Determination of glyphosate, AMPA and glufosinate in dairy farm water from Argentina using a simplified UHPLC-MS/MS method

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HIGHLIGHTS

- A simplified FMOC-UHPLC-MS/MS method for glyphosate, AMPA, glufosinate is presented.
- A rapid offline DCM cleaning after derivatization is used as unique cleanup procedure.
- LOQs were set at 0.6, 0.2, 0.1 µg/L for glyphosate, AMPA, glufosinate, respectively.
- The method was applied to the analysis of 158 water samples from 40 dairy farms.
- Glyphosate/AMPA were found in 15/53% of the samples at 0.6–11.3/0.2–6.5 µg/L levels.

GRAPHICAL ABSTRACT
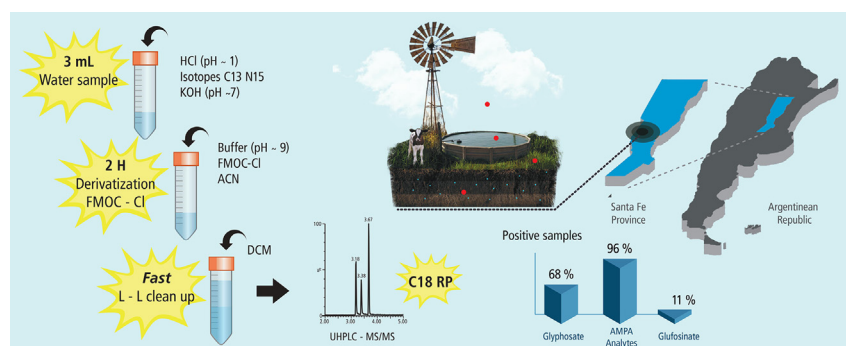
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ABSTRACT

Argentina, together with the USA and Brazil, produces approximately 80% of the total worldwide glyphosate loadings. The development of a simplified ultra-high performance liquid chromatographic tandem mass spectrometric method (UHPLC-MS/MS) for the determination of glyphosate, aminomethylphosphonic acid (AMPA) and glufosinate in water is described, including studies of several alternatives of 9-fluorenylmethylchloroformate (FMOC-Cl) derivatization and pretreatment steps. The proposed method includes acidification and neutralization of a low sample volume (3 mL), 2 hours derivatization step, cleanup with dichloromethane, followed by reverse phase UHPLC-MS/MS determination of the analytes. Figures of merit were satisfactory in terms of linearity, selectivity, accuracy and intermediate precision (%REC 70–105% with RSD < 15%). Limits of quantification (LOQ) were suitable for monitoring purposes (0.6, 0.2, 0.1 µg/L for glyphosate, AMPA and glufosinate respectively). The validated methodology was applied for the analysis of livestock wells waters from 40 dairy farms located in the central region of Argentina. Glyphosate and AMPA were quantified in 15% and 53% of the analyzed samples with concentrations ranging from 0.6–11.3 µg/L and 0.2–6.5 µg/L respectively. Greater concentrations of glyphosate were also verified in waters from open-reservoir tanks, which are directly exposed to the farm environment. In these cases glyphosate and AMPA occurrence increased, being quantified in the 33% and 61% of the samples with values ranging 0.6–21.2 µg/L and 0.2–4.2 µg/L respectively. Also in this case glufosinate was found in 52%

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samples at <LOQ levels and was quantified in one sample at 0.1 μg/L. This new information constitutes an important contribution to authorities and scientists for further research, control and risk analysis purposes.

1. Introduction

In 2016, the global area used for glyphosate and other agrochemicals highly demanding biotech crops was 185.1 million ha for a total of 26 countries, representing a 3% increase from 2015. Three countries account for 79% of this total global area (USA, 39%; Brazil, 27%; Argentina, 13%) (ISAAA, 2016). Argentina dramatically transformed its agriculture based on important technological innovations. These changes included the extensive adoption of Genetically Modified (GM) crops designed to be resistant to specific herbicides, mainly glyphosate (N-(phosphonomethyl) glycine) and glufosinate ((3-amino-3-carboxypropyl)methyl phosphinic acid). An average of approximately 200 million tons of glyphosate was loaded annually onto a confined surface that grew from 25 million ha in 1996 to >40 million ha in 2016, producing a total of >110 million tons of soybeans and other crops (Agroindustry Ministry of Argentina, 2016). The mentioned active ingredients (a.i.) are mainly used in agriculture as non-selective herbicides in pre-emergence weed control and as a pre-harvest desiccation agent, being other non-agricultural uses also registered. Chemical residues at low concentrations might be found in food, water, soils and other environmental samples, exposing both humans and livestock to toxicological risks through their diets.

Groundwater is the primary source used for livestock watering in the study region and no tolerable levels are specifically established for pesticides. For these compounds as well as for other pollutants, references to established levels for human consumption are usual (Charlón et al., 2005). The main reference on glyphosate levels in water recommends a value of 300 μg/L expressed in terms of the isopropylamine salt of glyphosate and applicable to the groundwater sources (Under Secretariat of Water Resources, Argentina, 2003).This same guideline establishes the environmental water quality criterion for aquatic biota protection applicable to fresh water (240 μg/L).

The analytical task involved in monitoring and studying the effects of the widespread use of these herbicides is still a challenge, because of their particular chemical properties including high polarity and water solubility, low volatility and molecular weight, amphoteric behaviour, and lack of chromophores. These are all characteristics that cause glyphosate and its related compounds to be not amenable to analysis by the multi-residue methods (MRMs) commonly employed in pesticide monitoring or enforcement plans (EFSA, 2016; GAO, 2014).

Analytical difficulties have been marked as an important factor to explain the lack of effective strategies to better understand glyphosate human dietary exposure, its environmental fate and its impact on ecosystems (Huhn, 2018).

Several analytical methodologies for the determination of glyphosate and its principal metabolite aminomethylphosphonic acid (AMPA) based on chromatographic-mass spectrometric techniques have been reviewed, including more recently the simultaneous determination of another phosphonate herbicide as glufosinate (Ding et al., 2015; Raina-Fulton, 2014). Methods based on derivatization strategies constituted the first approaches and continue to be used with the final determination using HPLC, LC-MS/MS and GC–MS/MS techniques (Arkan and Molnár-Perl, 2015).

For water analysis a variety of non-derivatization approaches was introduced (Bauer et al., 1999; Guo et al., 2016; Hao et al., 2011; Marek and Koskinen, 2014). Ionic chromatographic techniques still show some drawbacks. Thus, achieving simple and reliable methodologies with the use of these approaches is still a challenge.

Among the techniques that include derivatization, the case of the formation of FMOC derivatives and detection with LC-MS/MS clearly stands out. Variations of this approach using L-L cleanup and SPE concentration step with different online configurations and detection with LC-MS/MS systems, achieved ultra-trace levels of detection (Hanke et al., 2008; Ibáñez et al., 2005; Poiger et al., 2016; ISO 16308:2014). Mallet (2014) proposed an automated Open-Architecture UPLC system. Other authors made contributions about critical issues such as the interactions between the reagent and multivalent cations mainly observed in underground water (Freuze et al., 2007; Ibáñez et al., 2006), the derivation reaction time (Vreeken et al., 1998), and other matrix interference effects (Skeff et al., 2016; Toss et al., 2017). Some topics persist as complications in these approaches when FMOC-Cl is used. For example, the derivatization conditions that should be well established, the elimination of the reaction impurities, the improvement of the detection limits through preconcentration, special requirements on the mass spectrometry selectivity due to the FMOC affinity to amine moieties producing unspecific reactions. However, many of the its continue to be very valuable for water analysis (Adams et al., 2017). These include the improvement of retention in LC, the increase in molecular weight and sensitivity, the reproducibility of the reaction, the good recoveries working with Isotope Labelled Internal Standard (ILIS), and the very competitive LOQs. A final advantage is the suitability for using reverse phase chromatography (C18 and others) being important because it greatly simplifies operations and provides compatibility with other routine multi-residue applications using the same chromatographic systems. This study had two aims: first, to develop a simplified methodology for glyphosate, AMPA and glufosinate determination in water using the FMOC-Cl derivatization strategy and UHPLC-MS/MS detection; and second to apply it in field studies analysing selected groundwater and open-reservoir tanks water samples from dairy farms in the Argentinean central region.

2. Material and methods

2.1. Standards and reagents

Crystalline solid standards of glyphosate (97.0%), AMPA (98.0%), glufosinate ammonium (97.5%), glufosinate-FMOC (94.5%), glyphosate-FMOC (91.5%) and AMPA-FMOC (98.0%) were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Standard solutions in water of the isotope-labelled glyphosate (ILIS) 1,2-$^{13}C_2$ ^{15}N (100 μg/mL) and AMPA ^{13}C ^{15}N (100 μg/mL) were obtained from the same supplier.

Individual stock standard solutions of glyphosate, AMPA and glufosinate were prepared to give a final concentration of 1000 μg/mL in water, except for the standard solutions of FMOC derivatives which were prepared in MeOH at the same concentration. Intermediate standard solutions of 100 and 1 μg/mL were prepared by dilution of the stock solutions in water and MeOH. In the case of the isotope-labelled compounds, stock and intermediate solutions were prepared in water at 11 and 1 μg/mL, respectively. Finally, the working standard solutions were prepared on the day they were used by dilution of the 1 μg/mL intermediate solution in the mobile phase to reach the desired concentration level. All standard solutions were stored in glass vials at −18 °C.

Solutions of the derivatizing reagent 9-fluorenylmethylchloroformate (FMOC-Cl) (Sigma, St. Louis, MO, USA) and sodium tetraborate buffer (Anedra, San Fernando, BA, Argentina), were prepared separately by dissolving the reagents in acetonitrile and water, respectively, at the concentration levels required for each specific derivatization method. UHPLC-grade methanol, acetonitrile and water (Optima™, Fisher Scientific, NJ, USA) were used for the preparation of the mobile phase and as solvents in all the methods. For cleanup purposes, OASIS® HLB 6cc, 200 mg

Sorbent, 30 μm Particle Size extraction cartridges (Waters, Milford, MA, USA) and pesticide-grade dichloromethane (Sintorgan, Buenos Aires, Argentina) were employed. Hydrochloric acid (HCl), potassium hydroxide (KOH), EDTA and formic acid employed in derivatization reactions were from Cicarelli (San Lorenzo, SF, Argentina).

2.2. Chromatographic system and operating conditions

LC-MS/MS analyses were performed using an Acquity UPLC® liquid chromatograph (Waters, Milford, MA, USA) coupled to a triple quadrupole mass spectrometer equipped with an ESI source able to operate in either the positive or negative-ion mode (TQD, Waters Micromass, UK). Two different C18-based columns and two different mobile phases were tested to increase the instrumental response and to get better chromatographic parameters (resolution, asymmetry and peak shape). Column 1: Acquity UPLC® HSS C18 column (1.8 μm particle size, 100 × 2.1 mm i.d.) and column 2: Acquity UPLC® BEH C18 column (1.7 μm particle size, 100 × 2.1 mm i.d.) both from Waters, Milford, MA, USA. Both columns operated at a flow rate of 0.35 mL/min and at 40 °C.

Aliquots of 10 μL of standard and/or sample extracts were introduced by means of an auto-sampler (Waters, Milford, MA, USA). Mobile phase 1 consisted of water and acetonitrile (98:2) + 0.1% formic acid (solvent A) and acetonitrile + 0.1% formic acid (solvent B). Mobile phase 2 consisted of 5 mM ammonium acetate (solvent A) and methanol (solvent B).

The LC gradients for the separation for mobile phase 1 were from 0 to 0.25 min, isocratic (95% A:5% B); from 0.25 to 7 min, a linear increase of B from 5 to 100%; from 7 to 8 min, a linear decrease of B from 100% to 75%; from 8 to 8.5 min, a linear decrease of B from 75% to 40%; from 8.5 to 9 min, a linear decrease of B from 40% to 5%. The initial conditions were re-equilibrated in 1 min, resulting in a total run time of 10 min. For mobile phase 2 from 0 to 0.5 min, isocratic (90% A:10% B); from 0.5 to 1.5 min, a linear increase of B from 10 to 95%; from 1.5 to 1.6 min, a linear increase of B from 95 to 100%; and isocratic from 1.6 to 2.6 min (0% A:100% B). The initial conditions were re-established in 0.1 min and the column was re-equilibrated for 2.7 min, resulting in a total run time of 5 min.

Ionization was performed in positive-ion mode using nitrogen as the desolvation and cone gas at 600 L/h and 20 L/h, respectively. The source temperature was set at 140 °C and a capillary voltage of 1 kV was applied. The desolvation temperature was 500 °C. Argon at a pressure of 4.4×10^{-3} mbar and a flow of 0.1 mL/min was used at the collision cell to produce the respective ion fragments and acquisition was performed in the MRM mode. The m/z values for the precursor and fragment for each specific compound along with their respective cone voltages and collision energy values are shown in Table 1. A dwell time of 0.01 s was selected. Chromatographic and mass spectrometry data handling was performed using MassLynx software v 4.1 (Waters, Manchester, UK).

2.3. Analytical methods

Several alternatives for pre-column derivatization of glyphosate, glufosinate and AMPA with FMOC-Cl were submitted to a preliminary evaluation designing different procedures adapted to the aim of this work and based on several strategies reported in the literature. The main frame of the designed approaches studied in this work is laid out in Table 2. Method A is adapted from the method proposed by Hanke et al. (2008) reducing to a half the scale of volumes and adopting off line C18-SPE. Method B is based on the proposal of Ibáñez et al. (2006) changing to off line SPE, modifying the L-L partition and the pH control (1 and 6–7).

Method C is based on the work of Zelaya et al. (2010) introducing an off line SPE cleanup step and volume adjustments to achieve adequate pH with HCl and KOH and based on the work of the same authors

Table 1
Optimized MS/MS parameters for the FMOC derivatives of glyphosate, AMPA, glufosinate and internal standard.

Compound	Cone voltage (V)	Precursor ion (m/z)	Product ion (m/z)[a]	Collision energy (eV)
Glyphosate-FMOC	20	392.0	Q 88.1	30
			q 214.1	10
AMPA-FMOC	20	334.0	Q 179.1	20
			q 112.1	15
Glufosinate-FMOC	30	404.0	Q 136.1	25
			q 208.2	10
Isotope-labelled glyphosate-FMOC	20	395.0	Q 91.1	30
			q 217.1	10
Isotope-labelled AMPA-FMOC	20	336.0	Q 181.1	20
			q 114.1	15

[a] Q: transition used for quantification; q: transition used for confirmation.

Method D was designed introducing HCl and KOH volume adjustments and a reduction in reaction time.

Following the preceding scheme, different aspects of the experimental procedures were evaluated: conditioning of the sample before the derivatization step, the FMOC-Cl concentration, buffer volume and concentration, reaction time and cleanup alternatives. From the initial evaluation of the proposed approaches, method D was finally selected for further optimization and validation since it simplified the main operation steps and produced satisfactory results (Table 2) as justified in the next section.

Real water samples were used as blanks and for matrix matched calibrations, making sure that concentration of the three analytes were below the LODs and relatively low levels of divalent cations were present (total Ca^{++} and Mg^{++} below 200 mg/L).

2.4. Water samples

The validated methodology was applied to the study of the three analytes in waters that normally are supplied to milk-producing animals during four annual seasons (Spring 2012, and Summer, Autumn and Winter 2013). The sampling plan included 125 groundwater samples and other 33 surface-water of open-reservoir tanks samples, taken from 40 dairy farms located in the central region of Santa Fe Province in Argentina (Castellanos and Las Colonias departments). This region includes the most important dairy basin in Argentina, sharing the land with other agricultural activities such as extensive crop production (soybean, wheat, maize, sunflowers, and sorghum), most of them based on GM cultures that demand a great loading of glyphosate and other agrichemicals as a technological aid to no-till practices (Fig. 1).

Groundwater samples were obtained directly from the outlet tap of the well on each dairy farm, using standardized sampling procedures following an INTA protocol (INTA, 2011). Extraction of water from wells is carried out by mechanical pumps or mainly through windmills.

Regarding the aquifer system, the study area belongs to the Chaco-Pampeana plain, in which freshwater reservoirs correspond to geoforms that favour the infiltration and storage of meteoric waters. The soils are an Argiudoll type with a B2t horizon that contains a percentage of clays close to 60% (Tujchneider et al., 2002). The depth of the sampled wells varied in all cases between 20 and 25 m. In the Castellanos Department, the phreatic levels were 4.2 and 2.2 m during Spring 2012 and Summer 2013, respectively, and approximately 10.3 and 8.8 m during similar seasons in the Las Colonias Department. To characterize the chemical composition of the sampled waters, physical-chemical analyses were performed on samples obtained from the 40 farms at each sampling season. The range of concentrations found for representative parameters was as follows (expressed in mg/L): dissolved solids (696–5423), suspended solids (0–400), chloride (28–1552), sulphate (20–1637), calcium (6–168), potassium (6–2460), sodium (191–1610), magnesium (4–111) and pH values and conductivity in the range of 7.1–8.4 and 2.2–10.3 mS/cm, respectively.

Table 2
Detailed steps of the four procedures under study.

	Method A	Method B	Method C	Method D
Sample volume	40 mL	10 mL	3 mL	3 mL
Acidification	HCl 6 M (pH 1, 1 h)	HCl 6 M (pH 1, 1 min)	100 µL HCl 6 M (pH 1)	100 µL HCl 6 M (pH 1)
Internal standard	ILIS addition	ILIS addition	ILIS addition	ILIS addition
Neutralization	KOH 6 M (pH 6–7)	KOH 6 M (pH 6–7)	KOH 6 M (pH 6–7)	KOH 6 M (pH 6–7)
Derivatization	5 mL borate buffer 40 mM 5 mL FMOC-Cl 6.5 mM Shake gently and allow to stand for 2 h.	0.6 mL borate buffer 5% 0.6 mL FMOC-Cl 12 g/L Shake and allow to react overnight at ambient temperature (22 °C).	0.5 mL borate buffer 40 mM 0.5 mL FMOC-Cl 6 g/L 0.5 mL ACN Shake and allow to react overnight at ambient temperature (22 °C).	0.5 mL borate buffer 40 mM 0.5 mL FMOC-Cl 6 g/L 0.5 mL ACN Shake and allow to react for 2 h at ambient temperature (22 °C).
Acidification	FA (pH 3). Dilution: 50 mL water +2 mL EDTA 1 M.	Filtration 0.45 µm. HCl (pH 1.5).	Dilution: 50 mL water with 1% FA.	
Cleanup	**SPE**: Conditioning: 5 mL MeOH and 5 mL 0.1% FA. Extract derivatized sample at a flow of 2.5 mL/min. Dry the excess water under N_2 or air flow (30 min). Wash cartridge with 3.5 mL DCM and dry with N_2 or air flow (30 min). Elute analytes with 9 mL MeOH without vacuum aid. Evaporate MeOH to dryness and reconstitute with 500 µL of mobile phase. Pass through 0.2 µm filter and inject into LC-MS/MS system.	**Option 1 SPE**: Conditioning: 5 mL MeOH and 5 mL 0.1% FA. Extract derivatized sample at a flow of 2.5 mL/min. Dry the excess water under N_2 or air flow (30 min). Wash cartridge with 3.5 mL DCM and dry with N_2 or air flow (30 min). Elute analytes with 9 mL MeOH without vacuum aid. Evaporate MeOH to dryness and reconstitute with 500 µL of mobile phase. Pass through 0.2 µm filter and inject into LC-MS/MS system. **Option 2 Partition**: To 4 mL extract add 4.5 mL DCM and continue with partition method D.	**SPE**: Conditioning: 5 mL MeOH and 5 mL 0.1% FA. Extract derivatized sample at a flow of 2.5 mL/min. Dry the excess water under air flow (30 min). Wash cartridge with 10 mL water and 10 mL DCM and dry with air flow (30 min). Elute analytes with 5 mL MeOH without vacuum aid. Evaporate MeOH to dryness and reconstitute with 500 µL of mobile phase. Pass through 0.2 µm filter and inject into LC-MS/MS system.	**Partition**: Add 4.5 mL DCM, shake and extract an aliquot of aqueous phase. Pass through 0.2 µm filter and inject into LC-MS/MS system.
Recoveries[a] (%RSD)[b]	Level 0.25 and 2.5 µg/L: <60% (15–40) for three analytes.	**SPE**: Level 10 and 100 µg/L: <50% (10–50) for three analytes. **Partition**: Level 10 and 100 µg/L: 60–130% (10−20) for three analytes.	Level 100 µg/L: <45% (30–40) for three analytes.	Level 100 µg/L: 105 (12), 96 (14), 100 (8) for gly, AMPA and glu respectively.

FA: formic acid, ACN: acetonitrile, DCM: dichloromethane.
[a] n = 3.
[b] Relative standard deviations (%).

3. Results and discussion

3.1. Pre-conditioning of samples

Sample conditioning before the derivatization step is a critical point in the analysis of natural waters, which normally have a rich content of multivalent cations which interact with the amphoteric ionic behaviour of glyphosate and similar compounds to form stable complexes that will impede further reaction with the derivatizing agent. This is particularly important in the case of groundwater as has been clearly noted in the literature and is an important source of errors in former determinations using the FMOC derivatization (Freuze et al., 2007; Ibáñez et al., 2006). Strong acidification is the simplest and most effective way to avoid the presence of these interfering complexes being reported different waiting times to release glyphosate. Since this variable is also to be considered, we evaluated three different time periods at pH = 1 (Ibáñez

Fig. 1. Geo-referenced sampling sites corresponding to 40 selected dairy farms located in the Central Region of Santa Fe Province (Departments of Castellanos and Las Colonias), Argentina.

et al., 2006) prior to the procedures: 1 h, 1 min and no waiting time (Table 2). The addition of 100 uL of 6 M HCl with no waiting time (Method D) was sufficient to reach pH = 1 producing subsequent good results and reducing analysis time, so this procedure was adopted. Before continuing with the derivatization steps, the Isotope Labelled Internal Standard (ILIS) is added at this stage. The use of isotope-labelled glyphosate and AMPA (Glyphosate 1,2-13C2 15N; AMPA 13C 15N) is useful in following the behaviour of the derivatization reaction, minimizing the variations and the matrix effects. Recoveries calculated with the labelled compounds were used as quality control measurements in all runs.

After the acidic preconditioning it is very important to neutralize the medium in order to allow the subsequent buffering reagent to achieve and maintain an adequately alkaline medium for the derivatization reaction to occur. In our experience this neutralization step was demonstrated to be critical. Fixed volume of alkaline solution equivalent to the volume and concentration of the added HCl as described in most of the consulted methodologies were not sufficient to assure a good neutralization in all cases. For example, the addition of fixed volumes as proposed by Ibáñez et al. (2006) (200 μL of 6 M HCl + 200 μL of 6 M KOH) and Zelaya et al. (2010) (80 μl of each the same reagents) to neutralize the high HCl concentrations (pH = 1) led to obtain final solutions with variable pH since it strongly depends on sample composition. Moreover, incomplete derivatization reaction leading to low recovery values was observed and therefore a controlled adjustment of pH by addition of the 6 M KOH solution to reach pH = 6–7 was required.

3.2. Derivatization

An optimally alkaline medium of pH = 9 was early indicated for the derivatization reaction (Moye and Boning, 1979) and generally adopted in further literature. However, neither the type and conditions for the buffering step nor the proper reaction have been uniformly adopted. The concentration of sodium tetraborate has long been known to be critical because an excess may interfere with the solvents of the chromatographic system and at lower concentrations the buffering capacity may be insufficient to complete the derivatization. For that reason the previous neutralization step was necessarily adopted as mentioned above. In addition, in our study three different forms of using the borate buffer were evaluated (Table 2). The final concentrations of the buffering reagent tested with reference to the starting volume of the sample were 1.9 and 3 g/L for methods A and B, and 2.5 g/L for methods C and D respectively. This concentration range is in agreement with the referenced methods and others reported in literature. In our case the intermediate value assayed in selected method D was enough to achieve the good results obtained.

The required amount of derivatizing reagent is also critical to ensure a complete and reproducible reaction. This amount was experimentally determined in a range of concentrations between 0.3 and 1 g/L of FMOC-Cl, referred to the initial volume of sample considered in each tested procedure (Table 2). The stoichiometric molar ratio of 1:1 glyphosate: FMOC-Cl shows a low yield of derivative compounds. An excess of reagent must be used for the complete derivatization of the analytes present in a sample. For that reason a wide molar ratio analyte:reagent is used ranging 1:10–10^5. Thus although the excess of FMOC-Cl is common to all the techniques consulted its optimization is important since the unreacted reagent becomes an undesirable impurity and the formation of by-products occurs during the reaction. FMOC-Cl is highly reactive in water (the reaction of an acyl chloride with water) forming the product FMOC-OH. This by-product has the disadvantage that it is less soluble in water than the derivatives of the analytes and can precipitate, thus disturbing the performance of the chromatographic column. Such precipitation can also reduce the efficiency of ionization and consequently affect the robustness and sensitivity of the method in general (Hanke et al., 2008). To solve this problem a cleaning step is included in the procedure as it is described in Section 3.3. In our case three

molar ratios of glyphosate:FMOC-Cl were used, 1:0.8 · 10^5 (method A), 1:4.7 · 10^5 (method B) and 1:6.5 · 10^5 (method C and D) considering a concentration level of 10 μg/L of glyphosate in the water sample. The highest ratio corresponded to the selected method D which performed adequately no introducing further problems with the excess of reagent and the by-products impurities (Section 3.3).

Glyphosate and related compounds are soluble in water and only scarcely soluble in organic solvents. In contrast, the derivatizing reagent FMOC-Cl is almost insoluble in water, and for that reason, the addition of acetone or acetonitrile in different concentrations facilitate the reaction. Zelaya et al. (2010) experimented with a range of 10–65% of ACN to establish a suitable ratio ACN/water, observing no changes in the derivatization yields across this entire range. We considered appropriate the extra addition of ACN and considering the wide range suitable for that purpose we choose a relatively low proportion to favour the reaction but avoiding an undesirable solvent excess. Therefore for the method D finally selected 0.5 mL ACN (~20%) was added with reproducible results.

In addition to the medium conditions and the amount of reagent, the reaction time is another important variable because it has to allow a complete reaction and also take the least possible time to provide an acceptable duration of the analysis. However, a wide range of reaction times were reported being an overnight reaction period the most preferred option to assure complete reaction and also to adapt to a scheme of a two-day running period (Cullum and Schuhn, 2013; Ibáñez et al., 2006; Poiger et al., 2016; Vreeken et al., 1998; Zelaya et al., 2010). However other authors proposed a shorter reaction time of 2 h working at room temperature (Hanke et al., 2008) and at dark conditions (Daouk et al., 2013) and even as shorter as 30 min in dark conditions is indicated by ISO 16308:2014. To promote the completeness of the reaction heating during 30 min at 60 °C (Mallet, 2014) and 100 °C (Cullum and Schuhn, 2013) was also proposed. We experimented with reaction times of 2 h, 5 h and overnight, showing all of them to be adequate to achieve a complete and reproducible reaction. Satisfactory recoveries were obtained for the three analytes at two concentration levels (10 and 100 μg/L) when running Method D with the aforementioned reaction times (Table 3).Protection from light exposure was disposed for the storage of solutions during the reaction period. Considering the experimental results and the mentioned existing background from literature we selected 2 h of reaction time, as a reasonable time to minimize the total duration of the complete measurement, avoiding laborious heating and prolonged reaction periods.

3.3. Cleaning of reaction by-products

The elimination of the excess of reagent and the reaction by-products to obtain cleaner extracts, to be compatible with the chromatographic system, prompted the use of several alternatives of cleanup. Very extended is the use of a liquid-liquid pre-treatment with adequate solvents before submitting the extracts to a solid phase extraction (SPE) step. The online concentration and cleanup with C18 sorbents has been satisfactorily used in several methods (Hanke et al., 2008; Ibáñez et al., 2005, 2006; Poiger et al., 2016), with automated instrumental configurations (Cullum and Schuhn, 2013; Mallet, 2014) and more scarcely developed in offline approaches (Daouk et al., 2013; ISO 16308:2014). More differences have been observed between these methods regarding the L-L pretreatment with solvents when it is used, mainly with ethyl acetate and dichloromethane. Most of these procedures represented additional laborious and time-consuming steps. For this reason, with the aim of developing simpler, reliable and cost effective approaches modified cleaning alternatives were assayed based on classical off line SPE using C18 cartridges and L-L partition approaches as detailed in Table 2.

Briefly, for analyte concentration and cleanup using off line OASIS® HLB cartridges a 3.5 mL washing volume of dichloromethane was used after conditioning and loading the derivatized extract to remove

Table 3
Evaluation of the effect of derivatization reaction time on accuracy applying method D (n = 3)[a].

Compound	2 h		5 h		Overnight	
	10 µg/L	100 µg/L	10 µg/L	100 µg/L	10 µg/L	100 µg/L
	% Recoveries (%RSD)[b]	% Recoveries (%RSD)	% Recoveries (%RSD)	% Recoveries (%RSD)	% Recoveries (%RSD)	% Recoveries (%RSD)
Glyphosate	119 (1)	98 (9)	117 (4)	99 (2)	119 (8)	99 (6)
AMPA	118 (5)	100 (6)	112 (3)	105 (2)	110 (4)	95 (5)
Glufosinate	95 (3)	79 (8)	94 (8)	81 (4)	94 (2)	75 (2)

[a] Three water samples, spiked at 10 µg/L each.
[b] Relative standard deviations (%).

FMOC-Cl by-products impurities before the final elution with MeOH. This SPE procedure is necessary and effective for the analyte concentration to comply with the most demanding water regulations (e.g. 0.1 µg/L EU). However in our case this relatively labour intensive step did not give completely satisfactory results in terms of recoveries (Methods A and B option 1) (Table 2). An alternative procedure using 10 mL of water and 10 mL of dichloromethane did not improve results either (Method C). Therefore this preliminary results move us to explore the system behaviour without the SPE stage and the introduction of a single washing step with dichloromethane after the derivatization (Method B option 2 and Method D) with improved results in both cases (Table 2). As a consequence we continue the experimental work focused on the optimization and further validation of the procedure defined as Method D. This procedure consists of introducing a fast cleaning step based on an L-L partition with dichloromethane in a simplified way, since it uses only a small volume of solvent (4.5 mL), a very short time (10 s of vigorous shaking), and is able to achieve a complete elimination of reaction residues and other impurities. In our case, dichloromethane partition proved to be very effective providing very clean extracts with all the water sample types under study. The chromatographic system required little maintenance, being compatible with the use of the two types of C18 chromatographic columns tested (Section 2.2). In the case of the Acquity UPLC® HSS C18 chromatographic column, which was used continuously and exclusively for the determination of glyphosate for routine analysis in a variety of matrices using this method, no changes in the performance for >9150 injections were observed.

In the proposed strategy, avoiding the SPE pre-concentration compromises the detection and quantification capability of the method. However the LOQs achieved with values below the µg/L level for the three analytes, are suitable for routine control purposes for many countries that have water quality standards fixed above those concentration levels.

3.4. Chromatographic and mass spectrometric parameters

The chromatographic conditions of the two UHPLC columns and two mobile phases (Section 2.2) were assayed. With respect to the mobile phase selection, initially various combinations of water-methanol (Hanke et al., 2008; Poiger et al., 2016; Zelaya et al., 2010) and water-acetonitrile (Cullum and Schuhn, 2013; Daouk et al., 2013; Ibáñez et al., 2006; Mallet, 2014; Vreeken et al., 1998) with a conventional C18-BEH column (column 2) were tested. Secondly, the C18-HSS (column 1) performance was evaluated. Chromatograms in Figs. 2 and 3 show the responses of the injection of a mixture of glyphosate-FMOC, AMPA-FMOC and glufosinate-FMOC (100 µg/L) in solvent utilizing the two mobile phases and columns. The results indicated that good selectivity, relatively short retention time (<5 min), greater sensitivity, better peak shape and better peak resolution for glyphosate-FMOC, AMPA-FMOC and glufosinate-FMOC were obtained using water-acetonitrile and C18-HSS column. An increase in the areas of the two ion transitions (Q and q) and in the S/N ratio was observed. Instrumental conditions to achieve a better response to spectrometric parameters were studied based on a statistical Design of Experiment (DOE), Fractional Factorial Design. Parameters included in the model were: capillary voltage (CV), extractor voltage (EV), source temperature (ST), desolvation temperature (DT), desolvation gas flow (DF) and cone gas flow (CF). The number of experiments was given by 2^{k-p} (k: number of factors; p: times of fractioning or reducing the design). A design model 2^{6-2} was adopted with 3 repetitions of the central point. The final number of runs was 19. The final adjusted parameters adopted from the DOE results were: capillary voltage: 1 kV; extractor voltage: 1 kV; source temperature: 140 °C; desolvation temperature: 500 °C; desolvation gas flow: 600 L/h; cone gas flow: 20 L/h.

The sensibility achieved and the better performance of column 1 and mobile phase 1, led to the adoption of the conditions above mentioned for the further development and validation stages. Fig. 4 shows the chromatograms corresponding to a positive groundwater sample compared with water spiked with the three analytes at 1 µg/L level.

Figs. 2, 3, 4.

3.5. Validation parameters of the selected procedure

The set of studies and assays of the most relevant variables carried out during the development phase led to the acquisition of significant
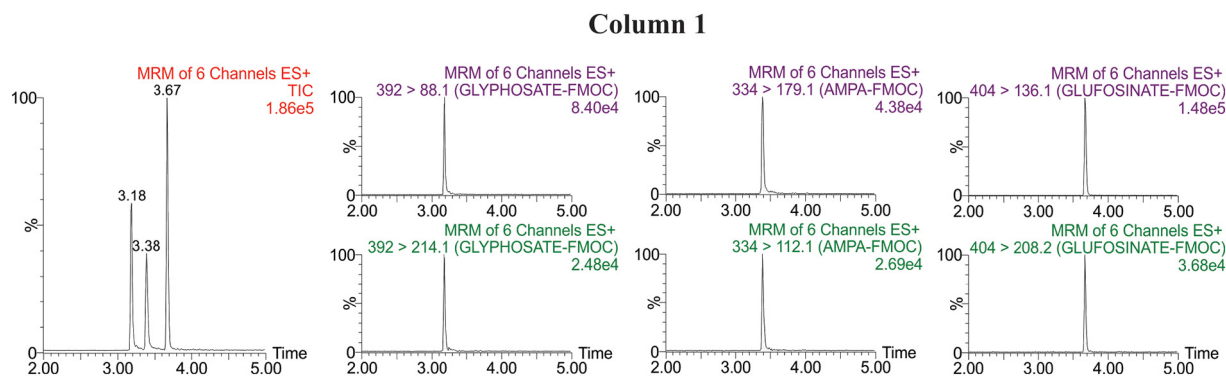
Column 1

Fig. 2. TIC and MRM chromatograms of glyphosate-FMOC, AMPA-FMOC and glufosinate-FMOC from a solvent mixed-standard solution (100 µg/L) using column 1 ACQUITY UPLC HSS® and the mobile phase 1: solvent A: H$_2$O:ACN 98:2 + 0.1% FA, solvent B: ACN + 0.1% FA. Base width: 15.1, 14.5 and 13.2, S/N ratios: 536, 537 and 2104 for glyphosate-FMOC, AMPA-FMOC and glufosinate-FMOC respectively.
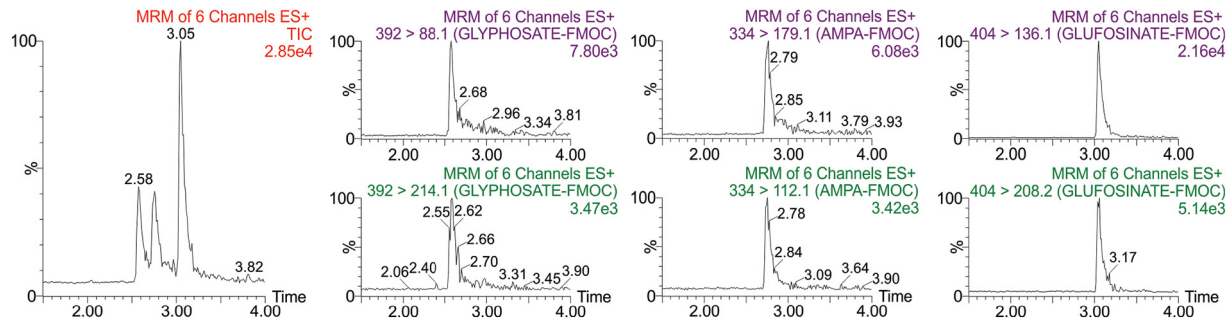
Column 2

Fig. 3. TIC and MRM chromatograms of glyphosate-FMOC, AMPA-FMOC and glufosinate-FMOC from a solvent mixed-standard solution (100 μg/L) using column 2 ACQUITY UPLC BEH® and the mobile phase 1: solvent A: H_2O:ACN 98:2 + 0.1% FA, solvent B: ACN + 0.1% FA. Base width: 26.5, 25.8 and 25.8, S/N ratios: 96, 86 and 437 for glyphosate-FMOC, AMPA-FMOC and glufosinate-FMOC respectively.

information and analytical data. These were further employed to orient and facilitate the final validation procedure, based on SANTE/11945/2015 guidance document (EC, 2015) to the adopted method D (Table 2). The main results of the figures of merit of this methodology are summarized as follows:

3.5.1. Linearity range

The linearity of the method was evaluated analysing five standard solutions of the three analytes in triplicate in the range of 0.1–100

μg/L. Linearity from regression analysis was considered satisfactory achieving a correlation coefficient higher than 0.99 and residuals lower than ±20% in all cases.

3.5.2. Matrix effect

Matrix interferences were studied by running the calibration experiment using the addition of standards to solvent (mobile phase) and different types of matrices such as surface water, tap water, groundwater and a reagent blank matrix, and further evaluation of
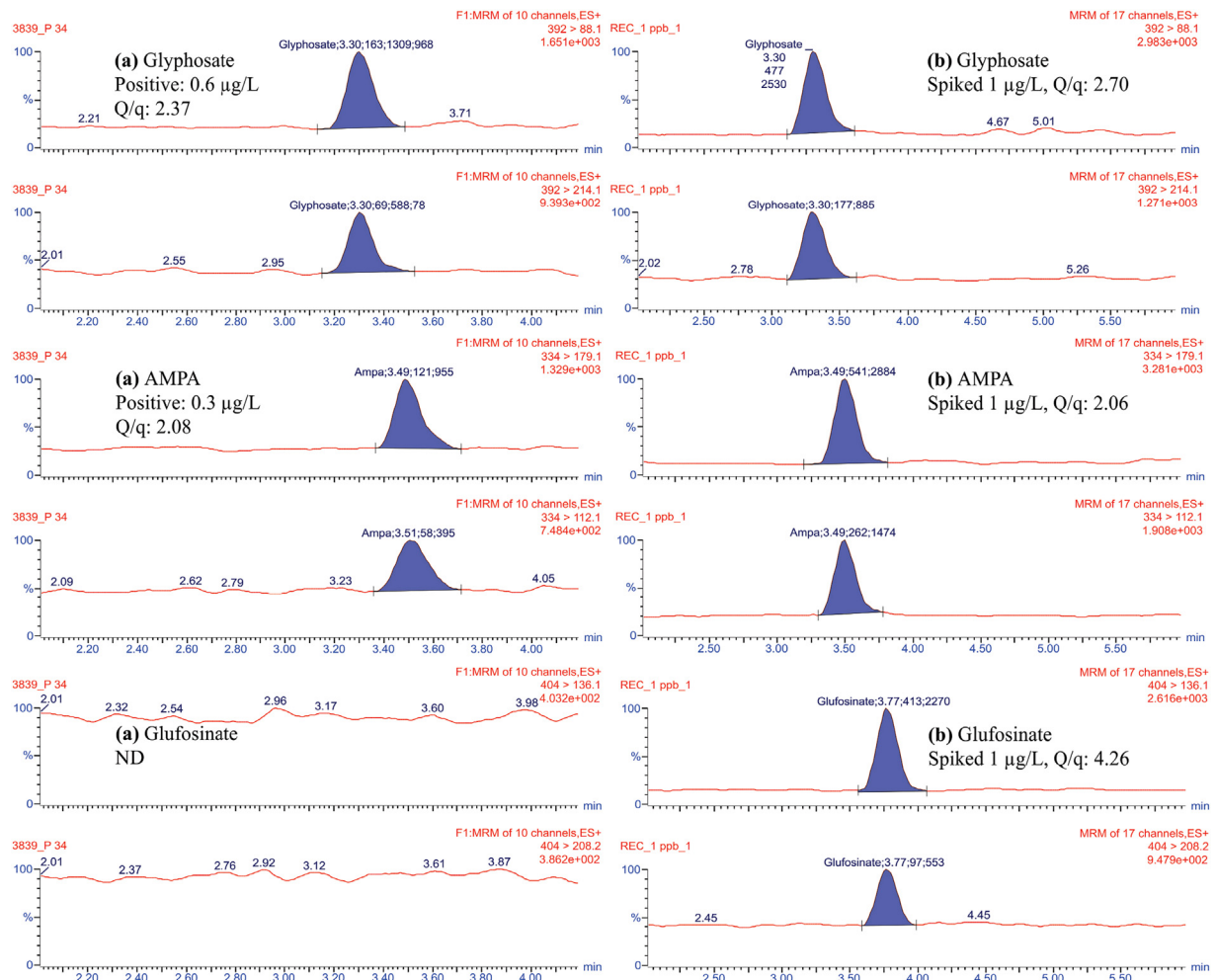

Fig. 4. Chromatograms from the analysis of groundwater samples using the validated method D with column 1 and mobile phase 1. a) Positive samples near the LOQ (glyphosate 0.6 μg/L, AMPA 0.2 μg/L and glufosinate 0.1 μg/L) and b) spiked water at a level of 1 μg/L for the three analytes.

Table 4
Method D analytical figures of merit (n = 5)[a].

Compound	Recoveries %		Repeatability % RSD	Intermediate precision % RSD	Repeatability % RSD	Intermediate precision % RSD	LOD µg/L	LOQ µg/L
	1 µg/L	100 µg/L	1 µg/L	1 µg/L	100 µg/L	100 µg/L		
Glyphosate	70–80	95–105	3	7	4	7	0.2	0.6
AMPA	80–90	90–95	4	11	2	5	0.1	0.2
Glufosinate	70–80	93–97	3	9	2	4	0.01	0.1

[a] Five water samples, spiked at 1 and 100 µg/L each.

slope ratio of the corresponding regression lines using the following formula (Kwon et al., 2012):

$$\%Matrix\ Effect = \frac{(slope\ MM - slope\ MP)}{slope\ MP} \cdot 100$$

where MM is the matrix-matched and MP is the mobile phase.

A moderate negative matrix interference between −20 to −40% for glyphosate, AMPA and glufosinate was verified with different sources of water. The major effect was observed for groundwater, probably because the water salinity and organic matter content are important variability sources, and for this reason it should be carefully taken into account. In contrast, a positive interference was observed in the responses from the three analytes when reagent blanks were used for calibration. This behaviour is less frequent in electrospray atomization detection. This might be explained by the relative lack of interferents competing in the ionization process and the presence of inorganic salts that could favour the atomization of the three analytes. For that reason all the quantification procedures were performed using as a reference the calibration curves constructed in matrices and taking into consideration the response of the corresponding matrix of the source of water under study (matrix-matched calibration).

3.5.3. Recovery study

Recoveries were determined both intraday and inter-day by analysing fortified blank samples at three levels of concentration (1, 10 and 100 µg/L) in quintuplicate with the results ranging between 70 and 105% and an RSD <15% in all cases (Table 4). Additional recovery experiment was performed at respective LOQ levels for the three analytes as it is described below.

3.5.4. Repeatability and inter-day intermediate precision

Repeatability within a day and the inter-day intermediate precision at two levels (1 and 100 µg/L) were determined in quintuplicate, obtaining RSD values <15% in all cases (Table 4).

3.5.5. Limits of detection and quantification

The LOD (0.2, 0.1 and 0.01 µg/L for glyphosate, AMPA and glufosinate respectively) and LOQ (0.6, 0.2 and 0.1 µg/L for glyphosate, AMPA and glufosinate respectively) were determined using S/N ratios of 3 and 10, respectively, from 1 µg/L spiked samples chromatograms. These values were experimentally verified by analysing matrix extracts at LOQs concentration levels to corroborate that diagnostic ions meet the conditions of relative abundances and the method criteria for trueness and precision. Five replicates of spiked matrix samples for each analyte at respective LOQs levels were prepared and analyzed. Recoveries of 78%, 97% and 89% with RSD 13%, 13% and 11% for glyphosate, AMPA and glufosinate were obtained respectively.

3.6. Analysis of water samples

The analysis of groundwater showed positive results for glyphosate and AMPA in 65.6% and 96% of the total of 125 samples, respectively. However, a minor incidence of quantified values (over the respective LOQs) of 15.2% and 52.8% of glyphosate and AMPA respectively was verified (Fig. 5). The quantified values found for glyphosate ranged from 0.6–11.3 µg/L, and for AMPA they ranged from 0.2–6.5 µg/L. No presence of glufosinate (all data <LOD) was observed in the analyzed groundwater samples.

A second group of 33 water samples was taken from the open-reservoir tanks where the animals drink. These waters are in contact with the environment of the farm, exposed to rain precipitation, dust and air from the surrounding agricultural fields. In this case, the results
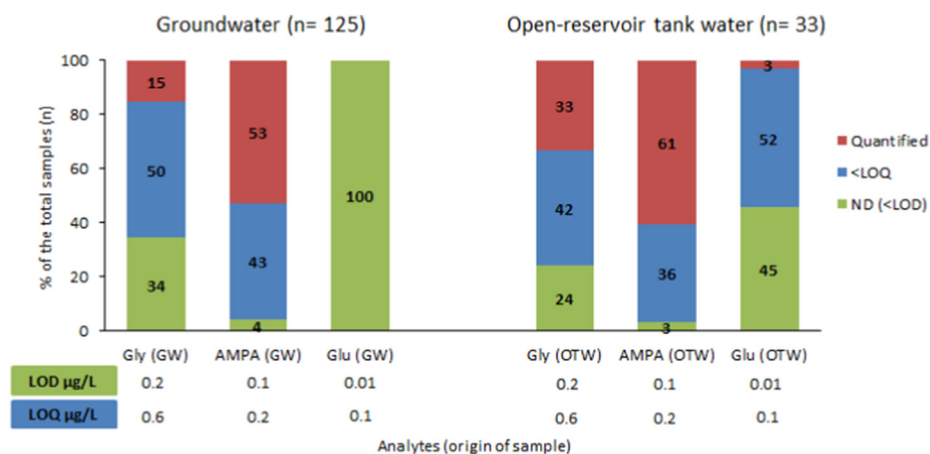

Fig. 5. Results corresponding to the determination of glyphosate, AMPA and glufosinate in samples of groundwater (GW) and open-reservoir tanks water (OTW) from 40 dairy farms in Santa Fe, Argentina, expressed as a percentage of the total samples. The results were grouped in: not detected (ND = lower than LOD) in green, less than the limit of quantification (<LOQ) in blue and quantified values in red. For the calculation of the mean, only the quantified values were considered (see Table 5). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 5
Mean of the quantified values and concentration range (LOQ to Max quantified values) of glyphosate, AMPA and glufosinate found in groundwater and drinking-tank water samples from 40 dairy farms near Santa Fe, Argentina (see Fig. 5).

	Groundwater			Drinking-tank water		
	Glyphosate	AMPA	Glufosinate	Glyphosate	AMPA	Glufosinate
n (total)	125	125	125	33	33	33
n (quantified)	19	66	–	11	20	1
Mean [μg/L]	2.1	0.5	–	5.6	0.8	0.1
Range [μg/L]	0.6–11.3	0.2–6.5	–	0.6–21.2	0.2–4.2	0.1–0.1

showed an increased presence of the studied herbicides. The positive samples rose to 75.8% (33.8% quantified) and 97% (60.6% quantified) for glyphosate and AMPA, respectively. Concentrations of glyphosate ranged from 0.6 to 21.2 μg/L, and for AMPA they ranged from 0.2 to 4.2 μg/L. Contrary to what was found in the groundwater, a trace presence of glufosinate was verified in these waters. Positive detections rose to 54.5% with the quantified values (3%) at levels of 0.1 μg/L (Table 5 and Fig. 5).

Increased occurrence of glyphosate and AMPA traces was verified with samples analyzed in spring and summer seasons (See supplementary material). These findings correspond to the exposure through the agricultural practices in the region. For example the soy planting begin in the month of October and extends until January. It is carried out under direct sowing, without prior soil tillage. Prior to this, a chemical fallow should be done to start the campaign with a clean lot. This is achieved through a chemical control of the weeds present in batch (broadleaf and annual grasses). Before the emergence of soybeans, an application can be made with pre-emergent herbicides including glyphosate.

Regarding the values found in our study, low levels were observed, which were in compliance with the applicable tolerances of the country and other foreign regulations as mentioned. However, the most important reflections to be made from our findings is the evaluation of the status of the influence of agricultural technical practice using these herbicides in an intense way and the resulting effect on the integrity of the water resources involved. Therefore, it is observed that there is evidence of the transfer of compounds from the external environment to functional aquifers with the potential risk of deepening contamination in the future. The presence of glyphosate and to a major degree its metabolite AMPA is detected with a high frequency of occurrence, although at trace levels. The good method performance achieved for glufosinate (LOQ 0.1 μg/L) allowed us to detected the presence of this less used herbicide in water sources.

4. Conclusion

After assessing different preliminary strategies, a method involving FMOC-Cl derivatization and LC-MS/MS determination of glyphosate, AMPA and glufosinate in water was developed. The proposed approach shows several differences and advantages with respect to other available methods mainly focused in its greater simplicity and utility for routine and research analysis at sub μg/L level (0.6, 0.2, 0.1 μg/L for the three analytes respectively), compatible with Argentinean regulations and other water quality standards. The method stands for its lower volume scale (3 mL water), rapid offline cleaning step consisting in a 10 s shaking with 4.5 mL of DCM after derivatization reaction (avoiding SPE cleanup), and direct injection into the LC-MSMS system. The method was applied to a case study of analysing 158 water samples from 40 dairy farms that contributed to obtain information about herbicide concentrations in water from an important agricultural region of the country. Results showed a high glyphosate occurrence frequency and to a major degree its metabolite AMPA at trace levels in groundwaters (0.6–11.3 μg/L and 0.2–6.5 μg/L respectively). With increased occurrence and found levels in open-reservoir tank waters (0.6–21.2 μg/L and 0.2–4.2 μg/L). Glufosinate is a less frequently used herbicide and

was only found in open-reservoir tank samples at <LOQ level (52% samples) and was quantified in one sample at 0.1 μg/L. This is the first study that reports levels of these herbicides in water from Argentinean rural areas, a useful information for environmental risk assessment and management purposes.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.06.340.

References

Adams, S., Guest, J., Dickinson, M., Fussell, R.J., Beck, J., Schoutsen, F., 2017. Development and validation of ion chromatography tandem mass spectrometry based method for the multi-residue determination of polar ionic pesticides in food. J. Agric. Food Chem. 65 (34):7294–7304. https://doi.org/10.1021/acs.jafc.7b00476.

Agro-industry Ministry of Argentina, 2016. Agroindustrial data, 2015–2016 agricultural estimates. https://datos.agroindustria.gob.ar/dataset/estimaciones-agricolas/archivo/95d066e6-8a0f-4a80-b59d-6f28f88eacd5, Accessed date: 20 December 2017.

Arkan, T., Molnár-Perl, I., 2015. The role of derivatization techniques in the analysis of glyphosate and aminomethyl-phosphonic acid by chromatography. Microchem. J. 121:99–106. https://doi.org/10.1016/j.microc.2015.02.007.

Bauer, K.-H., Knepper, T.P., Maes, A., Schatz, V., Voihsel, M., 1999. Analysis of polar organic micropollutants in water with ion chromatograpy-electrospray mass spectrometry. J. Chromatogr. A 837:117–128. https://doi.org/10.1016/S0021-9673(99)00048-5.

Charlón, V., Taverna, M.A., Herrero, M.A., 2005. The water in the dairy farm. In: Taverna, M.A. (Ed.), Manual of Technical References for the Achievement of Quality Milk, 2ª ed. INTA :pp. 43–54. http://www.aprocal.com.ar/wp-content/uploads/El_agua_en_el_tambo.pdf, Accessed date: 22 December 2017.

Cullum, N., Schuhn, B., 2013. Analysis of Glyphosate and AMPA in Drinking Water With the Agilent 1200 Infinity Series Online SPE Solution. Agilent Technologies, Application Note, Publication Number 5991-3208EN.

Daouk, S., Grandjean, D., Chevre, N., De Alencastro, L.F., Pfeifer, H.-R., 2013. The herbicide glyphosate and its metabolite AMPA in the Lavaux vineyard area, western Switzerland: proof of widespread export to surface waters. Part I: method validation in different water matrices. J. Environ. Sci. Health B 48:717–724. https://doi.org/10.1080/03601234.2013.780535.

Ding, J., Guo, H., Liu, W., Zhang, W., Wang, J., 2015. Current progress on the detection of glyphosate in environmental samples. J. Sci. Appl. Biomed. 3 (6):88–95. http://inter-use.com/uploads/soft/151001/1-151001092058.pdf, Accessed date: 20 December 2017.

European Commission (EC), 2015. Directorate-general for Health and Food Safety, Safety of the Food Chain Pesticides and Biocides, SANTE/11945/2015, guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. Supersedes SANCO/12571/2013. Implemented by 01/01/2016. https://www.bvl.bund.de/SharedDocs/Downloads/09_Untersuchungen/00_Rechtsgrundlagen/01_EU/Qualitätssicherung_Pestizidanalytik_SANTE_11945_2015.pdf?__blob=publicationFile&v=4, Accessed date: 14 December 2017.

European Food Safety Authority (EFSA), 2016. The 2014 European Union report on pesticide residues in food. EFSA J. 14 (10):4611. https://doi.org/10.2903/j.efsa.2016.4611.

Freuze, I., Jadas-Hecart, A., Royer, A., Communal, P.-Y., 2007. Influence of complexation phenomena with multivalent cations on the analysis of glyphosate and aminomethyl phosphonic acid in water. J. Chromatogr. A 1175:197–206. https://doi.org/10.1016/j.chroma.2007.10.092.

Government Accountability Office (GAO), 2014. Report to the ranking member, subcommittee on environment and the economy, committee on energy and commerce, house of representatives. https://www.gao.gov/assets/670/666408.pdf, Accessed date: 21 April 2018 (GAO-15-38, October).

Guo, H., Riter, L.S., Wujcik, C.E., Armstrong, D.W., 2016. Direct and sensitive determination of glyphosate and aminomethylphosphonic acid in environmental water samples by high performance liquid chromatography coupled to electrospray tandem mass spectrometry. J. Chromatogr. A 1443:93–100. https://doi.org/10.1016/j.chroma.2016.03.020.

Hanke, I., Singer, H., Hollender, J., 2008. Ultratrace-level determination of glyphosate, aminomethylphosphonic acid and glufosinate in natural waters by solid-phase extraction followed by liquid chromatography–tandem mass spectrometry: performance tuning of derivatization, enrichment and detection. Anal. Bioanal. Chem. 39: 2265–2276. https://doi.org/10.1007/s00216-008-2134-5.

Hao, C., Morse, D., Morra, F., Zhao, X., Yang, P., Nunn, B., 2011. Direct aqueous determination of glyphosate and related compounds by liquid chromatography/tandem mass spectrometry using reversed-phase and weak anion-exchange mixed-mode column. J. Chromatogr. A 1218:5638–5643. https://doi.org/10.1016/j.chroma.2011.06.070.

Huhn, C., 2018. More and enhanced glyphosate analysis is needed. Anal. Bioanal. Chem. 410 (13):304–3045. https://doi.org/10.1007/s00216-018-1000-3.

Ibáñez, M., Pozo, O.J., Sancho, J.V., López, F.J., Hernández, F., 2005. Residue determination of glyphosate, glufosinate and aminomethylphosphonic acid in water and soil samples by liquid chromatography coupled to electrospray tandem mass spectrometry. J. Chromatogr. A 1081:145–155. https://doi.org/10.1016/j.chroma.2005.05.041.

Ibáñez, M., Pozo, O.J., Sancho, J.V., López, F.J., Hernández, F., 2006. Re-evaluation of glyphosate determination in water by liquid chromatography coupled to electrospray tandem mass spectrometry. J. Chromatogr. A 1134:51–55. https://doi.org/10.1016/j.chroma.2006.07.093.

International Service for the Acquisition of Agri-biotech Applications (ISAAA), 2016. Global status of commercialized biotech/GM crops: 2016. Brief 52. https://www.isaaa.org/resources/publications/briefs/52/download/isaaa-brief-52-2016.pdf, Accessed date: 18 December 2017.

International Standarization Organization (ISO 16308), 2014. Water Quality–Determination of Glyphosate and AMPA–Method Using High Performance Liquid Chromatography (HPLC) With Tándem Mass Spectrometric Detection (September).

Kwon, H., Lehotay, S.J., Geis-Asteggiante, L., 2012. Variability of matrix effects in liquid and gas chromatography–mass spectrometry analysis of pesticide residues after QuEChERS sample preparation of different food crops. J. Chromatogr. A 1270: 235–245. https://doi.org/10.1016/j.chroma.2012.10.059.

Mallet, C., 2014. Analysis of glyphosate, glufosinate and AMPA in tap and surface water using open-architecture UPLC with 2D-LC technology. http://www.waters.com/webassets/cms/library/docs/720005169en.pdf, Accessed date: 15 April 2018.

Marek, L.J., Koskinen, W.C., 2014. Simplified analysis of glyphosate and aminomethylphosphonic acid in water, vegetation and soil by liquid chromatography-

tandem mass spectrometry. Pest Manag. Sci. 70 (7):1158–1164. https://doi.org/10.1002/ps.3684.

Moye, H.A., Boning, A.J., 1979. A versatile fluorogenic labelling reagent for primary and secondary amines: 9-fluorenylmethyl chloroformate. Anal. Lett. 12 (B1):25–35. https://doi.org/10.1080/00032717908082516.

National Institute of Agricultural Technology (INTA), 2011. Sampling protocol, transport and conservation of water samples for multiple purposes (human consumption, animal watering and irrigation). https://inta.gob.ar/sites/default/files/script-tmp-protocolo_de_muestreo_de_aguas_inta.pdf, Accessed date: 20 April 2018.

Poiger, T., Buerge, I.J., Bächli, A., Müller, M.D., Balmer, M.E., 2016. Occurrence of the herbicide glyphosate and its metabolite AMPA in surface waters in Switzerland determined with on-line solid phase extraction LC-MS/MS. Environ. Sci. Pollut. Res. 24 (2):1588–1596. https://doi.org/10.1007/s11356-016-7835-2.

Raina-Fulton, R., 2014. A review of methods for the analysis of orphan and difficult pesticides: glyphosate, glufosinate, quaternary ammonium and phenoxy acid herbicides, and dithiocarbamate and phthalimide fungicides. J. AOAC Int. 97 (4):965–977. https://doi.org/10.5740/jaoacint.SGERaina-Fulton.

Skeff, W., Recknagel, C., Schulz-Bull, D.E., 2016. The influence of salt matrices on the reversed-phase liquid chromatography behavior and electrospray ionization tandem mass spectrometry detection of glyphosate, glufosinate, aminomethylphosphonic acid and 2-aminoethylphosphonic acid in water. J. Chromatogr. A 1475:64–73. https://doi.org/10.1016/j.chroma.2016.11.007.

Toss, V., Leito, I., Yurchenko, S., Freiberg, R., Kruve, A., 2017. Determination of glyphosate in surface water with high organic matter content. Environ. Sci. Pollut. Res. 24 (9): 7880–7888. https://doi.org/10.1007/s11356-017-8522-7.

Tujchneider, O., Paris, M., D'Elía, M., Perez, M., 2002. Model of management of underground water resources in the center-west of the Province of Santa Fe (Argentina). Revista Latino-Americana de Hidrogeología (Latin-American Magazine of Hydrogeology). 2 :pp. 91–102. http://revistas.ufpr.br/hidrogeologia/article/viewFile/2626/2168, Accessed date: 20 December 2017.

Under Secretariat of Water Resources (Subsecretaría de Recursos Hídricos de la Nación), 2003. Development of national guide levels of environmental water corresponding to glyphosate. http://provincia25.gob.ar/obras-publicas/pdf/DOCUMENTO53.pdf, Accessed date: 21 December 2017.

Vreeken, R.J., Speksnijder, P., Bobeldijk-Pastorova, I., Noij, Th.H.M., 1998. Selective analysis of the herbicides glyphosate and aminomethylphosphonic acid in water by on-line solid-phase extraction-high-performance liquid chromatography-electrospray ionization mass spectrometry. J. Chromatogr. A 794:187–199. https://doi.org/10.1016/S0021-9673(97)01129-1.

Zelaya, M.J., Costa, J.L., Bedmar, F., Aparicio, V., Gianelli, V., Okada, E., Kloosterman, M.E., Gómez Ortiz, A.M., 2010. Analysis of glyphosate and AMPA in Balcarce: liquid chromatography and mass spectrometry. In: Camino, M., Aparicio, V. (Eds.), Environmental Aspects of the Use of Glyphosate :pp. 35–56. https://inta.gob.ar/sites/default/files/script-tmp-aspectos_ambientales_del_uso_de_glifosato__version_pa.pdf, Accessed date: 19 December 2017.