A novel fluorimetric method for glyphosate and AMPA determination with NBD-Cl and MCR-ALS

Ana L. Pérez[a,f], Giuliana Tibaldo[b], Germán H. Sánchez[b,c,f], Gabriel G. Siano[b,e,f], Nilda R. Marsili[b], Agustina V. Schenone[b,d,f] *

[a] *Universidad Nacional del Litoral, Facultad de Bioquímica y Ciencias Biológicas, Departamento de Física, Ciudad Universitaria, S3000ZAA Santa Fe, Argentina*

[b] *Universidad Nacional del Litoral, Facultad de Bioquímica y Ciencias Biológicas, Cátedra de Química Analítica II, Ciudad Universitaria, S3000ZAA Santa Fe, Argentina*

[c] *Universidad Nacional del Litoral, Facultad de Bioquímica y Ciencias Biológicas, Departamento de Química General e Inorgánica, Ciudad Universitaria, S3000ZAA Santa Fe, Argentina*

[d] *Instituto de Desarrollo Tecnológico para la Industria Química (INTEC), Ruta Nacional Nº 168, S3000ZAA, Santa Fe, Argentina*

[e] *Universidad Nacional del Litoral, Facultad de Ingeniería y Ciencias Hídricas, Instituto de investigación en señales, sistemas e inteligencias computacional, Ciudad Universitaria, S3000ZAA Santa Fe, Argentina*

[f] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Godoy Cruz 2290, C1425FQB, Ciudad Autónoma de Buenos Aires, Argentina*

* Corresponding author
E-mail: aschenone@fbcb.unl.edu.ar / aschenone@intec.unl.edu.ar,
Tel: + 54 342 451 1546 Ext.1058

Abstract

We report the development of a new analytical method for the quantification of N-(phosphonomethyl)glycine (glyphosate) and (aminomethyl)phosphonic acid (AMPA) by combining spectrofluorimetry and multivariate calibration. In this study, fluorescence spectroscopy was used to quantify glyphosate and AMPA, which were previously derivatized with the fluorogenic reagent: 4-chloro-7-nitrobenzofurazan (NBD-Cl). Fluorescence excitation-emission matrices (EEM) were recorded by exciting between 400 and 500 nm, and measuring the emission between 500 and 610 nm. The second-order data obtained were processed using the Multivariate Curve Resolution with Alternating Least Square (MCR-ALS) methodology. The developed method was used to predict different concentrations of glyphosate and AMPA in validation samples. In addition, the presence of the herbicide was evaluated in real samples: a commercial formulation and a water sample from a cultivated area. For this purpose, the standard addition method was used to study the matrix effect in each case. The ranges of working concentrations obtained for this new method are in agreement with the amounts found in surface water samples near a direct sowing soybean growing region in Argentina.

***Keywords*: Glyphosate; AMPA; NBD-Cl; Spectrofluorimetry; MCR-ALS**

1. Introduction

Glyphosate is a broad-spectrum, non-selective herbicide that controls annual weeds, perennials, herbs and broad-leaved weeds that compete with crops. This herbicide is a weak organic acid that contains a glycine group and a phosphonomethyl group. Glyphosate acts by inhibiting the 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS), an enzyme responsible of the formation of aromatic amino acids phenylalanine, tyrosine and tryptophan in weeds and pests [1]. (Aminomethyl)phosphonic acid (AMPA) is the main metabolite of glyphosate, with a structure similar to that of its precursor.

Currently, glyphosate is available throughout the world in different forms and under many registered names. Such products may have additional effects apart from those derived from glyphosate itself due to the presence of other ingredients, such as surfactants, which may reach up to 15% of the formulation. Its main function is to increase the permeability of plants. In general, they appear on labels as "inert elements", but their nature is not specified. Several studies have focused on the toxicity of these compounds. For example, it was confirmed that the surfactant polyoxyethylene amine (POEA) can cause gastrointestinal damage and respiratory distress, affects the central nervous system and destroys red blood cells in human blood [2, 3]. It was also shown that the POEA generally contains an impurity identified as 1,4-dioxane, whose carcinogenic effect in animals and damage to the liver and kidney in human were demonstrated [4].

According to a report by the World Health Organization (WHO) in 2015 [5], glyphosate was classified within group 2A as probably carcinogenic to humans. The teratogenic effects of glyphosate were studied by Paganelli et al. [6], in doses lower than those used for fumigation, showing the alteration of the expression of genes involved in the development of the head of amphibians. The changes described are effectively extrapolated to the development of any vertebrate organism, including humans [6].

The Maximum Residue Limits (MRL) are set in each country by the corresponding regulatory agencies, based on the recommendations of the Joint Meeting on Pesticide Residues (JMPR) and good agricultural production practices, which ensures that the pesticide residues in food are kept as low as possible. In 2016 the JMPR reaffirmed an Acceptable Daily Intake (ADI) for the sum of glyphosate and its metabolites of 0 - 1 mg kg^{-1} body weight [5, 7].

According to the amount of these compounds in different samples, there are several strategies for their instrumental detection. For concentrations of the order of mg %, the quantification is done by High Performance Liquid Chromatography (HPLC) with UV detection [8, 9] . On the other hand, to quantify them at low concentrations, residues or trace level, it is necessary to derivatize glyphosate and AMPA to increase the sensitivity of the method. Among the substances most commonly used as derivatizing agents, 9-fluorenylmethyl chloroformate (FMOC-Cl, CAS#28920-43-6) and O-phthalaldehyde (OPA, CAS# 643-79-8) can be found. The products of these reactions present fluorescence, so it is necessary to use a fluorometric detector [10-12].

The ion exchange columns are usually applied to eliminate the interfering substances present in the sample (cleaning procedure) [13]. Other cleaning techniques are based on the principle of matrix simplification similar to the QuEChERS technique (Quick, Easy, Cheap, Effective, Robust and Safe) [14]. Subsequently, the quantification is performed with HPLC coupled to Mass Spectrometer (MS), or with Liquid Chromatography (LC) coupled to MS/MS, with quantification limits for glyphosate in the order of 0.01 mg kg^{-1}. Usually, these methods do not require prior derivatization [15-17]. In addition, rapid screening assays, such as Enzyme Linked Immunosorbent Assay (ELISA) [18], electrochemical quantitative techniques [19], and capillary electrophoresis [20, 21] can also be found in the literature.

In Argentina, glyphosate and AMPA were detected in the surface waters and in the soil of the agricultural basins in the order of ppb [22]. The quantification was evaluated by UHPLC-MS/MS, including a previous derivatization with FMOC-Cl. Similarly, Lupi et al. [23] published the occurrence of both analytes in an agricultural basin, using a similar methodology. Higher concentrations were found in surface waters, sediments and soils near a soybean growing area [24]. In the case of water samples, the amount of glyphosate varied between 0.1 and 0.7 mg L^{-1}. The quantification technique used in the latter case was HPLC-UV, using FMOC-Cl as a derivatization agent.

Analytical instruments or methods can be classified according to the type of data they provide. In this sense, second-order instruments can generate a matrix (a second-order tensor) of data per sample [25]. Second-order data can be obtained by using “hyphenated” techniques (such as LC-DAD and GC-MS) or from a single second-order instrument, such as a spectrofluorometer capable of registering fluorescence Excitation–Emission Matrices (EEM), as in the present case.

When this kind of data must be used for quantitative purposes (besides qualitative ones), multivariate methodologies dedicated to the resolution of second-order information can exploit the so-called "second-order advantage" during the calibration procedure, even for the simultaneous determination of several analytes. This advantage, which is not available when working with data of order zero and one, means that it is allowed to analyze samples in the presence of any component that is not included in the calibration model [25], which can be done with information from pure standards.

Chemometrics tools are relevant in analytical chemistry due to their ability to analyze multivariate information from complex multicomponent mixtures without resorting to full separation procedures. Among different multivariate methodologies, the Multivariate Curve Resolution with Alternating Least Squares (MCR-ALS) methodology is suitable for modeling second-order data [26-29]. It has been widely applied to obtain the pure profiles (fluorescence emission/excitation spectra, absorption spectra, chromatographic elution, kinetics, etc) of components in mixtures and to predict the concentration of analytes in unknown samples, as it was performed in the present work with fluorescence EEM.

The aim of this work was to develop a new analytical method for the simultaneous quantification of glyphosate and AMPA, combining spectrofluorimetry and multivariate calibration. One of the most attractive features of molecular fluorescence is its inherent sensitivity, which is often one to three orders of magnitude greater than absorption spectroscopy. Typical detection limits are of the order of $\mu g\ L^{-1}$ (parts per billion) [30]. Since glyphosate and its metabolite AMPA do not have inherent fluorescence, it is necessary to apply a derivatization technique before its analytical detection. Both analytes react with NBD-Cl, in alcoholic medium and alkaline pH, resulting in compounds that emit fluorescence in acidic medium [31]. NBD-Cl reacts with aliphatic amines, amino acids, peptides, proteins or thiols and the generated fluorescence spectra are sensitive to pH and temperature.

Calibration and validation samples were analyzed with the proposed method. Also, glyphosate was quantified in a groundwater sample as well as in a commercial formulation. Matrix effect was evaluated trough the standard addition method in each real sample and these results were compared with those obtained by a reference method (HPLC).

2. Theory

2.1. MCR-ALS

MCR-ALS is one of the most applied second-order data analysis methodologies and has been extensively described in the literature [26, 27, 32]. In the context of simultaneous EEM processing, only a brief description of this soft-modelling technique is given here.

MCR-ALS relies on bilinearity, a property of some type of second-order data, such as EEM. When second-order data are bilinear, the responses (**D**) for pure compound *n* can be written as the outer product (called a bilinear component) [33]:

$$\mathbf{D} = \mathbf{a}_n \mathbf{b}_n \mathbf{c}_n^{\mathrm{T}} + \mathbf{E} \tag{1}$$

where $\mathbf{b}_n$ represents a column profile, c_n^T represents a row profile, a_n is a scaling factor related to the concentration of the constituent (optionally, this factor can be absorbed in one of the previous profiles), and **E** is the measurement noise. Both vector profiles must be independent of each other, such as in EEM. In this case, the emission spectrum does not depend on the excitation spectrum and the emission profiles are identical for all excitation wavelengths.

Taking into account a mixture of *N* bilinear components, if there is no interaction between them, then the contributions of each component in the two orders of the measurements can be added (i.e., rank additivity) [33] and the measured data can be described by:

$$\mathbf{D} = \mathbf{C}\mathbf{S}^{\mathrm{T}} + \mathbf{E} \tag{2}$$

where **C** ($K \times N$) and **S** ($J \times N$) contain, in columns, the column and row profiles, respectively, and the scaling constant was absorbed in either **C** or **S**.

MCR analysis can be empowered significantly when multiple data sets are simultaneously analyzed, which can be done by matrix augmentation. An advantage of this is that the good features for resolution presented by one or some of the included data matrices (for example, pure standard solutions) will have always a positive effect on the resolution of the most complex ones. To have a new meaningful data structure,

all individual matrices in the augmented matrix should share some information with the other appended matrices. When multiple samples have been analyzed by the same technique, the common approach is referred as column augmentation; the different data matrices are supposed to share their column vector space, and the row vector space of each data matrix is left unshared, to be independent of the other matrices [33].

In the case of EEM, considering a group of I samples, each one represented by a bilineal matrix ($\mathbf{D}_i$) of size $K \times J$ (emission wavelengths × excitation wavelengths), an augmented matrix ($\mathbf{D}_{\text{aug}}$, $KI \times J$) can be built by placing them one on top of each other. For this example, it means that the appended data matrices are assumed to share the excitation spectra. The corresponding MCR extended bilineal model can be described by:

$$\mathbf{D}_{aug} = \begin{pmatrix} \mathbf{D}_1 \\ \mathbf{D}_2 \\ \vdots \\ \mathbf{D}_I \end{pmatrix} = \begin{pmatrix} \mathbf{C}_1 \\ \mathbf{C}_2 \\ \vdots \\ \mathbf{C}_I \end{pmatrix} \mathbf{S}^{\mathrm{T}} + \begin{pmatrix} \mathbf{E}_1 \\ \mathbf{E}_2 \\ \vdots \\ \mathbf{E}_I \end{pmatrix} = \mathbf{C}_{aug}\mathbf{S}^{\mathrm{T}} + \mathbf{E}_{aug} \tag{3}$$

The bilinear decomposition of the augmented matrix $\mathbf{D}_{\text{aug}}$ is done according to the previous expression, taking into account N modeled bilinear components. If not known, this parameter must to be estimated, for example by means of Singular Value Decomposition (SVD) analysis of $\mathbf{D}_{\text{aug}}$. Also, it is worth noting a clear advantage of the simultaneous analysis of multiple data matrices using the last expression, that is, there is a net increase in the over determination of the linear system of equations to be solved and this produces a more stable and precise least-squares estimations in the general case [33].

After MCR-ALS resolution, $\mathbf{S}$ ($J \times N$) will contain the shared set of N excitation profiles and $\mathbf{E}_{\text{aug}}$ ($KI \times J$) will collect the experimental error and the variance not explained by the model. On the other hand, $\mathbf{C}_{\text{aug}}$ ($KI \times N$) will contain the N emission profiles scaled by corresponding concentration factors, which will be unique for each individual $\mathbf{D}_i$ matrix, on the basis of its own composition. Finally, MCR scores per modeled component and per sample can be obtained through area integration of the respective emission profiles. The scores of calibration samples can be regressed against nominal concentrations, and the obtained calibration curve can be used to predict concentrations in unknown samples.

The decomposition of $\mathbf{D}_{aug}$ is achieved by the iterative and alternating least-squares minimization of the $\mathbf{E}_{aug}$ Frobenius norm. MCR-ALS requires initialization profiles of $\mathbf{C}_{aug}$ or **S** as close as possible to the final results. In the present work, we use the SIMPLISMA methodology (simple to use interactive self-modeling mixture analysis) [34] in all cases, which is a useful algorithm for extracting spectra of pure components from mixtures of variable composition.

During the iterative recalculations of $\mathbf{C}_{aug}$ and **S**, some mathematical constraints can be applied to give physical meaning to the profiles obtained and to minimize rotational ambiguities. Some examples are non-negativity, unimodality, correspondence between species and trilinearity, among others. More details on the implementation of constraints can be found in previous works elsewhere [35, 36].

The estimation of the MCR-ALS figures of merit was performed according to Bauza and coworkers [37]. Therefore, the following expression was used to calculate the sensitivity (SEN):

$$\mathrm{SEN}_{\mathrm{MCR}} = m_n[J(\mathbf{S}^T\mathbf{S})^{-1}_{nn}]^{-1/2} \quad (4)$$

$$SEN_{MCR} = m_n\left[J\left(S^TS\right)^{-1}_{nn}\right]^{-1/2} \quad \textbf{(4 auxiliar)}$$

where n is the index for the analyte of interest in a multicomponent mixture, m_n is the slope of the MCR pseudounivariate calibration graph for this analyte, **S** is a matrix containing the profiles for all sample components in the non augmented MCR direction, and J is the number of channels in the test sample data matrix in the augmented MCR direction. The limit of detection (LOD) and quantification (LOQ), as well as other figures of merit, were also estimated [37].

3. Materials and methods

3.1. Chemicals and reagents

Analytical reagent-grade chemicals and ultrapure water were used. Glyphosate, AMPA and NBD-Cl were obtained from Sigma-Aldrich. Glyphosate standard solution 500.0 mg L^{-1} and AMPA standard solution 512.0 mg L^{-1} were prepared by dissolving appropriate amounts of each compound in 250.0 mL volumetric flasks and completing

to the mark with milliQ water. NBD-Cl solution 0.2 mM was prepared by placing 25 mg of the drug and dissolving it with methanol in a 25.0 mL volumetric flask.

Phosphate-borate buffer solution (pH= 9) was prepared by dissolving monobasic phosphate sodium (0.1199 g), dibasic phosphate sodium (6.9560 g) and boric acid (3.0915 g) in a 1000.0 mL volumetric flask and completing to the mark with milliQ water. The final concentration of the buffer was: H_2PO_4Na = 0.001 mol L^{-1}, HPO_4Na_2 = 0.05 mol L^{-1} and H_3BO_3 = 0.05 mol L^{-1}. All three drugs were purchased from Cicarelli (Argentina).

Methanol (HPLC grade) and hydrochloric acid were purchased from Cicarelli (Argentina). A solution of hydrochloric acid 6 mol L^{-1} was used in the derivatization experiments.

3.2. Instrumentation and software

All spectrofluorimetric measurements were performed using a Perkin-Elmer LS-55 luminescence spectrometer equipped with a xenon discharge lamp, Monk-Gillieson type monochromators and a gated photomultiplier connected. Slits for the excitation and emission monochromators were set at 5 nm and the detector voltage was 650 V.

The resolution of fluorescence signals with the MCR-ALS methodology and the study of rotation ambiguities with MCR-BANDS were implemented through the graphical user interface and command line tools provided by R. Tauler in http://www.mcrals.info. All the algorithms were implemented using MATLAB 8.5 [38].

3.3. Calibration and validation samples

In order to study the linearity of the method, a calibration set of five glyphosate solutions was prepared in duplicate, in a concentration range of 100 to 500 µg L^{-1}, and a calibration set of four AMPA solutions in duplicate, in a range of 200 to 800 µg L^{-1}. Blanks solutions in the absence of both analytes were also prepared. All solutions were processed according to the developed quantification method, which is detailed below.

Nine artificial samples, which contained both glyphosate and AMPA, were prepared and processed in duplicate. This validation set was made following a composed central design of 2 factors, full type and with one central point. The final concentrations varied from 110 to 360 µg L^{-1} and from 420 to 770 µg L^{-1}, for glyphosate and AMPA, respectively.

A 27-sample precision set was prepared at three different concentration levels of both, glyphosate (200, 300 and 400 μg L^{-1}) and AMPA (200, 400 and 600 μg L^{-1}), in triplicate, and in three different days.

All samples were processed according to the developed quantification method.

3.4. Real samples

A Credit® solution of 36 mg L^{-1} of glyphosate (approximated value based on the product label information) was prepared from the commercial product, by dissolving the appropriated volume in a 100.0 mL volumetric flasks and completing to the mark with milliQ water.

A groundwater sample from a cultivated area in the city of Córdoba, Argentina, was also analyzed. Prior to analysis, the water sample was filtered through 0.45 mm filters and stored at 4 ºC in the refrigerator.

3.5. Developed Quantification Method

All working solutions (blanks, glyphosate and AMPA calibration, artificial and real samples) were derivatized with 0.2 mL of NBD-Cl (0.2 mM) at 90°C for 15 minutes in alkaline medium with 2 mL of phosphate-borate buffer solution (pH = 9) and 2 mL of methanol. Then, the solutions were cooled for 5 minutes in an ice bath (4°C). After that, the medium was acidified with HCl 6 mol L^{-1}. Finally, a final volume of 5.0 mL was reached with methanol, which increases the fluorescence signal.

The excitation-emission matrices of all solutions were obtained by exciting in a wavelength range from 400 to 500 nm every 0.5 nm, while the emission was recorded in a range from 500 to 610 nm every 10 nm. Then, the size of each matrix was 201 × 12.

3.6. Study of matrix effect

To study the existence of the matrix effect in the analyte signals caused by the presence of interferences in the Credit® commercial solution and in the groundwater sample, glyphosate standard addition curves were prepared. In the case of Credit®, 30 μL of diluted solution was added to each 5.00 mL flask in the derivatization stage and concentrations between 0 and 500 μg L^{-1} of pure glyphosate were incorporated. A volume of 0.5 mL of sample and concentrations of 0 to 500 μg L^{-1} of pure glyphosate were used to prepare the standard addition curve for groundwater.

3.7. HPLC method

HPLC (Waters) equipped with a UV–Vis dual detector (Waters 2489) was used for the quantification of glyphosate in a diluted solution (36 mg L^{-1} of glyphosate) of the commercial product Credit® and also in a groundwater sample. A reference method published by Kawai et al. [39] was applied for the analysis. This technique consists in derivatizing glyphosate with p-toluenesulfonyl chloride (TsCl) prior to the chromatographic run. The detection was made at 240 nm.

4. Results and Discussion

4.1. Calibration curves: Linearity Study and Figures of Merit

The linearity of the instrumental response was studied by performing a calibration curve with five glyphosate standard solutions, in duplicate, in a concentration range from 100 to 500 μg L^{-1}. Similarly, a calibration curve was prepared with four standard solutions of AMPA, in duplicate, in a concentration range of 200 to 800 μg L^{-1}. For each solution, the fluorescence EEM was measured.

All matrices were restricted in both modes (excitation and emission wavelengths) before constructing the models. Specifically, the excitation wavelengths were restricted between 429.5 and 494.5 nm and the emission wavelengths between 520 and 610 nm, generating matrices of 131 × 10 per sample. Due to the fact that there is a greater difference between the excitation spectra of the three components of the mixtures, than in the emission spectra, we decided to work with the transposed matrices (10 × 131, emission × excitation).

The excitation-emission matrices corresponding to the glyphosate and AMPA calibration sets were column-wise augmented, creating two different augmented $\mathbf{D}_{aug}$ matrices, respectively. Two components were estimated for each augmented matrix by SVD: glyphosate and NBD-Cl on one side and AMPA and NBD-Cl on the other. The initial estimates were obtained by SIMPLISMA [34], and then the analysis of each $\mathbf{D}_{aug}$ matrix was made by MCR-ALS. The constraints applied were non-negativity in both profiles, normalization in excitation spectra and trilinearity.

The area under each resolved emission profile was plotted as a function of glyphosate and AMPA concentrations, respectively. The calibration curves can be seen in Fig. 1.

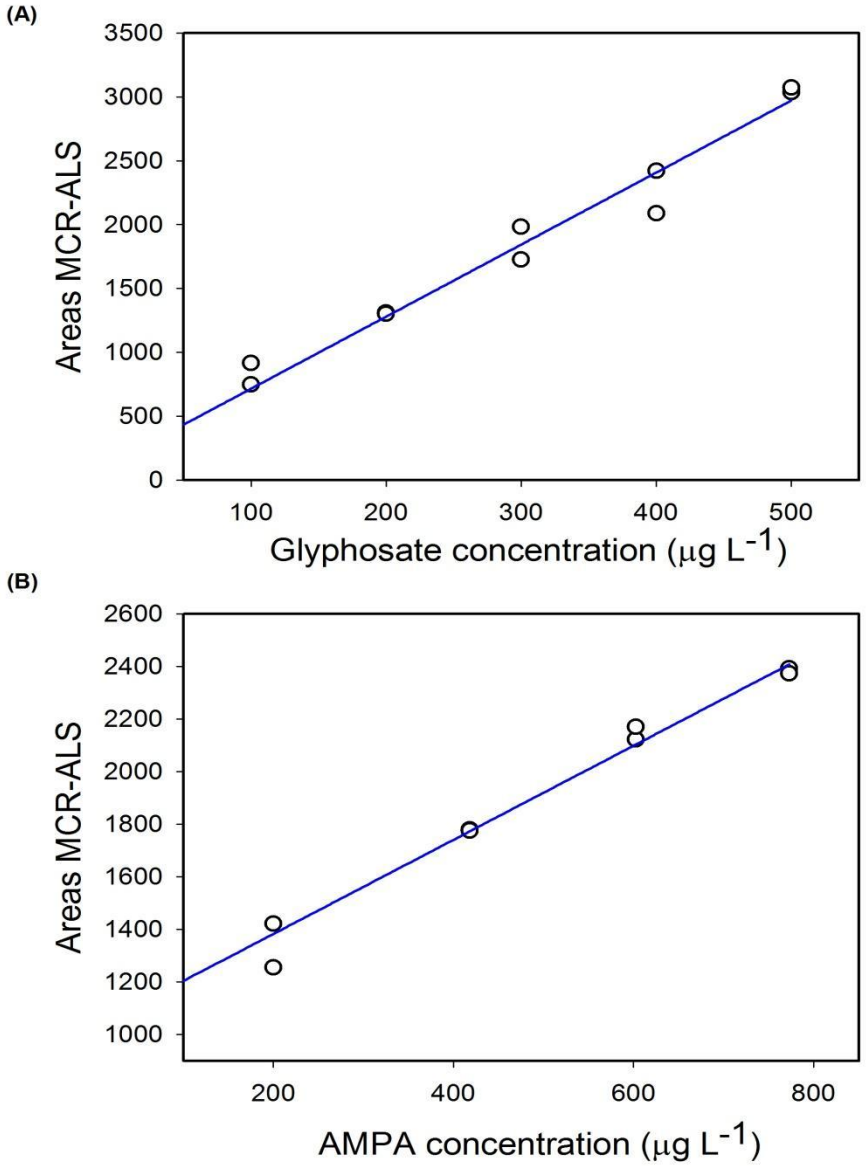

Fig. 1. (A) Glyphosate calibration curve in concentrations between 100 and 500 μg L^{-1} and (B) AMPA calibration curve in concentrations between 200 and 800 μg L^{-1}.

A simple linear regression method was applied for each curve. The statistical parameters obtained are shown in Table 1. In addition, the ANOVA test was carried out. In the case of glyphosate, a *F-ratio* of 1.21 and an associated *p*-value of 0.40 were obtained due to lack of fit. Since the latest is greater than 0.10, the model is adequate for the experimental data. The same conclusion was reached for AMPA, with a *F-ratio* and a *p*-value of 0.99 and 0.45, respectively.

Table 1.

Linear regression parameters for glyphosate and AMPA calibration curves.

Regression analysis		
	Glyphosate	**AMPA**
Slope[a]	5.4 (0.3)	1.8 (0.1)
Intercept[a]	242 (110)	991 (50)
R^2	0.97	0.98
S_{fit}[b]	149	62

[a] In parentheses it is the standard deviation.

[b] Residual standard deviation.

Since the most important procedure for the comparison of analytical methods is the determination of the figures of merit, these parameters were calculated after applying the MCR-ALS methodology. Table 2 shows the analytical figures of merit obtained according to Bauza et al. [37].

Table 2.

Figures of merit for the determination of glyphosate and AMPA with MCR-ALS.

	MCR-ALS	
	Glyphosate	AMPA
SEN (AFU L μg^{-1})	0.10	0.07
γ (L μg^{-1})[a]	19	14
γ^{-1} (μg L^{-1})	0.05	0.07
LOD (μg L^{-1})[b]	43	60
LOQ (μg L^{-1})[c]	103	182

AFU: arbitrary fluorescence units.

[a] Analytical sensitivity.

[b] LOD, limit of detection calculated according to Bauza et al. [37], considering 95 % of probability.

[c] LOQ, limit of quantification calculated as LOD×(10/3.3).

Considering that no previous pre-concentration/cleaning techniques were applied, the figures of merit obtained are considered satisfactory. In Table 3, several related quantification methods and their limits of detection and quantification are presented for several types of samples.

Table 3.

Limits of detection and quantification for the determination of glyphosate and AMPA with different analytical methods.

Matrix	Derivatization Agent[a]	Method[b]	LOD[c]	LOQ[c]	Ref.
Soil Groundwater	TFAA and TFE	GC-MS-SIM	3 0.05	6 0.1	[40]
Lake water	TFAA and TFE	GC-MS-SIM	0.09-65	1.15-180	[41]
Waters[d]	TFAA and HFB	GC-MS-MS	0.025	0.050	[42]
Surface water	AA and TMOA	GC-NPD	5	17	[43]
Drinking, surface, waste-water	FMOC-CL	LC-MS-MS	0.03	0.05	[44]
Water Soil	FMOC-CL	LC-MS-MS	0.005 5	0.05 50	[45]
Water	FMOC-CL	LC-FD	0.1	0.3-10	[46]
Ground, surface, river waters	FMOC-CL	LC-MS-MS	0.0002-0.0006	0.0007-0.0023	[47]
Soil	FMOC-CL	LC-MS/MS	43-120.3	22.7-88.9	[11]
Water	Post column, OPA-MCE	LC-FD	0.22	0.72	[48]
Soil, sediment Surface water	FMOC-CL	LC-MS/MS	1-5 0.1	5-10 0.5	[22]
Soil, sediments Streamwater	FMOC-CL	LC–MS/MS	0.05 0.05	1 0.5	[23]
Soil, sediments	FMOC-CL	LC–UV	100	250	[24]

Streamwater	40	100	

[a] TFAA = trifluoroacetic acid anhydride; TFE = trifluoroethanol; HFB = heptafluorobutanol; FMOC-CL=9-fluorenylmethyl chloroformate; OPA-MCE = o-phthalaldehyde-2-mercaptoethanol; AA = acetic acid anhydride; TMOA = trimethylorthoacetate.

[b] GC = gas chromatography; LC= liquid chromatography; MS: mass spectrometry; SIM= Selected ion monitoring; UV= UV detection; FD = fluorescence detection; NPD =nitrogen-phosphorous detection.

[c] LOD= limit of detection and LOQ= limit of quantification, expressed in μg L^{-1} for liquid samples and μg Kg^{-1} for solid samples.

[d] Model waters of various hardnesses.

As can be seen, most of the analytical methods found in the literature involve a chromatographic technique, a derivatization step and a more sophisticated detection system, such as MS-MS. In those cases, the LOD and LOQ were lower than those presented in this work. On the other hand, it should be highlighted that in our case no separation method was used, and also that the analytical signal was obtained from a less complex instrument. In addition, the values of LOD and LOQ were similar to those published by Peruzzo et al. [24], in which a separation method was used with UV detection to quantify glyphosate.

4.2. Validation Samples: glyphosate and AMPA prediction with MCR-ALS

MCR-ALS was applied to simultaneously analyze nine validation samples in duplicate. For this purpose, an augmented $\mathbf{D}_{aug}$ matrix was constructed with calibration and validation EEM.

Before beginning the resolution by MCR-ALS, the determination of the number of components was made by SVD and the presence of three components (corresponding to glyphosate, AMPA and NBD-Cl) was established. The initial **S** estimates were created through SIMPLISMA.

During the ALS optimization, the following constraints were applied: non-negativity in the spectral and concentration profiles, normalization in spectra, correspondence between species and trilineality for all analytes. Convergence was achieved and lack of fit percentages of 1.343 % and 3.83 % were obtained for glyphosate and AMPA, respectively. The explained variances were 99.98% (glyphosate) and 99.93% (AMPA).

In Fig. 2, excitation profiles extracted by MCR-ALS for NBD-Cl (solid blue line), glyphosate (dotted green line) and AMPA (dashed red line) are shown.

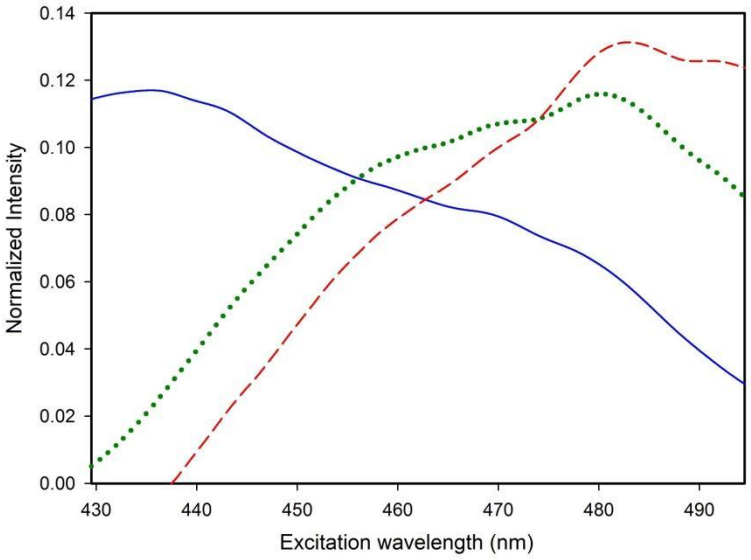

Fig. 2. Excitation profiles contained in **S** and extracted by MCR-ALS. Solid blue line: NBD-Cl, dotted green line: derivatized glyphosate and dashed red line: derivatized AMPA.

The concentration information contained in $\mathbf{C}_{aug}$ (the area under resolved emission profiles) was used to construct the pseudounivariate graphs. The results of the predicted concentrations for glyphosate and AMPA in the validation samples, together with their nominal concentrations, are presented in Table 4. As can be observed, there is a good agreement between them since the recovery values as well as the relative error of prediction were satisfactory.

Fig. 3 shows some emission profiles in $\mathbf{C}_{aug}$ obtained after applying MCR-ALS. The first band corresponds to a blank sample containing only NBD-Cl, the second to a validation sample containing glyphosate and AMPA, and the following bands correspond to several glyphosate calibration samples. All samples show the NBD-Cl spectrum as expected. Although the existence of significant overlap, the spectra corresponding to the analytes and the derivatizing agent were adequately recovered by MCR-ALS.

Table 4.

Determination of glyphosate and AMPA in the validation samples.

	Glyphosate (µg L^{-1})		AMPA (µg L^{-1})	
Sample	**Nominal**	**Predicted [a, b]**	**Nominal**	**Predicted [a, b]**
1	232.5	225.5 (2)	416.7	384.2 (3.3)
2	315.5	254.2 (2.4)	700.8	691 (5)
3	147.9	144.8 (1.9)	709.8	699.3 (4.5)
4	349.7	331.4 (2.3)	576.6	581.1 (3.6)
5	146.5	136.4 (1.3)	470	434.6 (2.8)
6	232.5	220.6 (3)	760.2	826 (7)
7	110.3	86.7 (1.1)	589.1	601.8 (6.5)
8	233.0	216.0 (2.8)	586.9	579.4 (2.6)
9	318.9	239.4 (1.9)	470.7	503 (3)
Average recovery [b]		**89 (9)**		**99.9 (6.2)**
REP(%)[c]		**5.1**		**4.7**

[a] Average of duplicate analysis.

[b] Between parentheses it is the standard deviation.

[c] REP(%): Relative error of prediction, $REP = 100 \times \left[\frac{1}{I} \sum_{1}^{I} (c_{nom} - c_{pred})^2 \right]^{1/2} \times \overline{c}$, where $\overline{c}$ is the mean calibration concentration and I=18.

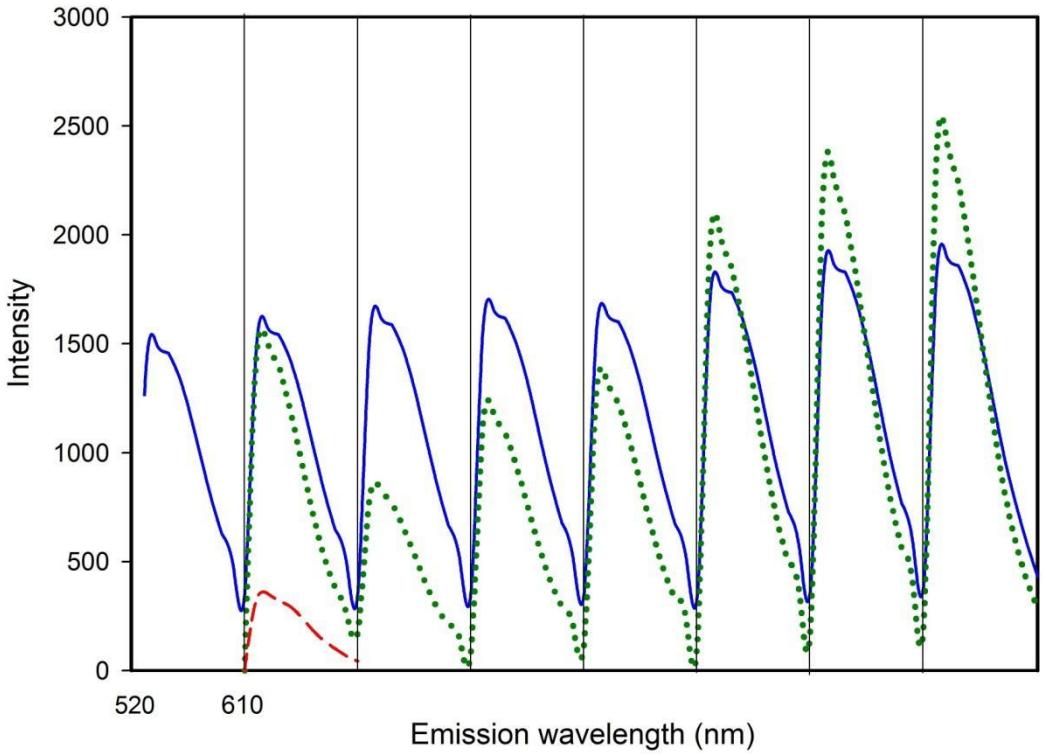

Fig. 3. Emission profiles extracted with MCR-ALS when a blank sample (first band), a validation sample (second band) and several calibration samples (last six bands) were analyzed simultaneously. Solid blue line: NBD-Cl, dotted green line: derivatized glyphosate and dashed red line: derivatized AMPA.

In order to study the extent of rotation ambiguity and boundaries associated to the obtained MCR resolution, MCR-BANDS was applied [49, 50]. For this purpose, the obtained profiles were analyzed applying the same constraints used during the ALS optimization: non-negativity in both profiles, normalization in excitation spectra and trilinearity. The results (not shown) suggest that rotation ambiguities were practically eliminated, since for the three components (NBD-Cl, glyphosate and AMPA) the maximum and minimum optimization function values were very close to each other (mean difference lower than 0.003 for all tested samples and species). When the difference between those values is close to zero, it means that practically there is no remaining rotation ambiguity.

4.3 Precision and accuracy study

The results of the precision study for glyphosate and AMPA, carried out on the precision test samples, are summarized in Table 5. For this purpose, repeatability and intermediate precision were calculated. In the first case, three different concentration levels were analyzed in triplicate. Then, the same three levels were evaluated in triplicate during three different days in order to compute intermediate precision.

Table 5.

Results obtained when analyzing the precision set, coefficients of variation and ANOVA probabilities.

Glyphosate (µg L^{-1})	**Day 1**		**Day 2**		**Day 3**		**ANOVA[b] (*p*-value)**
	Prediction	**CV%[a]**	**Prediction**	**CV%[a]**	**Prediction**	**CV%[a]**	
200	198.7		198.7		203.1		
	201.8	0.8	197.5	0.8	192.4	2.9	0.504
	199.4		195.4		194.2		
300	299.8		297.2		294.6		
	293.8	1.3	295.4	0.5	305.3	1.9	0.620
	301.5		294.5		296.7		
400	401.5		395.9		399.3		
	396.9	0.6	400.0	1.0	393.9	0.9	0.783
	397.8		403.6		400.5		
AMPA (µg L^{-1})	**Prediction**	**CV%[a]**	**Prediction**	**CV%[a]**	**Prediction**	**CV%[a]**	
200	195.4		202.3		203.7		
	202.4	2.1	189.9	4.1	208.7	1.5	0.248
	202.9		205.5		209.1		
400	408.2		407.5		402.3		
	403.6	1.6	399.5	1.3	410.1	1.0	0.748
	395.8		409.6		403.8		
600	593.5		602.3		604.6		
	601.2	0.8	588.5	1.6	600.7	0.3	0.729
	603.2		606.4		603.1		

[a] CV%: Percentage of variation coefficient. CV% = (SD/average conc.) × 100, in which SD is the standard deviation for $n - 1$ degree of freedom.

[b] Analysis of variance: all the means are statistically equivalent when $p > 0.05$.

As can be appreciated, results can be considered satisfactory for the herbicide and its metabolite, since the obtained CV% values were lower than 5% in every case.

An analysis of variance was performed to compare the predictions obtained in each day and at every concentration level. In the three cases, the obtained *p*-value were higher than 0.05 (Table 5). Consequently, there are not significant differences between the means, i.e. the precision of the proposed method is acceptable.

In addition, accuracy was assessed by applying the joint statistical test for the slope and the intercept of the linear regression between the nominal and predicted analyte concentrations [51], using the same set of samples previously utilized to perform the precision study. As can be seen in Fig. 4, both ellipses, built with a confidence level of 95%, contain the theoretically expected values for the intercept (0) and the slope (1). This fact is indicative of the absence of both proportional and constant errors, in spite of the high spectral overlapping between glyphosate, AMPA and the derivatizing agent.

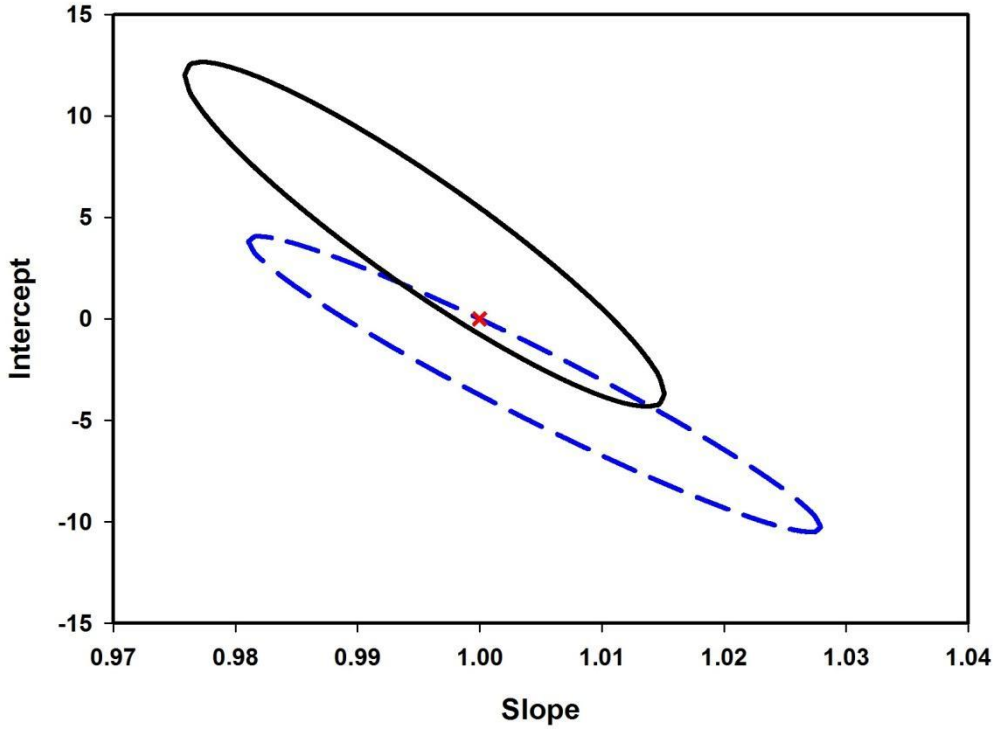

Fig. 4. Elliptical joint confidence regions (EJCR) for the slope and intercept of the regression of predicted concentrations versus nominal values for glyphosate (blue dashed line) and AMPA (black line) (95% of confidence). The cross shows the theoretically expected values of the intercept (0) and the slope (1).

4.4 Real Samples

4.4.1 Study of matrix effect and glyphosate quantification

The commercial glyphosate is formulated with additives, substances that reduce the surface tension (detergent), which can produce a change in the analytical signal. For this reason, the effect of the matrix was studied by comparing the slope of the glyphosate calibration curve with the slope of a Credit® standard addition curve. For this purpose, an augmented $\mathbf{D}_{aug}$ matrix was created by appending the EEM of both curves. Two components were estimated by SVD, and initial estimates were obtained by SIMPLISMA. After applying MRC-ALS, the concentration information contained in $\mathbf{C}_{aug}$ was used to plot both curves (Fig. 5) and ANOVA statistical analysis was performed to compare the slopes.

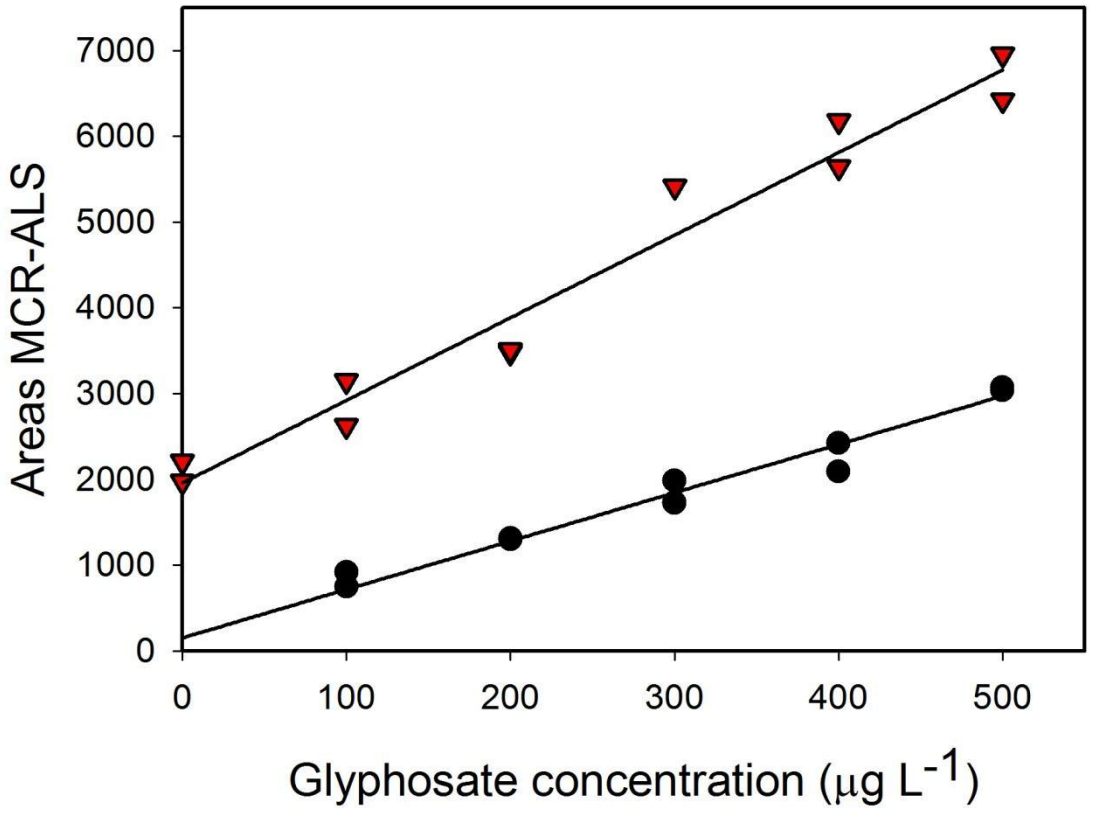

Fig. 5. Glyphosate calibration curve (black circles) and standard addition curve (red triangles).

Before the ANOVA, the Levene´s test was carried out in order to study the equality of variances between the calibration curve and the standard addition curve. Since the *p*-value was 0.06 (greater than the significance level 0.05), the null hypothesis of equal variances was accepted.

As a result of the ANOVA test, there exists a statistically significant difference between the slopes with a 99% confidence level since the *p*-value for the slopes was less than 0.01. This result shows that the additives added to the commercial formulation produce a change in the analytical signal, thus preventing the quantification of glyphosate in the commercial sample using pure standards.

Consequently, the prediction was made by the standard addition method. Furthermore, the sample was also analyzed by a liquid chromatographic method and results were compared. The sample was processed in duplicate and the predicted concentrations are shown in Table 6. It can be seen that the results obtained by both methods are comparable and consistent with the prepared solution concentration.

In the case of the groundwater sample, firstly, the EEM were analyzed simultaneously with the glyphosate and AMPA calibration matrices. Three components were estimated by SVD, and initial estimates were obtained by SIMPLISMA. After the application of MCR-ALS, it was observed that the sample did not contain neither the herbicide nor its metabolite (or they were under respective LOD).

Additionally, the sample was added with 400 μg L^{-1} of glyphosate, in duplicate, and the same analysis was performed. In this case, the prediction results were 144.8 and 93.3 μg L^{-1} of glyphosate. These values were not consistent with the added amount. For this reason, the presence of matrix effect was suspected and a standard addition curve was made for successful analyte quantification. Increasing concentrations of glyphosate (0 to 500 μg L^{-1}) were added to the same volume of sample (0.5 mL). The regression parameters obtained after plotting the areas gathered by MCR-ALS *vs.* the added glyphosate concentrations were: slope 11.3, intercept 921.5, R^2 0.948 and S_{fit} 675.9.

Before comparing the slopes of the pure standard curve and the standard addition curve, the variances were analyzed. The Levene's test resulted in a *p*-value > 0.05, verifying that the variances of each regression line were comparable. Then, the ANOVA statistical analysis was performed. The resulting *p*-value was lower than 0.01, confirming the existence of a statistically significant difference between the slopes with a confidence level of 99%, therefore, matrix effect was demonstrated. Due to this, the

quantification was performed with the standard addition curve combined with MCR-ALS.

The groundwater obtained from an area cultivated in the city of Córdoba (Argentina) presented 0.85 mg L^{-1} of the herbicide. This value was in agreement with the quantities found by Peruzzo et al. [24] in surface water samples near a direct sowing soybean growing region in Argentina. Furthermore, the water sample was analyzed by the HPLC method following the procedure proposed by Kawai et al. [39]. Results obtained are displayed in Table 6, showing an acceptable level of agreement with the MCR-ALS methodology.

Table 6.

Predicted concentration of glyphosate in the diluted Credit® solution and in groundwater.

Sample	MCR- ALS (mg L^{-1})[a,b]	HPLC (mg L^{-1}) [a,b]
Credit®	33.9 (3.3)	29.0 (1.7)
Water sample	0.85 (0.55)	0.8 (0.3)

[a] Average of duplicate analysis.

[b] Between parentheses it is the standard deviation.

5. Conclusions

The use of spectroscopic techniques in combination with chemometrics through the application of multivariate methods, allowed the determination of glyphosate and AMPA in validation samples and in different real samples (commercial formulation and groundwater). The proposed method resolved both excitation and emission fluorescence spectra of the derivatizing reagent (NBD-Cl) and also of the reaction products (Glyphosate-NBD-Cl and AMPA-NBD-Cl).

The range of work concentrations handled by this new method is 100 to 500 μg L^{-1} for glyphosate and 200 to 800 μg L^{-1} for AMPA. Acceptable figures of merit, comparable to those found in the literature, were obtained. Adequate predictions were obtained for validation samples, with mean recovery values of 89% for glyphosate and 99.9% for AMPA, and REP% of 5.1 and 4.7 for the herbicide and its metabolite, respectively. In addition, the precision study was satisfactory, indicated by the obtained

values of CV% (lower than 5%) and the results of the ANOVA test. Furthermore, the elliptical joint confidence regions for the slope and intercept of the regression of predicted concentrations versus nominal values were used to evaluate the accuracy of the proposed method, indicating that bias was not significant because its respective ellipse contains the ideal point for slope and intercept.

The quantification of glyphosate in real samples (commercial formulation and groundwater) was possible by applying the standard addition method, combined with MCR-ALS. The results were satisfactory and comparable with those delivered by HPLC.

The combination of the fluorescence technique with the chemometric method is simple to apply and the data could be obtained with low complexity laboratory equipment. The developed analytical method was successfully implemented to determine glyphosate and AMPA in the presence of unknown sample matrix components.

Acknowledgments

The authors are grateful to Universidad Nacional del Litoral (UNL) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), for financial support. G.H.S. and A.L.P. thank CONICET for their fellowships.

References

[1] C.A. Mallory-Smith, E. James Retzinger Jr, Revised classification of herbicides by site of action for weed resistance management strategies, Weed Technol, 17 (2003) 605-619.
[2] M. Chłopecka, M. Mendel, N. Dziekan, W. Karlik, The effect of glyphosate-based herbicide Roundup and its co-formulant, POEA, on the motoric activity of rat intestine – In vitro study, Environ Toxicol Phar, 49 (2017) 156-162.
[3] N. Defarge, J. Spiroux de Vendômois, G.E. Séralini, Toxicity of formulants and heavy metals in glyphosate-based herbicides and other pesticides, Toxicology Reports, 5 (2018) 156-163.
[4] D.J. Sharon Wilbur, John F Risher, Jewell Crawford, Brian Tencza, Fernando Llados, Gary L Diamond, Mario Citra, Mark R Osier, Larry O Lockwood., Toxicological profile for 1,4 Dioxane, in: A.f.T.S.a.D.R. (ATSDR) (Ed.), Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, 2012.
[5] W.H.O. World Health Organization, IARC Monographs Volume 112: evaluation of five organophosphate insecticides and herbicides, in: I.A.f.R.o.C. (IARC) (Ed.), 2015.
[6] A. Paganelli, V. Gnazzo, H. Acosta, S.L. López, A.E. Carrasco, Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling, Chem Res Toxicol, 23 (2010) 1586-1595.

[7] C. Portier, B. K Armstrong, B. Baguley, X. Baur, I. Belyaev, R. Bellé, F. Belpoggi, A. Biggeri, M. C Bosland, P. Bruzzi, L. Budnik, M. Bugge, K. Burns, G. M Calaf, D. Carpenter, H. M Carpenter, L. López-Carrillo, R. Clapp, P. Cocco, Z. Sf, Differences in the carcinogenic evaluation of glyphosate between the International Agency for Research on Cancer (IARC) and the European Food Safety Authority (EFSA), 2016.
[8] F. Parrot, R. Bedry, J.C. Favarel-Garrigues, Glyphosate herbicide poisoning: Use of a routine aminoacid analyzer appears to be a rapid method for determining glyphosate and its metabolite in biological fluids, Clin Toxicol, 33 (1995) 695-698.
[9] B.M. Da Silva, P.R. Das Dores Da Silva, M.O.O. Rezende, Development of green HPLC/UV methodology for the determination of glyphosate in soil environmental samples, Eclet Quim, 40 (2015) 106-116.
[10] S. Goscinny, H. Unterluggauer, J. Aldrian, V. Hanot, S. Masselter, Determination of Glyphosate and Its Metabolite AMPA (Aminomethylphosphonic Acid) in Cereals After Derivatization by Isotope Dilution and UPLC-MS/MS", Food Anal Method, 5 (2012) 1177-1185.
[11] G.R. Todorovic, A. Mentler, M. Popp, S. Hann, G. Köllensperger, N. Rampazzo, W.E.H. Blum, Determination of Glyphosate and AMPA in Three Representative Agricultural Austrian Soils with a HPLC-MS/MS Method, Soil Sediment Contam, 22 (2013) 332-350.
[12] P. Kaczyński, B. Łozowicka, Liquid chromatographic determination of glyphosate and aminomethylphosphonic acid residues in rapeseed with MS/MS detection or derivatization/fluorescence detection, in: Open Chem, 2015.
[13] L. J Marek, W. C Koskinen, Simplified analysis of glyphosate and aminomethylphosphonic acid in water, vegetation and soil by liquid chromatography–tandem mass spectrometry, Pest Manag Sci, 70 (2014) 1158-1164.
[14] M. Anastassiades, S.J. Lehotay, D. Stajnbaher, Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) approach for the determination of pesticide residues, in: 18th Annual Waste Testing and Quality Assurance Symposium, WTQA 2002 - Proceedings, 2003, pp. 231-241.
[15] S. Adams, J. Guest, M. Dickinson, R.J. Fussell, J. Beck, F. Schoutsen, Development and Validation of Ion Chromatography–Tandem Mass Spectrometry-Based Method for the Multiresidue Determination of Polar Ionic Pesticides in Food, J Agr Food Chem, 65 (2017) 7294-7304.
[16] H. Guo, L.S. Riter, C.E. Wujcik, D.W. Armstrong, 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 (2016) 93-100.
[17] S.J. Lehotay, K. Maštovská, A.R. Lightfield, Use of buffering and other means to improve results of problematic pesticides in a fast and easy method for residue analysis of fruits and vegetables, J AOAC Int, 88 (2005) 615-629.
[18] H.Z. Şenyuva, J. Gilbert, Immunoaffinity column clean-up techniques in food analysis: A review, J Chromatogr B, 878 (2010) 115-132.
[19] S. Pintado, R.R. Amaro, M. Mayén, J.M.R. Mellado, Electrochemical determination of the glyphosate metabolite aminomethylphosphonic acid (AMPA) in drinking waters with an electrodeposited copper electrode, Int J Electrochem Sc, 7 (2012) 305-312.
[20] M. Tomita, T. Okuyama, Y. Nigo, B. Uno, S. Kawai, Determination of glyphosate and its metabolite, (aminomethyl)phosphonic acid, in serum using capillary electrophoresis, J Chromatogr B, 571 (1991) 324-330.

[21] M. Corbera, M. Hidalgo, V. Salvadó, P.P. Wieczorek, Determination of glyphosate and aminomethylphosphonic acid in natural water using the capillary electrophoresis combined with enrichment step, Anal Chim Acta, 540 (2005) 3-7.
[22] V.C. Aparicio, E. De Gerónimo, D. Marino, J. Primost, P. Carriquiriborde, J.L. Costa, Environmental fate of glyphosate and aminomethylphosphonic acid in surface waters and soil of agricultural basins, Chemosphere, 93 (2013) 1866-1873.
[23] L. Lupi, K.S.B. Miglioranza, V.C. Aparicio, D. Marino, F. Bedmar, D.A. Wunderlin, Occurrence of glyphosate and AMPA in an agricultural watershed from the southeastern region of Argentina, Sci Total Environ, 536 (2015) 687-694.
[24] P.J. Peruzzo, A.A. Porta, A.E. Ronco, Levels of glyphosate in surface waters, sediments and soils associated with direct sowing soybean cultivation in north pampasic region of Argentina, Environ Pollut, 156 (2008) 61-66.
[25] K.S. Booksh, B.R. Kowalski, Theory of Analytical Chemistry, Anal Chem, 66 (1994) 782-791.
[26] R. Tauler, Multivariate curve resolution applied to second order data, Chemometr Intell Lab, 30 (1995) 133-146.
[27] A. De Juan, R. Tauler, Comparison of three-way resolution methods for non-trilinear chemical data sets, J Chemometr, 15 (2001) 749-772.
[28] R. Tauler, Calculation of maximum and minimum band boundaries of feasible solutions for species profiles obtained by multivariate curve resolution, J Chemometr, 15 (2001) 627-646.
[29] M.C.G. Antunes, J.C.G. Esteves Da Silva, Multivariate curve resolution analysis excitation-emission matrices of fluorescence of humic substances, Anal Chim Acta, 546 (2005) 52-59.
[30] D.A. Skoog, Fundamentos de química analítica : novena edición, Cengage Learning, México, D.F., 2015.
[31] I. Durán Merás, T. Galeano Díaz, M. Alexandre Franco, Simultaneous fluorimetric determination of glyphosate and its metabolite, aminomethylphosphonic acid, in water, previous derivatization with NBD-Cl and by partial least squares calibration (PLS), Talanta, 65 (2005) 7-14.
[32] J. Saurina, C. Leal, R. Compañó, M. Granados, M.D. Prat, R. Tauler, Estimation of figures of merit using univariate statistics for quantitative second-order multivariate curve resolution, Anal Chim Acta, 432 (2001) 241-251.
[33] A.M.d.l. Peña, H.C. Goicoechea, G.M. Escandar, A.C. Olivieri, Fundamentals and Analytical Applications of Multiway Calibration, Elsevier, 2015.
[34] W. Windig, J. Guilment, Interactive Self-Modeling Mixture Analysis, Anal Chem, 63 (1991) 1425-1432.
[35] M.C. Antunes, J.E. J. Simão, A.C. Duarte, R. Tauler, Multivariate curve resolution of overlapping voltammetric peaks: Quantitative analysis of binary and quaternary metal mixtures, Analyst, 127 (2002) 809-817.
[36] T. Azzouz, R. Tauler, Application of multivariate curve resolution alternating least squares (MCR-ALS) to the quantitative analysis of pharmaceutical and agricultural samples, Talanta, 74 (2008) 1201-1210.
[37] M.C. Bauza, G.A. Ibañez, R. Tauler, A.C. Olivieri, Sensitivity equation for quantitative analysis with multivariate curve resolution-alternating least-squares: Theoretical and experimental approach, Anal Chem, 84 (2012) 8697-8706.
[38] MATLAB, MATLAB R2015a, The MathWorks Inc., Natick, Massachussets, USA, in, 2015.
[39] S. Kawai, B. Uno, M. Tomita, Determination of glyphosate and its major metabolite aminomethylphosphonic acid by high-performance liquid chromatography

after derivatization with p-toluenesulphonyl chloride, J Chromatogr A, 540 (1991) 411-415.
[40] E. Börjesson, L. Torstensson, New methods for determination of glyphosate and (aminomethyl)phosphonic acid in water and soil, J Chromatogr A, 886 (2000) 207-216.
[41] C.D. Stalikas, G.A. Pilidis, Development of a method for the simultaneous determination of phosphoric and amino acid group containing pesticides by gas chromatography with mass-selective detection: Optimization of the derivatization procedure using an experimental design approach, J Chromatogr A, 872 (2000) 215-225.
[42] A. Royer, S. Beguin, J.C. Tabet, S. Hulot, M.A. Reding, P.Y. Communal, Determination of Glyphosate and Aminomethylphosphonic Acid Residues in Water by Gas Chromatography with Tandem Mass Spectrometry after Exchange Ion Resin Purification and Derivatization. Application on Vegetable Matrixes, Anal Chem, 72 (2000) 3826-3832.
[43] J. Struger, D. Thompson, B. Staznik, P. Martin, T. McDaniel, C. Marvin, Occurrence of Glyphosate in Surface Waters of Southern Ontario, B Environ Contam Tox, 80 (2008) 378-384.
[44] R.J. Vreeken, P. Speksnijder, I. Bobeldijk-Pastorova, T.H.M. Noij, 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 (1998) 187-199.
[45] M. Ibáñez, Ó.J. Pozo, J.V. Sancho, F.J. López, F. Hernández, 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 (2005) 145-155.
[46] M. Corbera, M. Hidalgo, V. Salvadó, Extraction and Preconcentration of the Herbicide Glyphosate and its Metabolite AMPA Using Anion-Exchange Solid Phases, Microchim Acta, 153 (2006) 203-209.
[47] I. Hanke, H. Singer, J. Hollender, 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, 2008.
[48] M. Piriyapittaya, S. Jayanta, S. Mitra, N. Leepipatpiboon, Micro-scale membrane extraction of glyphosate and aminomethylphosphonic acid in water followed by high-performance liquid chromatography and post-column derivatization with fluorescence detector, J Chromatogr A, 1189 (2008) 483-492.
[49] J. Jaumot, R. Tauler, MCR-BANDS: A user friendly MATLAB program for the evaluation of rotation ambiguities in Multivariate Curve Resolution, Chemometr Intell Lab, 103 (2010) 96-107.
[50] A. Golshan, H. Abdollahi, S. Beyramysoltan, M. Maeder, K. Neymeyr, R. Rajko, M. Sawall, R. Tauler, A review of recent methods for the determination of ranges of feasible solutions resulting from soft modeling analyses of multivariate data, 2016.
[51] A.C. Olivieri, Practical guidelines for reporting results in single- and multi-component analytical calibration: A tutorial, Anal Chim Acta, 868 (2015) 10-22.