolving 50 mg, accurately weighted, in 100 mL of acetone, obtaining a final concentration around 500 mg/L. For LC-MS analysis, the stock solutions were ten-fold diluted with ACN to prepare individual solutions around 50 mg/L. From these, mixed solutions were prepared by diluting with

ACN to obtain a final concentration of 5 mg/L. Working mix solutions of all pesticides were prepared from the 5 mg/L solutions by dilution with ACN.

In order to remove solid particles, nylon syringe filters (0.22 µm) were tested (Supelco, Bellefonte, PA, USA).

2.2   Sampling area

Samples were collected from 12 experimental fields of Instituto Nacional de Tecnología Agropecuaria (INTA) (**Figure 1 SI**), where no agricultural activity took place in the last years. En each sampling plot, a composite sample from 50 sub-samples (from 0 to 5 cm depth) was prepared.

The probe was cleaned by discarding several extractions in order to avoid any contamination between samples. The samples were conditioned using a hot-air heater set at 30 ºC, and then dry milled. The mill was used only for untreated samples in order to avoid contamination, and it was cleaned between samples by washing with abundant water. The dried samples were then passed through a 2 mm sieve.

In the INTA laboratory at Balcarce, soil texture of all the samples was determined, as well as cation-exchange capacity (CEC), pH and total organic carbon (**Table 1, Supplementary Information**).

2.3   Selected pesticides

Pesticides selected as target analytes were chosen among the most widely used in agricultural practices of Argentina [30]. In the list of the 30 most-consumed pesticides, the 3 top compounds are herbicides, specifically glyphosate, 2,4-D and atrazine. The second and third compounds were selected for this work, but unfortunately glyphosate could not be included in this multi-reside methodology due to its particular physico-chemical characteristics, which require the use of specific methodology for this compound [7]. Other compounds from the list of most-consumed pesticides included in

the present work were chlorpyrifos, picloram, metolachlor, imidachloprid and fipronil. Those pesticides from the top-list that were more appropriate for GC analysis were excluded from this study, and finally another eleven LC-MS amenable compounds, frequently used in agricultural practices around the world, were also considered making a total of 18 target pesticides including herbicides (8), fungicides (2) and insecticides/acaricides (8).

2.4   Liquid chromatography/mass spectrometry

A Waters Alliance 2795 LC system was interfaced to a Quattro micro triple quadrupole mass spectrometer (Waters Milford, MA, USA) using an orthogonal Z-spray–electrospray interface. The LC separation was performed using a Brisa C18 column (3 µm, 5 cm × 2.1 mm; Teknokroma) at a flow rate of 0.2 mL/min. The mobile phase was water (0.1 mM NH_4Ac)/MeOH with a gradient where the percentage of MeOH changed as follows: 0 min, 20%; 1 min, 20%; 1.8 min, 35%; 6 min, 80%; 11 min 95%; 11.5 min 95%; 12 min to 15 min, 20%.

The drying gas as well as the nebulizing gas was nitrogen. The desolvation gas and cone gas flows were adjusted to 600 and 60 L/h, respectively. Infusion experiments were performed using the built-in syringe pump, directly connected to the interface. For operation in MS/MS mode, the collision gas was argon (99.995%; Praxair, Valencia, Spain) at a pressure of $2×10^{-3}$ mbar in the collision cell. Capillary voltages of 3 kV in negative ionization mode and 3.5 kV in positive mode were used.

The interface temperature was set to 350 °C and the source temperature to 120 °C. In order to assure at least 10 points per chromatographic peak, compounds were distributed in different functions at dwell times of 0.1 s except for picloram (0.2 s). Two solvent delays were selected to give an additional clean-up using the built-in divert valve controlled by the Masslynx v.4.1 software, the first one from 0 to 2.3 min and the

second one from 12 to 15 min. The application manager TargetLynx was used to process the quantitative data obtained from calibration standards and from samples.

2.5   Recommended procedure

5.0 g of soil sample (previously dried at room temperature and homogenized) were weighted into a 50-mL centrifuge tube and extracted with 5 mL water and 25 ml ACN in a mechanical shaker for 1h followed by ultrasonic bath for 15 min. Then, it was centrifuged at 4600 rpm for 10 min. A 500 µL aliquot of the supernatant was diluted with 500 µL HPLC-grade water into a glass tube. Then, 10 µL formic acid was added to adjust its final content to 1%. After that, the extracts were filtered through a 0.22 µm nylon membrane. Analyses were performed by injecting 20 µL of the final extract in the LC-ESI-MS/MS system.

Calibration curves in solvent (between 1 and 100 ng/mL) were prepared by taking 440 µL water, and adding 10 µL HCOOH, 100 µL of corresponding standard mix solutions and 400 µL of acetonitrile. QCs were also analyzed in every set of samples for quality control in order to ensure the recoveries were within the tolerance range (60-140%) following SANCO guidelines.

Fortification of samples for recovery experiments (in both method validation and preparation of QCs) was performed by delivering 1 mL of 0.25 or 2.5 µg/mL standard mix solutions in acetonitrile to 5 g homogenized soil sample in order to yield fortification levels of 0.05 or 0.5 mg/kg, respectively. The fortified samples were aged for 1 h prior extraction.

2.6   Method validation

In the absence of specific guidelines for soil analysis, validation of the method was made on the basis of the European Union SANCO 12571/2013 guideline for pesticide

residues analysis in food and feed [31]. In addition, the SANCO 825/00, Rev.1 guidance document on residue analytical methods was also taken into account [32]. Linearity was studied by injecting (in triplicate) standards in solvent (ACN:water) and also in the soil extract used for method development at seven concentrations in the range 1– 100 ng/mL (equivalent to 0.012–1.2 mg/kg in sample). Precision (repeatability, % relative standard deviation) and accuracy (% recoveries) were estimated by recovery experiments in soil at two fortification levels, 0.05 and 0.5 mg/kg (analyzed in quintuplicate). The limit of quantification (LOQ) objective was set as the lowest concentration validated in fortified samples with satisfactory precision (RSD<20%) and recovery (70 -120%).

The specificity of the method was evaluated from the quantification transition by analysis of a procedure blank, a processed blank sample, and a processed blank sample spiked at the LOQ level.

Confirmation of the identity of the compound in the samples was carried out by acquisition of two MS/MS transitions and the compliance of the q/Q ratio (where q, Q are the confirmation and quantification transitions, respectively) between samples and reference standards, with maximum tolerance of ± 30%. The agreement in retention time was also required, with maximum deviation of ± 0.2 min between the analyte in sample and the reference standard [31].

3. RESULTS AND DISCUSSION

Soil samples selected in this work presented wide range of organic matter content (between 0.5 and 10.3%), clay (between 6.4 and 69.5%), CEC (between 9 and 40.5 cmol kg^{-1}), and pH (from 5.7 to 7.5) (**see Table 1, Supplementary Information**). Therefore, the analytical methodology was tested in very different soil types, with a notable variation in their physico-chemical characteristics. This allowed us to support the robustness of the method and its applicability to a large variety of soil matrices.

3.1 MS optimization

MS/MS parameters were optimized by infusion of 2.5 mg/L methanol: water (50:50, v/v) individual solutions of each compound, at a flow rate of 10 μL/min. Full-scan mass spectra were acquired to select the precursor ion and optimum cone voltage. Once the precursor ion was selected, product ion scan acquisitions were performed at different collision energies in order to select product ions and optimum collision energies.

The majority of the analytes were determined by positive ionization mode, with a few exceptions for acidic compounds that gave a better response in negative mode (2,4-D, MCPA and fipronil). The formation of sodium adducts (cyanazine, dimethoate, metolachlor, carbaryl) was minimized adding 5mM of NH_4Ac in the vial, increasing in this way the abundance of the protonated molecule. Under the experimental conditions finally selected, all precursor ions corresponded to $[M+H]^+$ in positive ESI, or $[M-H]^-$ in negative ESI.

Two transitions were selected for each compound for analysis under selected reaction monitoring (SRM) mode. The most sensitive (Q) was used for quantification purposes, while the second transition (q) was used for confirmation of the identity, avoiding those transitions corresponding to non-specific losses such as H_2O and CO_2.

The presence of atoms with abundant characteristic isotope distribution (e.g., Cl) in the chemical structure was used to improve the identification process of some analytes by selecting the transitions corresponding to both ^{35}Cl and ^{37}Cl. This occurred for picloram and cyanazine (measured in ESI+) and for 2,4-D and MCPA (negative mode) **(Figure 2SI).**

The mass spectrometry parameters selected, i.e. precursor and product ions, cone voltage and collision cell energy, together with the q/Q intensity ratio (a relevant parameter in the confirmation process) are shown in **Table 1.**

3.2   Chromatographic conditions

Two organic solvents (ACN and MeOH) as well as two modifiers (HCOOH and NH_4Ac) were tested to optimize the chromatographic separation and mass spectrometry signal (increase of sensitivity and satisfactory peak shape). The study was made with mix standard solutions (100 μg/L each compound) in solvent. Under the final conditions selected, matrix-matched standards at 100 μg/L were also injected to test for possible variations in retention time and peak shape.

As expected, most of compounds determined in positive mode presented better ionization yield when MeOH was used as organic modifier due to its protic character. On the contrary, for those compounds determined under negative ionization, the use of an aprotic solvent in the mobile phase, as acetonitrile, favored their ionization. In order to improve their chromatographic retention, the acidification of the mobile phase, by adding low amounts of HCOOH, was necessary. Therefore, the use of acetonitrile with an acidic additive seemed a good option for compounds ionized in negative mode trying to reach a compromise between chromatographic retention and ionization.

The effect of adding NH_4Ac as modifier was also evaluated, with the result that sensitivity and peak shape improved for most compounds when a small amount (0.1 mM) was present in the aqueous phase. The addition of NH_4Ac in the organic solvent did not produce a significant improvement; therefore, NH_4Ac was added only to the aqueous phase. As the majority of analytes (15 out of 18) were determined in positive ionization mode, we finally selected methanol as organic solvent in the mobile phase and NH_4Ac as modifier in the aqueous phase.

In order to obtain better retention for the most polar compounds, 10% of methanol was tested as initial percentage. However, an excessive band broadening was observed for some compounds. This situation was improved by increasing the initial content of methanol. Starting the gradient at 20% of MeOH led to satisfactory retention and peak shape for nearly all analytes. Nevertheless, under these conditions acidic analytes like MCPA, fipronil or 2,4-D, showed lower sensitivity and poor chromatographic retention. HCOOH (0.01 and 0.1 %) was tested in both water and MeOH phases in order to increase their retention, but it was discarded because sensitivity decreased and worse peak shapes were obtained. Therefore, the addition of 0.5 or 1% (v/v) HCOOH in the sample vial was tried, avoiding in this way the continuous entrance of this additive into the ionization source. Using 1% HCOOH in the sample vial provided better peak shape and acceptable reproducibility, increasing significantly the sensitivity in negative mode.

The chromatographic conditions finally selected are indicated in "Experimental". Under these conditions, the compounds eluted as shown in **Table 1**, with retention times between 3.1 min (picloram) and 12 min (pendimethalin).

The two SRM transitions per compound were distributed in eleven functions according to the retention times in order to achieve adequate number of points per chromatographic peak.

3.3   *Matrix effects evaluation*

A detailed study of matrix effects was made by comparison of standards in solvent and in soil matrix at the same concentration after application of different sample treatments. Firstly, three extracting solvents were studied (MeOH, ACN and acetone), following the recommended procedure (see section 2.5). For each solvent, the effect of 2-, 5- and 10-fold dilution with water of the initial soil extract was assayed in order to reduce potential matrix effects.

To evaluate matrix effects (%ME), the response/signal obtained for each analyte in the soil extract (S_{matrix}) was compared with that in solvent ($S_{solvent}$) at the same concentration. To perform this study, soil extracts spiked at 50 ng/mL (n=3) were used. The ratio $[(S_{matrix} - S_{blank})/S_{solvent}) \times 100]$ was taken as absolute matrix effect, where S_{blank} corresponded to the analyte signal of the non-spiked extract of soil. Thus, ME 100% means that no matrix effect was observed. Values below or above 100% indicate ionization suppression or enhancement, respectively. No significant matrix effects were considered to be present when ME ranged between 70% and 120% (i.e. the same range used as acceptable recoveries in method validation).

A summary of the results can be found in **Supplementary Information**. Both ionization suppression and enhancement were observed although most analytes were influenced by suppression. Acetone was found to be less suitable for extraction in terms of matrix effects, surely due to the larger amount of co-extractive compounds in this solvent. The use of MeOH and ACN led in general to lower matrix effects for most of compounds selected. As expected, the effect of diluting the extracts with water resulted in a minimization of matrix effects, in such a way that a dilution x10 led to satisfactory values (between 70 and 120%) for almost all compounds **(Figure 3 SI).** We finally selected a 2-fold dilution as a compromise between minimization of matrix effects and sensitivity required to reach the limit of quantification objective in this work (0.05 mg/kg).

A comparison of the matrix effects observed with the three extracting solvents after 2-fold dilution of the soil extract with water is shown in **Figure 4 SI**.

Moreover, a slight modification of QuEChERS (CEN) standard method EN 15622 (extraction of 5 g soil sample with water (5 mL) and acetonitrile (10 mL), followed by a salting-out with 4 g MgSO4, 1 g NaCl, 1 g sodium citrate dihydrate and 0.5 g di-sodium hydrogen citrate sesquihydrate) was tested. In order to minimize matrix effects observed, 2-fold dilution (final analyte concentration 50 µg/L), 4-fold dilution (20

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µg/L) and 5-fold dilution (10 µg/L) were checked (**Figure 5 SI)**. Matrix effects were irrelevant (between 70-120%) at dilution x 5, except for 2,4D and MCPA that presented signal enhancement. As expected, matrix effects become more important in more concentrated extracts (i.e. 2-fold dilution), where signal suppression was mostly observed for analytes measured in positive ionization whereas a notable signal enhancement occurred for those determined in negative mode (MCPA, 2,4D and Fipronil). Moreover, different clean-up reagents, as octadecyl-silanized silica gel (C18), Florisil, graphitized carbon black (GCB), and $MgSO_4$/C18 and $MgSO_4$/C18/GCB mixtures, were evaluated in the QuEChERS procedure. After the clean-up step, a 2-fold extract dilution was carried out without any improvement of the results obtained. The commonly applied dispersive-SPE step with primary-secondary amine (PSA) was not tested in this work due to the low recoveries reported for acidic compounds when using PSA for clean-up [33]. The retention of acidic analytes, as picloram, 2,4-D, fipronil or imazapic, on PSA material has been reported as the main reason of the low recoveries [15,17,34,35]. In this work, the results after application of different clean-up procedures did not substantially improve. It seemed that the highly concentrated soil extract (1:2) employed, led to strong matrix effects in all cases for this type of matrices.

After all experiments performed, the extracting system finally selected was acetonitrile with two-fold dilution of the soil extract with water. Using this procedure, signal enhancement or signal suppression was not much significant for most compounds (ME for the soil tested varied between 60-120% for the analytes under study).

Nowadays, the use of isotope-labelled internal standards (ILIS) is widely accepted as an efficient and simple way to correct matrix effects. However, in multi-residue methods the use of ILIS is problematic due to the high cost and the unavailability of commercial ILIS for all analytes. Although only three ILIS were available at our laboratory (dimethoate-d6, MCPA-d3, chlorpyrifos-d10), we compared

the results for the corresponding analytes (dimethoate, MCPA, chlorpyrifos) using calibration in solvent, with and without ILIS correction. MCPA showed recoveries at the LOQ level above 120% without applying correction. However, the use of its deuterated internal standard allowed correcting its recovery (working with relative areas), highlighting the relevance of using ILIS for each compound for an efficient matrix effects correction. The other two ILIS tested (dimethoate-d6, chlorpyrifos-d10) seemed unnecessary as the recoveries for dimethoate and chlorpyrifos were satisfactory without ILIS correction **(Table 2).**   Therefore, the use of MCPA-d3 as ILIS is recommended, if available to the laboratory.

Due to the lack of ILIS availability in our laboratory for every analyte included in the method, we applied an alternative approach to assure correct quantification. It consisted on the analysis of all soil samples with and without fortification at 0.5 mg/kg. This implies that a quality control (QC) was analyzed for every soil. Therefore, a correction factor for quantification might be applied depending on the QC recovery in those cases that it was out of the tolerance window (see Analysis of Soil Samples section)

3.4   *Method validation*

Linearity was satisfactory in the range 1– 100 ng/mL, in both solvent and in matrix-extract resulting after application of the recommended procedure. Correlation coefficients were higher than 0.99 and residuals lower than $\pm$ 30% for all pesticides.

The method was validated using a soil sample that was spiked at two concentrations (analysis in quintuplicate). An estimation of matrix effects for this soil was made previously to method validation because it was different to the soil used in matrix effects evaluation section. Data obtained revealed a rather similar behavior, except for the three compounds measured in negative mode **(Figure 1).**

The results for precision and accuracy at the two concentrations tested (0.05 and 0.5 mg/kg) are shown in **Table 2**. Data were obtained performing quantification with standards in solvent (ACN:water). The method presented satisfactory precision (RSD<20%) and accuracy (recoveries between 70 and120%) for all analytes, at the two levels of fortification, with the exception of the herbicide picloram, whose recoveries were around 50%. Some authors also reported low recoveries for this weak acid herbicide when extracted from soils at pH lower than 7 [6]. The QC samples for picloram in the soils tested had average recoveries between 47 and 60%, which was consistent with data obtained in method validation. Although not tested in this work, the use of picloram ILIS, added as surrogate, seems the easiest and best solution to correct the analytical errors for this herbicide.

The LOQ objective was established to be 0.05 mg/kg, as this was the lowest analyte spiked level in samples that was fully validated with satisfactory precision and accuracy [31]. This value of 0.05 mg/kg is typically fixed as LOQ for pesticide residue methods in soil [32]. In order to illustrate the capability of the method to detect low analyte concentrations, the limit of detection (LOD) was estimated for a signal-to-noise ratio of 3 from the lowest point of the calibration prepared with standards in soil matrix (equivalent to 0.012 mg/kg) (be aware that the term LOD in this case is not used as limit of determination, as in certain guidances[32]). The estimated LODs varied between 0.1 and 4 µg/kg (**Table 2**). The method was found specific as no relevant signals were observed in the blank extracts analyzed at the analyte retention time **(see Figure 2** as an example for selected compounds)**.

3.5   *Analysis of soil samples*

Soils may strongly differ in their physico-chemical characteristics, which mean that a method satisfactorily validated for one soil sample only may be questionable for other soils. Therefore, with this type of matrices is important to ensure the robustness

of the method and that data are satisfactory for different types of soils. This implies testing the method in a notable number of soil samples. Keeping this objective in mind, after method validation in the soil selected the methodology developed was assayed in eleven soil samples from different physico-chemical characteristics analyzing each soil before and after fortification (at 0.5 mg/kg) with the pesticides mixture, i.e. preparing individual QCs for each soil. Recoveries of the 11 individual QC samples served as an additional validation and supported the robustness and applicability of the method.

Soil samples used in this work (**Figure 1SI**) were considered as "blank" samples as no pesticides were expected to be found. Besides the eleven QCs analyzed additional QCs were prepared with the same soil used in validation experiments at 0.05 and 0.5 mg/kg fortification levels (in triplicate).

The results are shown in **Table 3**. As it can be seen, recoveries for the eleven QCs were highly satisfactory, leading to average values between 70-120%, with the only exception of picloram (60%). RSDs were excellent taking into account that all were individual values from different soils. Similarly, the QCs prepared from the soil used in the validation experiments (in triplicate) were in general satisfactory, with values from 60-120% for nearly all compounds at the LOQ and 10xLOQ level (i.e. 0.05 and 0.5 mg/kg). Again, picloram showed low recoveries (47%) at both concentrations. Recoveries for MCPA and carbaryl were satisfactory at 0.5 mg/kg, but they were higher than 120% at the LOQ level. This behavior was consistent with data obtained in method validation.

Despite that soil samples were collected from experimental fields without agricultural activities for several years, up to five pesticides were detected in 8 out of 11 soils analyzed: ametryn, atrazine, chlorpyrifos, dimethoate, imidacloprid **(See S.I. Table 2).** The herbicide atrazine was the most detected (4 soil samples). Ametrine, dimethoate, imidacloprid and chlorpyrifos were detected in two soil samples each. However, pesticide concentrations were mostly below the LOQ objective (0.05 mg/kg),

and did not exceed 0.1 mg/kg in any sample. The presence of some pesticides in the soils at very low concentrations was not considered much significant, and might be due to some drift when applying pesticides in nearby areas.

Figure 3 shows selected LC-MS/MS chromatograms for Pergamino soil sample, where three pesticides were detected. The identity of the compounds in the sample was confirmed by retention time and ion ratio agreement in comparison with the reference standard. As it can be seen, q/Q ratio deviation in all positives was within the maximum tolerance of $\pm$ 30% (SANCO/12571/2013).

4. CONCLUSIONS

A simple and fast LC-MS/MS multiresidue method has been developed for determination of pesticides of environmental concern in soil samples. After soil extraction with acetonitrile (1 hour mechanical shaking followed by 15 min ultrasounds) and two-fold dilution of the extract with water, satisfactory recoveries were obtained at 0.05 mg/kg and 0.5 mg/kg, with LODs between 0.1 and 4 μg/kg. No clean-up steps were necessary, minimizing in this way sample manipulation, solvents and reagent consumption, and analysis time. The applicability of the method to very different types of soils from Argentina was demonstrated by analysis of QCs for each soil analyzed. Therefore, the analytical methodology proposed in this paper can be easily implemented to routine laboratories for monitoring pesticide residues in different types of soils.

Matrix effects were carefully studied and, with few exceptions, were not much relevant, allowing quantification of the compounds using standards in solvent. Despite the soils used in this study had not been employed for agricultural purposes for many years, two herbicides (atrazine, ametryn) and three insecticides (chlorpyrifos, imidachloprid, dimethoate) were detected at low concentrations (mostly below 0.07 mg/kg). This might

be due to their use in the surrounding areas or as a result of uncontrolled runoff processes.

REFERENCES

1   Argenbio, 2012. "Gracias a los transgénicos, la Argentina ya está entre los primeros 10 países agrícolas." http://argenbio.org/index/notas/5923 (accessed April 2015).

2   Osaro, 2015. http://bcr.com.ar/default.aspx (accessed April 2015).

3   Cesafe, 2013. http://www.casafe.org/ (accessed April 2015).

4   V. C. Aparicio, E. De Gerónimo, D. Marino, J. Primost, P. Carriquiriborde and J. L. Costa, *Chemosphere*, 2013, **93**, 1866–73.

5   V. Andreu and Y. Picó, *TrAC Trends Anal. Chem.*, 2004, **23**, 772–789.

6   E. Assis, A. Silva, L. Barbosa, M. Queiroz, L. D´Antonino and V. GonCalves, *Planta Da*, 2011, 683–696.

7   A. M. Botero-Coy, M. Ibáñez, J. V Sancho and F. Hernández, *J. Chromatogr. A*, 2013, **1292**, 132–41.

8   S. S. Albaseer, R. Nageswara Rao, Y. V. Swamy and K. Mukkanti, *J. Chromatogr. A*, 2010, **1217**, 5537–5554.

9   S. Net, A. Delmont, R. Sempéré, A. Paluselli and B. Ouddane, *Sci. Total Environ.*, 2015, **515-516**, 162–180.

10   K. Ahmadi, Y. Abdollahzadeh, M. Asadollahzadeh and A. Hemmati, *Talanta*, 2015, **137**, 167–173.

11   F. Hernández, J. Beltran, F. J. Lopez and J. V. Gaspar, *Anal. Chem.*, 2000, **72**, 2313–2322.

12   M. R. Rigi, M. Farahbakhsh and K. Rezaei, *J. Brazil Chem Soc.*, 2015, **26**, 156–164.

13   C. Tadeo, Jose., Perez, Rosa., Albero, Beatriz., Garcia-Valcácer, Ana., Sanchéz-Brunete and A. L. N. van Nuijs, *J. AOAC Int.*, 2012, **14**, 1258 – 1271.

14   M. Anastassiades, S. J. Lehotay, D. Stajnbaher and F. J. Schenck, *J. AOAC Int.*, 2003, **86**, 412–31.

15   J. L. D. O. Arias, C. Rombaldi, S. S. Caldas and E. G. Primel, *J. Chromatogr. A*, 2014, **1360**, 66–75.

16   M. C. Bruzzoniti, L. Checchini, R. M. De Carlo, S. Orlandini, L. Rivoira and M. Del Bubba, *Anal. Bioanal. Chem.*, 2014, **406**, 4089–116.

17   Y. Cheng, F. Dong, X. Liu, J. Xu, W. Meng, N. Liu, Z. Chen, Y. Tao and Y. Zheng, *Anal. Methods*, 2014, **6**, 1788 – 1795.

18   N. Homazava, C. Gachet Aquillon, E. Vermeirssen and I. Werner, *Int. J. Environ. Anal. Chem.*, 2014, **94**, 1085–1099.

19   N. Ivdra, S. Herrero-Martín and A. Fischer, *J. Chromatogr. A*, 2014, **1355**, 36–45.

20   A. Masiá, K. Vásquez, J. Campo and Y. Picó, *J. Chromatogr. A*, 2015, **1378**, 19–31.

21   S. S. Caldas, C. M. Bolzan, M. B. Cerqueira, D. Tomasini, E. B. Furlong, C. Fagundes and E. G. Primel, *J. Agric. Food Chem.*, 2011, **59**, 11918–11926.

22   T. Chai, Q. Jia, S. Yang and J. Qiu, *J. Sep. Sci.*, 2014, **37**, 595–601.

23   S. Li, X. Liu, Y. Zhu, F. Dong, J. Xu, M. Li and Y. Zheng, *J. Chromatogr. A*, 2014, **1358**, 46–51.

24   V. C. Fernandes, S. J. Lehotay, L. Geis-Asteggiante, H. Kwon, H. G. Mol, H. van der Kamp, N. Mateus, V. F. Domingues and C. Delerue-Matos, *Food Addit Contam Part A Chem Anal Control Expo Risk Assess*, 2014, **31**, 262–270.

25   F. Hernández, M. Ibañez and T. Portolés, in *Comprehensive Environmental Mass Spectrometry*, ed. A. L. I. Publications, 2012, pp. 309 – 328.

26   F. Hernández, M. I. Cervera, T. Portolés, J. Beltrán and E. Pitarch, *Anal. Methods*, 2013, **5**, 5875.

27   A. Kruve, R. Rebane, K. Kipper, M.-L. Oldekop, H. Evard, K. Herodes, P. Ravio and I. Leito, *Anal. Chim. Acta*, 2015, **870**, 8–28.

28   P. Herrero, F. Borrull, E. Pocurull and R. M. Marcé, *TrAC Trends Anal. Chem.*, 2014, **62**, 46–55.

29   H. V Botitsi, S. D. Garbis, A. Economou and D. F. Tsipi, *Mass Spectrom. Rev.*, 2011, **30**, 907–39.

30   Minagricultura, Insumos agrícolas, http://www.minagri.gob.ar/site/agricultura/contratistas_rurales/index.php (accessed April 2015).

31   SANCO/12571/2013,Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed, European Commission, Health and Consumer Protection Directorate General

32.   SANCO 825/00, rev. 8.1. 2010, Guidance document on pesticide residue analytical methods, European Commission, Health and Consumer Protection Directorate General

33.   Acidic pesticides using QuEChERS method; http://quechers.cvua-stuttgart.de/pdf/acidicpesticides.pdf (accessed January 2015)

34.   R. Romero-González, M.M. Aguilera-Luiz, P. Plaza-Bolaños, A.Garrido Frenich, J.L.Martinez Vidal, *J. Chromatogr. A.,* 2011, **1218**, 9353–9365.

35.   F.J. Schenck, A.N. Brown, L.V. Podhorniak, A. Parker, M. Reliford, J.W. Wong, *J. AOAC Int.*, 2008, **91**, 422–438.

Figure captions

Figure 1. Matrix effects in the soil used for method validation after application of the recommended analytical procedure

Figure 2. UHPLC–MS/MS chromatograms for selected compounds: (a) blank soil (b) standard 50 µg/L, (c) Soil extract spiked at 50 µg/L.

Figure 3. UHPLC–MS/MS chromatograms for Pergamino soil, where three pesticides were identified: ametryn (0.09 mg/kg), dimethoate (0.03 mg/kg*), imidacloprid (0.02 mg/kg*). Quantification transition (Q), confirmation transition (q)
estimated concentration from a peak response above S/N=10 (but below the LOQ objective of 0.05 mg/kg).

1	Picloram
2	Imidacloprid
3	Dimethoate
4	2,4 D
5	MCPA
6	Carbendazim
7	Cyanazine
8	Carbofuran
9	Carbaryl
10	Atrazine
11	Ametryn
12	Malathion
13	Metolachlor
14	Epoxiconazole
15	Fipronil
16	Diazinon
17	Chlorpyrifos
18	Pendimethalin

Figure 1

Figure 2

Ametryn — Dimethoate — Imidacloprid
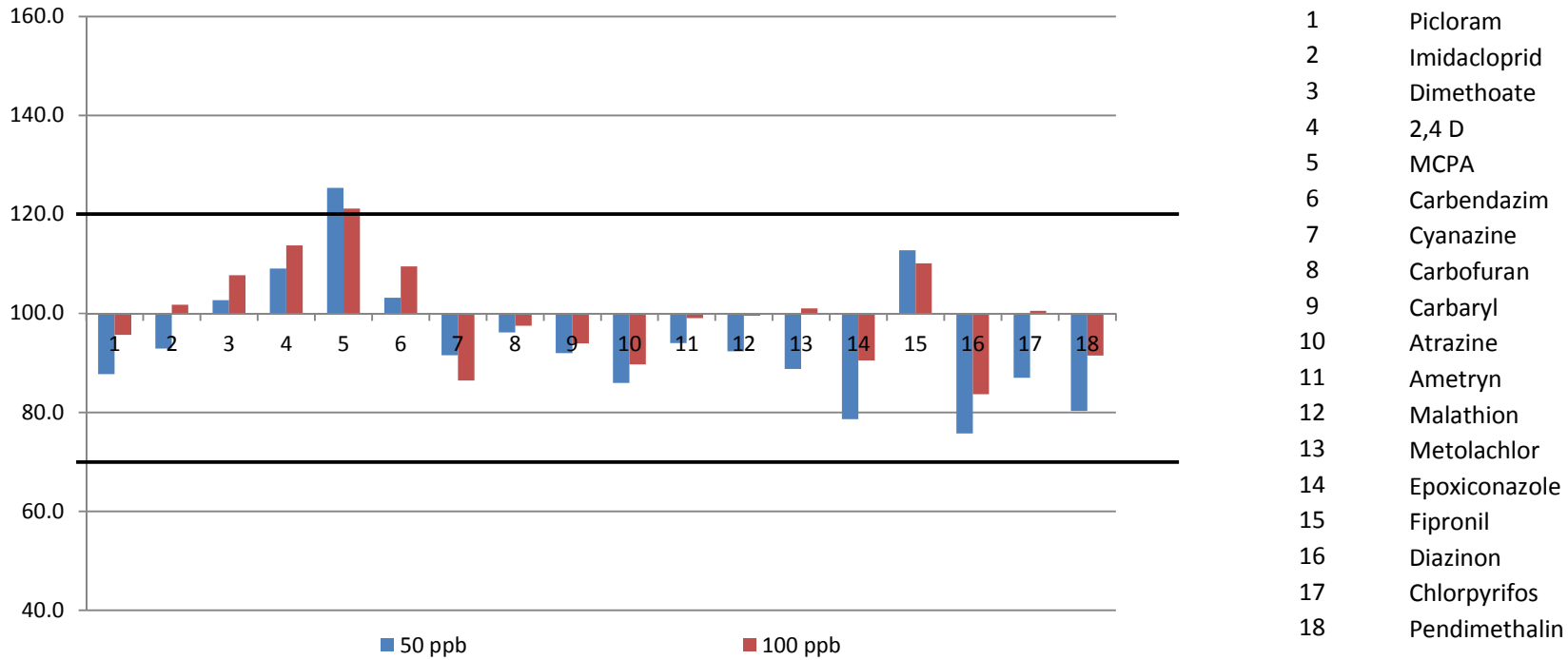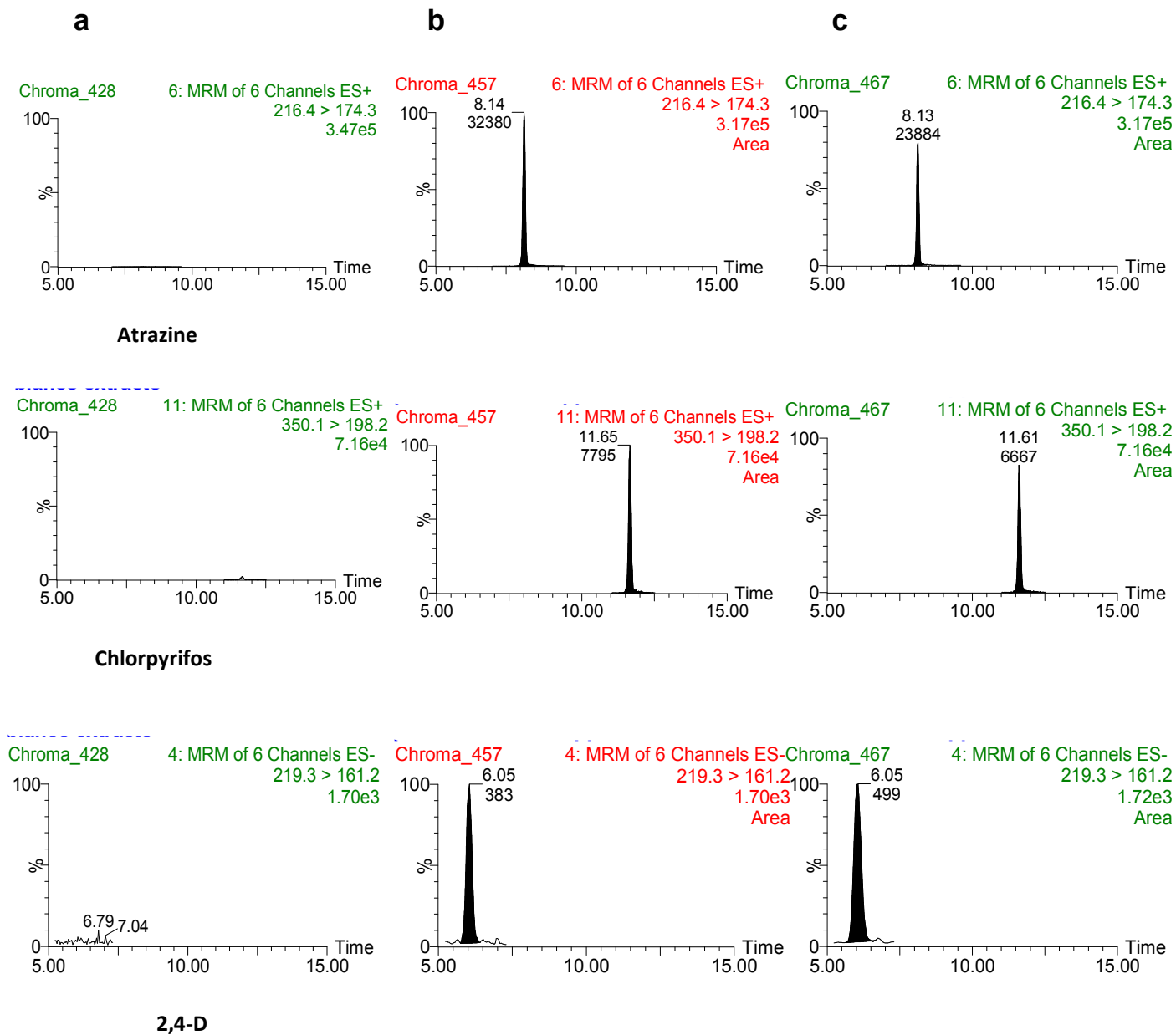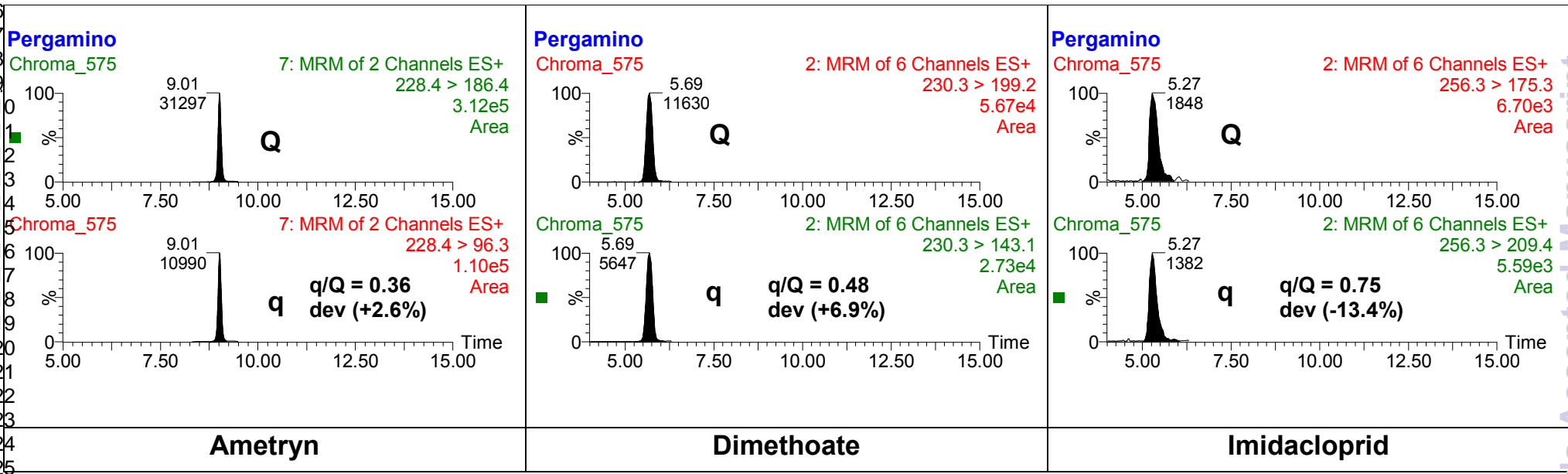
Figure 3

Table 1. Mass spectrometric conditions for selected compounds.

No	Compound	Use	Mode	RT (min)	Precursor ion (*m/z*)	Cone (V)	Collision energy (eV)	Product ion (*m/z*)[a]	q/Q (RSD)
1	Picloram (PIC)	Herbicide	ES+	3.1	241.2 243.2	25	20 20	195.2 197.2	0.94(0.02)
2	Imidacloprid (IMI)	Insecticide	ES+	5.3	256.3	25	15 15	175.3 209.4	0.87(0.03)
3	Dimethoate (DIM)	Insecticide	ES+	5.7	230.3	15	10 20	199.2 143.1	0.45(0.03)
4	2,4-D	Herbicide	ES-	6.3	219.3 221.3	20	15 15	161.2 163.2	0.84(0.04)
5	MCPA	Herbicide	ES-	6.4	199.3 201.3	20	15 15	141.2 143.2	0.28(0.03)
6	Carbendazim (CAR)	Fungicide	ES+	6.5	192.3	25	15 30	160.2 132.2	0.18(0.01)
7	Cyanazine (CYN)	Herbicide	ES+	7.3	241.4 243.4	30	15 15	214.3 216.3	0.32(0.01)
8	Carbofuran (CRB)	Insecticide	ES+	7.4	222.4	25	20 10	123.2 165.2	0.71(0.04)
9	Carbaryl (CBL)	Insecticide	ES+	7.9	202.4	15	10 25	145.1 117.2	0.16(0.01)
10	Atrazine (ATZ)	Herbicide	ES+	8.3	216.4	35	15 30	174.3 104.2	0.24(0.01)
11	Ametryn (AME)	Herbicide	ES+	9.0	228.4	35	20 25	186.4 96.3	0.35(0.01)
12	Malathion (MAL)	Insecticide	ES+	9.2	331.3	20	10 25	127.1 99.1	0.67(0.10)
13	Metolachlor (MEC)	Herbicide	ES+	9.7	284.4	20	15 25	252.4 176.3	0.48(0.01)
14	Epoxiconazole (EPZ)	Fungicide	ES+	9.8	330.3	30	20 20	121.1 162.2	0.12(0.05)
15	Fipronil (FPN)	Insecticide	ES-	9.9	435	20	15 25	330.0 250.2	0.28(0.03)
16	Diazinon (DZN)	Insecticide	ES+	10.2	305.3	30	20 20	169.2 153.2	0.77(0.12)
17	Chlorpyrifos (CHLOR)	Insecticide	ES+	11.9	350.1	25	20 20	198.2 115.1	0.36(0.02)
18	Pendimethalin (PEN)	Herbicide	ES+	12.0	282.4	15	10 20	212.4 194.3	0.12(0.01)
	Dimethoate-d6		ES+	5.7	236.3	15	10 15	205.2 131.1	0.51(0.03)
	MCPA-d3		ES-	6.4	202.3 204.3	20	15 15	144.2 146.2	0.30(0.06)
	Chlorpyrifos-d10		ES+	11.8	360.1	25	20 20	199.2 115.1	0.09(0.01)

[a] The first transition (top) was used for quantification and the second transition (bottom) was used for confirmation.

Table 2. Method validation*. Percentage recoveries and relative standard deviation (in brackets). Limits of detection (LOD)

Compound	0.05 mg/kg	0.5 mg/kg	LOD (µg/kg)
Picloram (PIC)	50 (2)	51 (4)	3.9
Imidacloprid (IMI)	96 (3)	102 (5)	0.8
Dimethoate (DIM)	95 (6)	107 (6)	0.1
2,4 D (2,4D)	120 (10)	100 (6)	1.0
MCPA (MCPA)**	134 (7)**	115 (11)**	0.9
Carbendazim (CAR)	90 (4)	94 (5)	3.0
Cyanazine (CZN)	74 (4)	85 (4)	0.1
Carbofuran (CRB)	98 (5)	93 (9)	0.1
Carbaryl (CBL)	138 (3)	107 (5)	0.1
Atrazine (ATZ)	86 (6)	91 (4)	0.1
Ametryn (AME)	100 (6)	100 (6)	0.2
Malathion (MAL)	99 (6)	99 (5)	0.1
Metolachlor (MEL)	101 (5)	99 (7)	0.2
Epoxiconazole (EPZ)	78 (3)	84 (6)	0.2
Fipronil (FPN)	114 (5)	112 (4)	0.3
Diazinon (DZN)	86 (8)	78 (7)	0.4
Chlorpyrifos (CHLOR)	91 (6)	91 (5)	0.3
Pendimethalin (PEN)	83 (5)	86 (8)	0.6

* Data corresponding to a soil sample collected from Balcarce

** Recoveries for MCPA were 95% (0.05 mg/kg) and 102% (0.5 mg/kg) when MCPA-d3 was used as ILIS
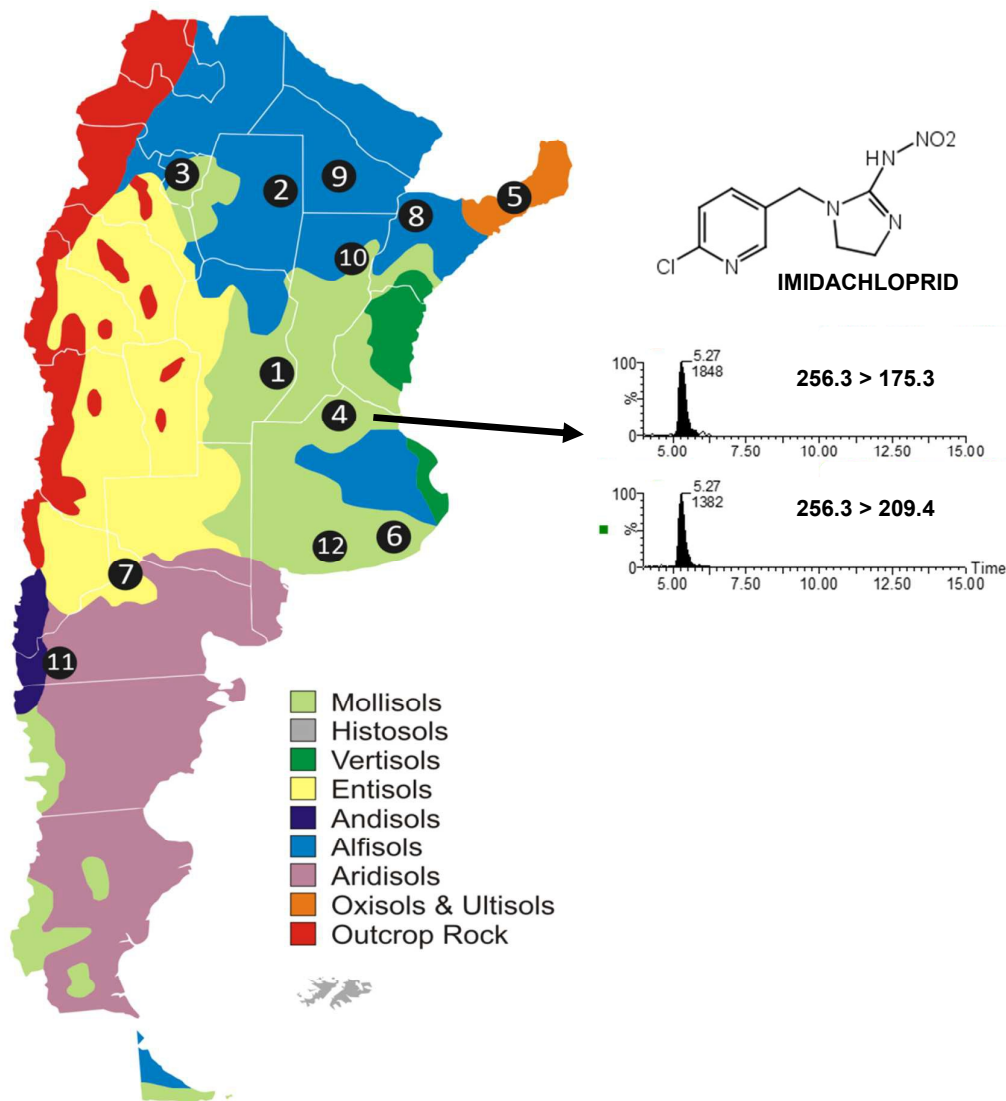
Table 3. Recovery for Quality Controls prepared from different soil samples

COMPOUND	Analysis of eleven soil samples*		QC LOQ (n=3)**		QC 10xLOQ (n=3)**	
	QC Rec	RSD	QC Rec	RSD	QC Rec	RSD
Picloram	60	16	47	4.6	47	1.0
Imidacloprid	98	6.1	88	2.8	94	1.9
Dimethoate	103	4.4	92	8.3	95	1.6
2.4-D	105	7.7	117	7.9	102	1.3
MCPA	111	9.2	132	2.5	110	1.7
Carbendazim	104	8.4	92	1.6	93	2.7
Cyanazine	84	7.0	65	5.8	74	2.1
Carbofuran	99	7.2	92	9.3	92	0.2
Carbaryl	105	4.3	164	10	98	0.0
Atrazine	88	7.7	66	4.1	74	0.9
Ametryn	98	4.6	92	3.1	90	0.4
Malathion	107	7.5	96	7.3	105	0.8
Metolachlor	100	5.7	89	1.4	88	0.3
Epoxiconazole	88	8.1	65	7.5	71	0.1
Fipronil	110	13	113	1.2	103	0.7
Diazinon	76	13	63	6.9	63	6.0
Chlorpyrifos	91	5.8	79	6.0	79	1.0
Pendimethalin	86	6.7	72	4.3	70	0.1

* Average recovery and relative standard deviation for 11 QCs prepared from different soils (0.5 mg/kg fortification level)

**Average recovery and relative standard deviation for the QCs prepared from the soil used in validation experiments (n=3) at 0.05 mg/kg (LOQ) and 0.5 mg/kg (10xLOQ) fortification level

GRAPHICAL ABSTRACT

IMIDACHLOPRID

256.3 > 175.3

256.3 > 209.4

Mollisols
Histosols
Vertisols
Entisols
Andisols
Alfisols
Aridisols
Oxisols & Ultisols
Outcrop Rock