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A QUICK, SIMPLE, SENSITIVE AND SELECTIVE LC-MS/MS METHOD USED FOR THE SCREENING OF ETHEPHON, GLYPHOSATE AND AMINOMETHYLPHOSPHONIC ACID FROM WATER AND FOOD SAMPLES

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Abstract: Pesticide use has increased steadily over the years in both industrial farming and local agriculture. One of the most widely used pesticides at a global level, glyphosate, has been controversial for many years and many studies have focused on the effects of this compound on human health. Ethephon is also a controversial ripening agent used to ripen crops more quickly. A basic but crucial step in the study of the effects of their use on human health is the development of adequate analytical methodologies for the quantification of the compounds in relevant samples. For this study a quick, simple and selective method which uses LC-MS/MS was developed for the determination of ethephon, glyphosate and AMPA. The method uses selective fragment monitoring for each analyte and the internal standard, without prior analytical separation. Mobile phase used consisted of aqueous ammonium formate and methanol in isocratic elution and the sample cleanup was made using solid phase extraction (SPE). The method was validated with regards to selectivity, sensitivity, accuracy and precision in accordance with applicable guidelines. After validation the LC-MS/MS method was successfully used to determine ethephon, glyphosate and AMPA residues in ground and surface water, as well as vegetable samples.

Keywords: glyphosate, AMPA, ethephon, LC-MS, QTOF, biomonitoring, pesticide.

1. Introduction

Glyphosate (N-(phosphonomethyl)glycine) (**Fig. 1**) was first synthetized to be used as a chelating agent and chemical intermediate for other chemical compounds (US Patent Office, 1964; Nandula, 2010), and due to its chemical properties it was studied as a possible water softening agent (Nandula, 2010).

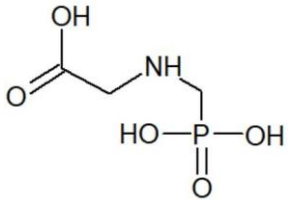

Fig. 1. Chemical structure of glyphosate

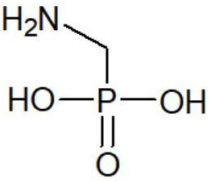

Fig. 2. Chemical structure of aminomethylphosphonic acid (AMPA)

While studying its water softening properties it was discovered that glyphoste along with other, similarly structured chemical agents, have a potential use in herbicidal products and it started being the focus in the development of a commercial herbicide instead (Nandula, 2010).

Glyphosate is an effective, broad-spectrum weedkiller and works by inhibiting an enzyme with a pivotal role in the biosynthesis of certain amino acids which play a role in the structure of plant tissue, and which are not present in animals and humans. The loss of the biological activity of glyphosate occurs when it is metabolized to aminomethylphosphonic acid (AMPA) (**Fig. 2**), which can be used as a marker of glyphosate use in biomonitoring (Schuette, 1998). In order to increase sales and profits, Monsanto also created resistant, genetically modified plants able to withstand the use of glyphosate, thus providing farmers with the opportunity to destroy weeds without affecting the crop, despite glyphosate being absorbed into the crops (Pollegioni et al., 2011).

Glyphosate is a highly water soluble, polar molecule, highly stable in solutions of various pH values and not photosensitive, making it very advantageous for farmers as a herbicide, but at the same time increasing the likeliness of its presence in crops where glyphosate is used, even after longer periods of time. Glyphosate is usually metabolized by certain types of bacteria and other microorganisms, as such its decomposition is highly dependent on the microbiota of the soil on which it is applied, the water source which it gets dissolved into or other factors (Schuette, 1998).

Although glyphosate has been shown to be present in food, groundwater and drinking water, amid a highly controversial move, the European Union extended the license for glyphosate containing products which expired in 2017 until 2022 (Myers et al, 2016; Rendón-von Osten and Dzul-Caamal, 2017; Askew, 2017). Interestingly, the limit for human exposure in the EU was increased in 2015 from 0.3 mg/kg bw/day to 0.5 mg/kg bw/day (Myers et al, 2016; Székács and Darvas, 2018), while indeed this limit is still more than three times lower compared to the USA (Myers et al, 2016).

Ethephon, 2-chloroethylphosphonic acid (**Fig. 3**), is a plant growth regulator used in to artificially accelerate ripening of fruit and vegetables.

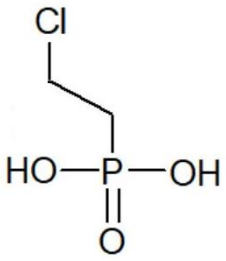

Fig. 3. Chemical structure of ethephon

It has become the most widely used ripening agent due to its positive effect on fruit coloration and ripening. It is also used in some parts of the world as an insecticide. After absorption into the plants it is metabolized into ethylene, which is a natural growth hormone for many plant species (Bhadoria, 2018).

Although ethylene is generally considered to have low toxicity in humans, the EU has currently set an acceptable limit for human exposure of 0.03 mg/kg bw/day (EFSA, 2008). In the USA however the Environmental Protection Agency (EPA) considers that there is no chronic dietary risk that might be of concern (EPA, 1995).

Due to their chemical properties and widespread use debate regarding the effects on human health of glyphosate, AMPA, ethephon and other similar substances used in agriculture have been ongoing for many years. Study results have been divisive and sometimes ambiguous, and the divisiveness has also affected the stance of regulatory agencies around the world, some regulators considering glyphosate to be safe for human use (EFSA, 2015; EPA 2020), while other organizations have labelled it as "probably carcinogenic" to humans (IARC, 2015). Due to the risk of glyphosate and its metabolite reaching the edible parts of crops and thus being consumed by the population regulatory authorities in most regions of the world limited acceptable human intake. At the same time studies on its cytotoxicity, involvement in genetic damage, tumor-cell growth and other negative health effects are being carried out (George, 2010; Suárez-Larios, 2017). There have been studies showing that the use of ethephon can also have negative health effects on humans, ranging from loss of appetite, diarrhea, stomach aches, but in some cases can also result in dermatotoxicity and hepatotoxicity (Bhadoria, 2018; EFSA, 2008).

Among the controversies it has become growingly important to have adequate methodologies to be able to measure human exposure to these compounds. The development of such methods poses a number of challenges however with regards to analytical performance and sample preparation depending on the sample matrices studied (soil, ground water, food samples, drinking water, blood/plasma, urine, tissues etc.).

The aim of the present study is to propose a fast, simple and selective LC-MS/MS method for the determination of ethephon, glyphosate and AMPA in ground and surface water, as well as vegetable samples, for quick screening purposes.

2. Materials and methods

Reagents and solutions

Glyphosate, AMPA and ethephon were acquired from LGC (Augsburg, Germany), glyphosate-2-^{13}C,^{15}N was acquired from MilliporeSigma (Burlington, USA). HPLC grade methanol was acquired from Honeywell (Seetze, Germany), ammonium formate was acquired from VWR International (Radnor,

USA). Ultrapure water was sourced from a Millipore Direct-Q 3 (Milford, USA).

Equipment

An LC-MS system composed of a Perkin Elmer (Waltham, USA) FX-10 UHPLC coupled with an AB Sciex (Framingham, USA) quadrupole-time-of-flight (QTOF) 4600 mass spectrometer was used. Other equipments: Eppendorf (Hamburg, Germany) 5430R centrifuge; Radwag (Radom, Poland) XA 523Y analytical scale; Velp Scientifica (Usmate Velate, Italy) vortex mixer; JP Selecta (Barcelona, Spain) Ultrasons H-D ultrasonic bath; Eppendorf Research Plus (Hamburg, Germany) automatic pipettes. Solid phase extraction was performed using Macherey-Nagel Chromabond HLB 1ml/100mg (30µm) cartridges (Düren, Germany).

Sample collection

A total number of 8 food samples (tomatoes and bell peppers) and 10 water samples were collected during a two week period in April-May 2021 in order to be analyzed for residual glyphosate, AMPA and ethephon content.

A number of four samples of tomatoes (*Solanum lycopersicum*) of different varieties were acquired: two were bought from supermarkets, having originated from Spain and Italy respectively according to the labels; one was bought from a local farmer's market in Targu Mures (Romania), being labeled as locally grown; one was collected in a local vegetable garden in Bistrita-Nasaud county (Romania). Out of the four pepper (*Capsicum annuum*) samples, two were bought from supermarkets, with origins being labeled as Italy and Romania; one was bought from a local farmer's market in Targu Mures, being labeled as locally grown; one was collected in a local vegetable garden in Bistrita-Nasaud county. All samples were stored at 5 ^{o}C until analyzed.

For the water sample analysis, a total ten samples were collected, with one being local Targu Mures tap water as reference, another being water from a local creek in Targu Mures collected near the evacuation point of a nearby farmer's market. The other eight samples were collected near a local farm and vegetable garden in Bistrita-Nasaud county, with four collection points: well upstream from the vegetable garden; creek collecting rainwater immediately adjacent to the vegetable garden and bordering it; well downstream from the vegetable garden; collecting creek at the limits of the property approximately 500 m distance from the vegetable garden. For each of these four collection points a sample was taken within 48 hours of using glyphosate-based products and after one week from using the herbicide. After collection, all samples were stored at -20 ^{o}C until analyzed.

LC-MS analysis

Stock solutions of glyphosate, AMPA and ethephon were prepared with concentrations of 10 µg/ml with aqueous 20 mM ammonium formate as solvent. These stock solutions were used to prepare a mixed standard solution with a concentration of 1µg/ml of each analyte, also in aqueous 20 mM ammonium formate, further used to prepare the working calibration solutions in the same solvent with concentrations ranging between 0.5-100 ng/ml of each analyte, with a total of 8 calibration levels. Using the mixed standard solution quality control working solutions of three different concentration levels were prepared for validation purposes with concentrations of 1.5 ng/ml (QCA), 40 ng/ml (QCB) and 80 ng/ml (QCC), respectively. A solution of isotopically labeled glyphosate-2-^{13}C,^{15}N was prepared to be used as internal standard (ISTD) for all solutions, with a concentration of 6.5 µg/ml

using aqueous 20 mM ammonium formate as solvent.

Calibration standards for the calibration curves were prepared by mixing 990 µl of the appropriate working solution with 10 µl of internal standard solution and performing solid phase extraction similarly to the sample solutions. Blank ISTD solutions containing only internal standard were also prepared by mixing 990 µl of mobile phase with 10 µl of internal standard solution. The concentration of internal standard in the final solutions was 65 ng/ml.

For the water samples cleanup was performed by mixing 990 µl of sample with 10 µl of internal standard solution and performing solid phase extraction using HLB cartridges. The cartridges were conditioned with methanol followed by a conditioning step with a mixture of 1:1 methanol:1% aqueous formic acid (v/v), after which the sample was loaded onto the cartridge and the eluent collected and filtered through 0.45 µm polypropylene syringe filters. Tomato and bell pepper food samples were each mashed up, and 100 mg of the mashed and homogenized samples were extracted in 990 µl of aqueous 20 mM ammonium formate mixed with 10 µl of internal standard through ultrasonication for 15 minutes, centrifuged at 10000 rpm for 3 minute and the supernatant extracted using the same SPE method as for water samples.

Detection of analytes was carried out in multiple reaction monitoring (MRM) mode for all analytes and the internal standard, after negative electrospray ionization. The ionization parameters were used for the ionization source were: spray voltage: -4500V, vaporizer temperature: 550 ^{o}C, ion gas source 1: 30, ion gas source 2: 30, curtain gas: 10, declustering potential: -10. The following fragment ions were monitored for each compound: fragments m/z 150.05, 123.04, 79.05 and 63.05 from parent m/z 167.98 at a collision energy (CE) of -14 V for glyphosate; 79.05 and 63.05 from parent m/z 109.95 at a collision energy (CE) of -20 V for AMPA; fragments m/z 107.02, 99.05 and 79.04 from parent m/z 142.90 at a collision energy (CE) of -9 V for ethephon; and fragment ions m/z 152.05, 125.05, 81.05 and 65.05 from m/z 169.95 at CE -14 V for the detection of the internal standard. An injection volume of 10 µL of each solution was injected into the LC-MS system with a total sample run-time of 3 minutes per solution. No chromatographic separation was used and the mobile phase was composed of methanol and aqueous 20 mM ammonium formate solution with a ratio of 40:60 (v/v) in isocratic conditions, at a constant flow rate of 0.1 mL/min.

3. Results and discussions

Method validation

The method was validated with regards to sensitivity, selectivity, linearity, accuracy, precision, carryover and post preparative stability in accordance with current regulatory guidelines (EMA, 2012). A bioanalytical method can be considered selective and sensitive enough for the application it was designed for if any interfering peaks in blank samples have peak areas lower than 20% of the peak areas at the lower limit of quantification (LLOQ) for the analytes and lower than 5% of of the peak area of the internal standard. To prove the selectivity of the method blank samples containing only mobile phase were analyzed. For ethephon, although initially the LLOQ was set at the same concentration as for glyphosate and AMPA (0.5 ng/ml), due to issues obtaining acceptable selectivity, the first calibration standard was eliminated from each analytical run and the final LLOQ for ethephon was set at 1ng/ml.

Table 1. Selectivity and sensitivity for glyphosate, AMPA, ethephon and internal standard

Analyte	**Mean Blank Area**	**LLOQ Area**	**Selectivity (%)**
Glyphosate	17.287	460.856	3.75
AMPA	22.222	266.448	8.34
Ethephon	0.000	10955.702	0.00
Glyphosate-2-^{13}C,^{15}N	338.675	12539.783	2.70

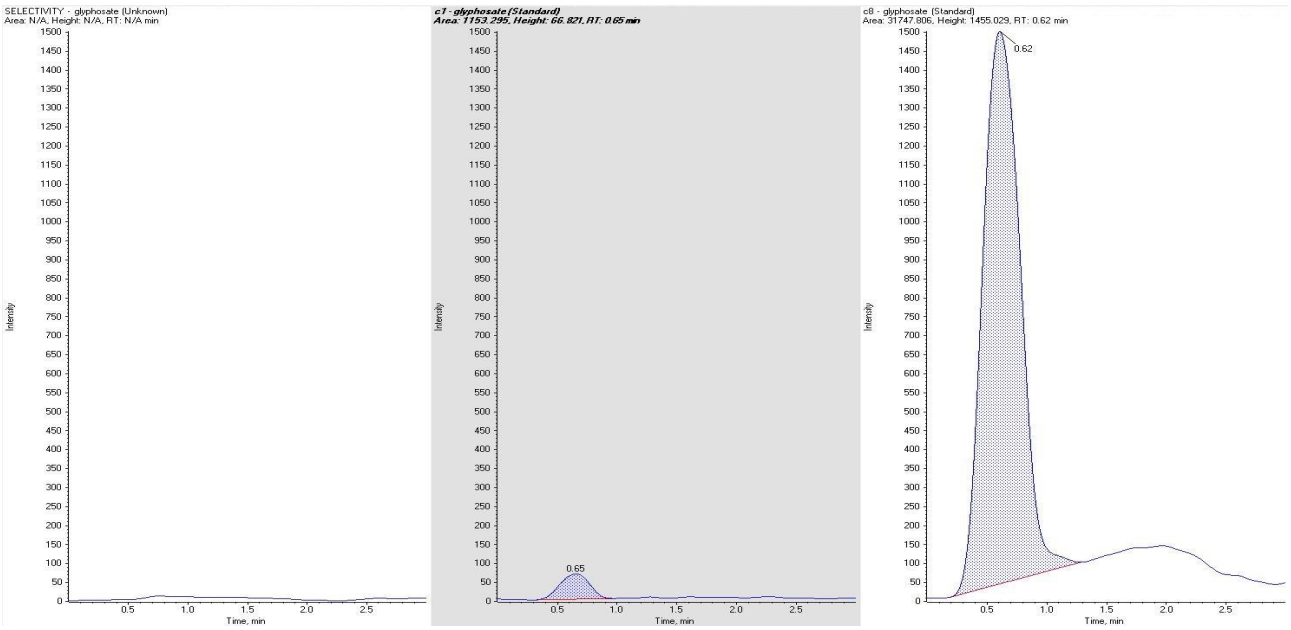

Fig. 4. Extracted chromatograms for glyphosate - blank solution, LLOQ, ULOQ solution

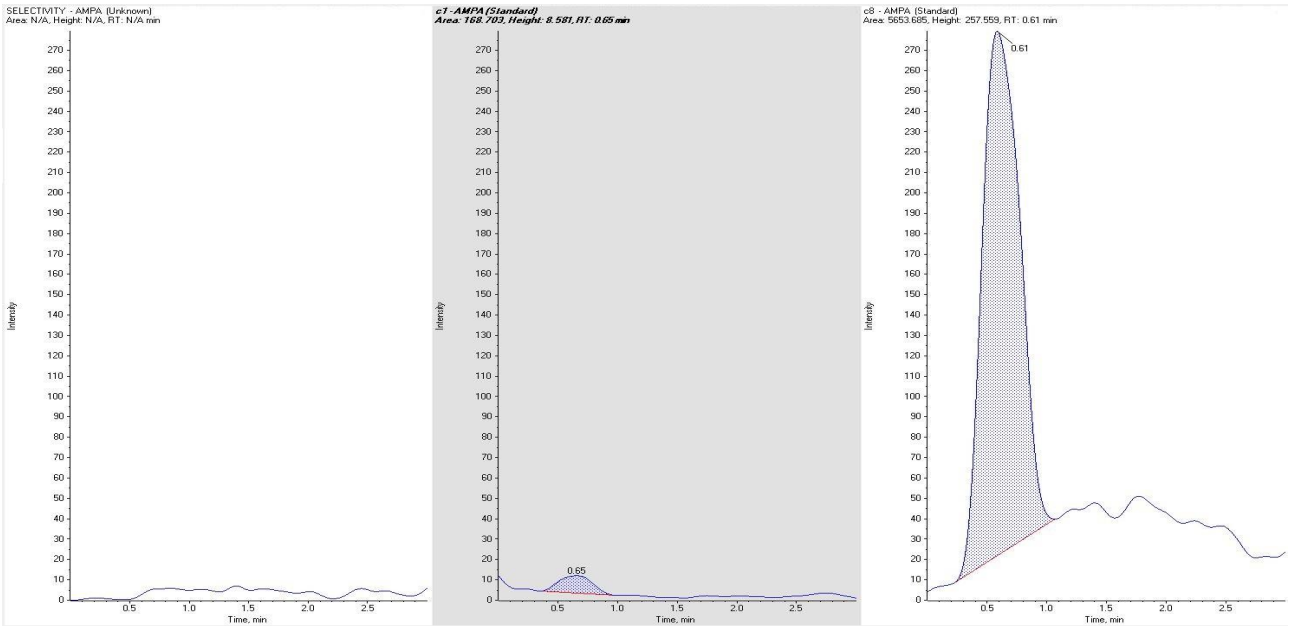

Fig. 5. Extracted chromatograms for AMPA - blank solution, LLOQ, ULOQ solution

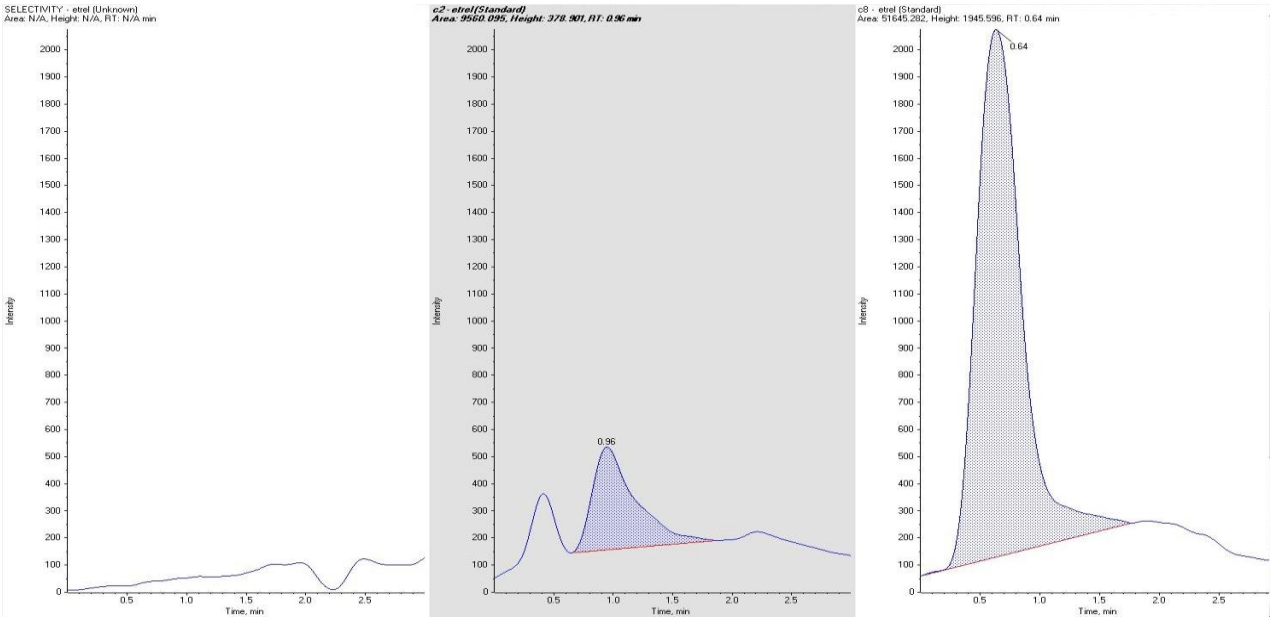

Fig. 6. Extracted chromatograms for ethephon - blank solution, LLOQ, ULOQ solution

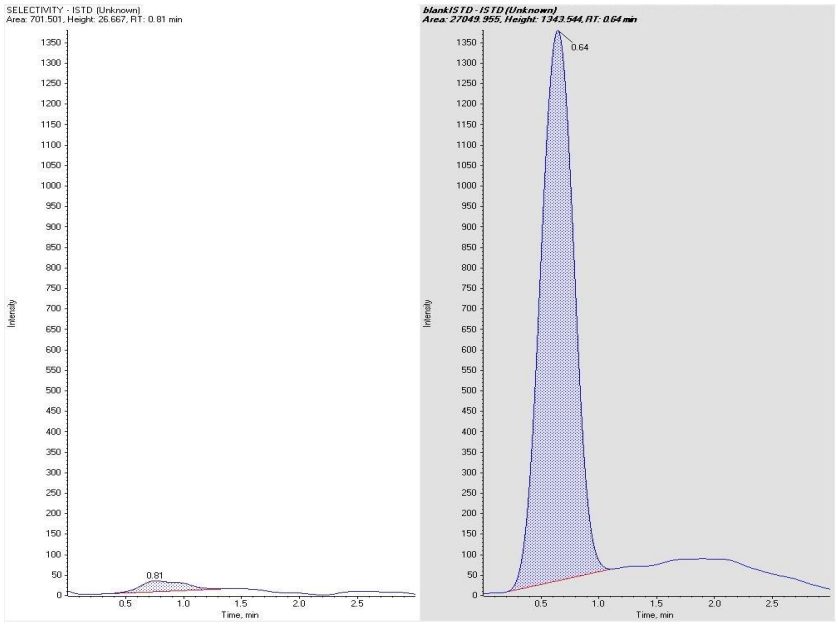

Fig. 7. Extracted chromatograms for internal standard - blank solution, blankISTD solution

No interfering peaks with area greater than 20% of analyte peak areas at the limit of quantification (LLOQ) were detected (**Fig. 4-7**) for any of the analytes. Results for selectivity testing are presented in **table 1**. A freshly prepared calibration curve was injected into the LC-MS system for each analytical run, both during validation and sample analysis, in order to assess linearity of the method. For each run of the analytical method during method validation the calibration curves were linear for all analytes, with no more than 2 of the 8 calibration standards needing to be excluded due to low accuracy residuals (**Fig. 8**). The coefficient of correlation (R) was higher than 0.99 for all calibration curves during both validation and sample analysis, and the values for coefficients of correlation and residuals are presented in **tables 2-4**. Accuracy and precision of the method were verified at three different

concentration levels (QCA, QCB and QCC), both within a single analytical run and between different analytical runs, by analyzing five different quality control samples at each concentration level. The results for accuracy, calculated as percentage bias from the theoretical value, and precision, calculated as coefficient of variation, were within acceptance limits of ±15% and are presented in **tables 5-7**. The carryover of the analytical method when injecting a blank sample after a high concentration standard solution (ULOQ) was below the threshold provided by validation guidelines, with interfering peaks areas in the blank solutions injected for carryover testing being lower than 20% of the area of analytes at LLOQ and lower than 5% of the area of the internal standard. Thus it was concluded that no significant carryover is present when using the analytical method. Results for carryover testing are presented in **table 8.**

Table 2. Coefficient of correlation for validation calibration curves and residuals - glyphosate

	Accuracy %				
Nominal concentration ng/ml	**Analytical run 1**	**Analytical run 2**	**Analytical run 3**	**Analytical run 4**	**Analytical run 5**
0.500	-17.2	8.3	-19.2	-12.2	4.3
1.000	9.2	-9.4	*70.4**	0.5	*-77.6**
5.000	7.4	5.9	5.1	13.6	-10.4
10.000	0.2	*-25.2**	6.5	7.3	12.5
25.000	-4.7	3.0	14.5	0.1	-0.7
50.000	2.5	7.4	11.3	3.7	3.4
75.000	-2.3	-12.0	-8.1	-5.1	5.0
100.000	0.8	4.6	-11.5	-7.8	-6.3
Coefficient of correlation					
R	0.9991	0.9964	0.9940	0.9974	0.9972

** excluded due to inaccuracy >15% (20% for LLOQ)*

Table 3. Coefficient of correlation for validation calibration curves and residuals - AMPA

	Accuracy %				
Nominal concentration ng/ml	**Analytical run 1**	**Analytical run 2**	**Analytical run 3**	**Analytical run 4**	**Analytical run 5**
0.500	-11.3	-3.0	-9.7	4.0	9.9
1.000	*97.5**	*-56.4**	*115.5**	-6.2	-11.6
5.000	13.5	4.4	6.2	-7.8	-3.0
10.000	14.4	-3.8	10.8	13.5	12.1
25.000	12.5	3.9	11.6	8.6	11.2
50.000	9.2	8.6	6.6	10.4	12.5
75.000	-11.3	*-33.6**	-9.8	-12.6	-3.6
100.000	-13.8	-8.1	-10.2	-1.4	-13.8
Coefficient of correlation					
R	0.0992	0.9977	0.9950	0.9946	0.9936

** excluded due to inaccuracy >15% (20% for LLOQ)*

Table 4. Coefficient of correlation for validation calibration curves and residuals - ethephon

	Accuracy %				
Nominal concentration ng/ml	**Analytical run 1**	**Analytical run 2**	**Analytical run 3**	**Analytical run 4**	**Analytical run 5**
0.500	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
1.000	5.8	-7.0	-5.6	-18.3	7.1
5.000	*-98.2**	5.0	-10.0	-8.9	-4.8
10.000	4.9	*-15.6**	*-36.8**	1.1	3.4
25.000	-3.0	-2.7	12.1	2.7	*23.0**
50.000	-11.4	8.7	-3.3	7.0	-5.3
75.000	4.2	-12.7	-2.4	-3.6	8.5
100.000	9.8	10.6	0.9	-1.3	-2.6
Coefficient of correlation					
R	0.9957	0.9929	0.9985	0.9989	0.9981

** excluded due to inaccuracy >15% (20% for LLOQ)*

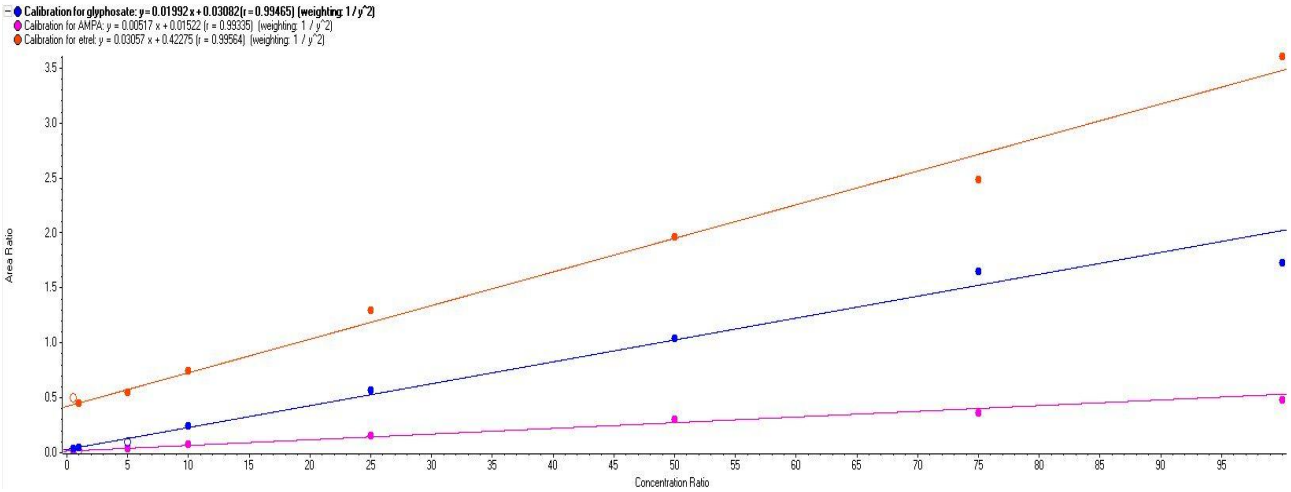

Fig. 8. Calibration curves for glyphosate (blue), AMPA (magenta) and ethephon (orange)

Table 5. Accuracy and precision of the analytical method - glyphosate

Within run accuracy and precision				**Between run accuracy and precision**			
Nominal conc. ng/ml	**Mean measured conc. ng/ml**	**Precision %**	**Accuracy %**	**Nominal conc. ng/ml**	**Mean measured conc. ng/ml**	**Precision %**	**Accuracy %**
1.500	1.460	12.5	-2.6	1.500	1.628	6.9	8.5
40.000	40.380	1.6	0.9	40.000	42.023	9.6	5.1
80.000	79.342	1.3	-0.8	80.000	84.522	7.3	5.7

The stability of analyte containing solutions, tested and proven by reinjecting quality control samples prepared on a previous day after being kept in the autosampler at room temperature, allows for preparation of standard calibration solutions and samples at any time and injecting them into the LC-MS system within 24 hours after being prepared. The results of the autosampler stability testing were within the ±15% acceptance limits and are presented in **table 9**.

Table 6. Accuracy and precision of the analytical method - AMPA

Within run accuracy and precision				Between run accuracy and precision			
Nominal conc. ng/ml	**Mean measured conc. ng/ml**	**Precision %**	**Accuracy %**	**Nominal conc. ng/ml**	**Mean measured conc. ng/ml**	**Precision %**	**Accuracy %**
1.500	1.698	4.1	13.2	1.500	1.644	5.5	9.6
40.000	44.890	4.9	12.2	40.000	43.356	11.7	8.4
80.000	73.936	4.8	-7.6	80.000	77.046	4.0	-3.7

Table 7. Accuracy and precision of the analytical method - ethephon

Within run accuracy and precision				Between run accuracy and precision			
Nominal conc. ng/ml	**Mean measured conc. ng/ml**	**Precision %**	**Accuracy %**	**Nominal conc. ng/ml**	**Mean measured conc. ng/ml**	**Precision %**	**Accuracy %**
1.500	1.549	8.5	3.2	1.500	1.658	4.2	10.5
40.000	43.692	13.7	9.2	40.000	36.428	8.7	-8.9
80.000	86.530	7.1	8.2	80.000	89.561	2.0	12.0

Table 8. Carryover for glyphosate, AMPA, ethephon and internal standard

Analyte	**Mean Blank Area**	**Mean LLOQ Area**	**Carryover (%)**
Glyphosate	0	639.907	0.00
AMPA	8.042	173.054	4.65
Ethephon	0	8535.563	0.00
Glyphosate-2-^{13}C,^{15}N	184.653	16216.661	1.14

Table 9. Autosampler stability for glyphosate, AMPA and ethephon

Analyte	**QC sample conc. ng/ml**	**Mean calculated conc. ng/ml**	**Accuracy (%)**
Glyphosate	1.5 (QCA)	1.56	3.7
	40 (QCB)	44.46	11.1
	80 (QCC)	86.52	8.2
AMPA	1.5 (QCA)	1.57	4.7
	40 (QCB)	45.47	13.7
	80 (QCC)	82.05	2.6
Ethephon	1.5 (QCA)	1.42	-5.1
	40 (QCB)	42.74	6.8
	80 (QCC)	83.46	4.3

Sample analysis

All food and water samples collected were analyzed for glyphosate, AMPA and ethephon content. Results of the analysis showed that some of the samples analyzed had quantifiable amounts of glyphosate and AMPA residue, but at the same time ethephon was not identified in any of the samples. The values measured in each of the samples for the studied analytes are presented in **tables 10-11**.

As a proof-of-concept study, the current research fulfills its purpose as the LC-MS method developed can successfully be used to analyze water and food samples for residues after glyphosate use. Glyphosate and its metabolite, AMPA, were detected, although at low concentrations, in some commercially acquired food samples, but also in food samples cultivated with previous use of glyphosate-based herbicides. As expected, in the immediate vicinity of crops where glyphosate is sprayed to herbicide residues of the compound and its metabolite can be found in ground and surface waters, even a few days after use, due to the slow decomposition in the environment. Ethephon was not detected in any of the samples, which in the case of food samples might be either due to the substance not being used, or due to the quick metabolization once absorbed into plants. In the case of the collected water samples, as no ethephon is known to have been used in the area of collection, it was not expected to detect the substance, however the research offers a method which can be used to detect ethephon in ground or surface waters, in cases when the substance is used, as stability in these types of samples should be higher and detection possible after a longer period of time.

Table 10. Results for food samples analyzed for glyphosate, AMPA and ethephon residues

Sample	Glyphosate ng/mg	AMPA ng/mg	Ethephon ng/mg
Food sample 1	-	-	-
Food sample 2	56.4	54.7	-
Food sample 3	11.0	-	-
Food sample 4	148.1	130.1	-
Food sample 5	31.5	37.3	-
Food sample 6	15.4	10.8	-
Food sample 7	-	-	-
Food sample 8	-	-	-

Table 11. Results for water samples analyzed for glyphosate, AMPA and ethephon residues

Sample	Glyphosate ng/ml	AMPA ng/ml	Ethephon ng/ml
Water sample 1	-	-	-
Water sample 2	5.69	13.63	-
Water sample 3	21.77	26.89	-
Water sample 4	11.89	8.92	-
Water sample 5	-	-	-
Water sample 6	1.44	-	-
Water sample 7	-	-	-
Water sample 8	17.38	18.48	-
Water sample 9	1.08	-	-
Water sample 10	-	-	-

As finding truly blank matrices can be difficult in certain situations, due to the widespread and rarely reported use of glyphosate and ethephon, it was not possible to test for matrix effect during the study, however the matrix effect can be determined on a case-by-case basis using the matrix of a sample and spiking it with standard solution, analyzing the spiked sample together with the unspiked matrix and standard solution. This enables a more accurate quantitation of the analytes, in case it is deemed necessary, and sufficient amounts of the appropriate matrix are available.

While many analytical methodologies described in literature use derivatization in order to achieve quantification (Trass, 2014), similar LC-MS methods for the underivatized determination of glyphosate and its metabolite, as well as ethephon, have been described in literature, in some cases being showcased by equipment and reagent vendors and manufacturers (Shimelis, 2019; Schreiber and Jin, 2016; Ulrich and Ferguson, 2021). Compared to existing methods, the lack of analytical separation for the method developed during this study, although also having some drawbacks, has the advantage of a very short runtime of only 3 minutes per sample analyzed. This enables the quick screening of a large number of samples in a very short timeframe, while using a quick and simple solid phase extraction which is used by most other methods also. The method developed thus has similar performances, in some cases even higher sensitivity, compared to other similar existing methods, while enabling a slightly faster analysis.

Conclusions

The method developed can be used for screening purposes in the analysis of food and water samples for glyphosate and ethephon use. Although the method uses no chromatographic separation, an aspect which could be improved in order to avoid interferences in certain cases, the highly sensitive and selective QTOF mass spectrometric detection offers the performance needed for such an application.

While after use the detection of ethephon might be more difficult due to the rather quick metabolization to ethylene after absorption into plants, it could still be useful to perform screening in water samples and even food samples, since the method enables the detection of very low residual concentrations. At the same time, the use of glyphosate in certain crops leaves behind a detectable trace of either glyphosate or its main metabolite, or both compounds, which can ultimately easily be detected and quantified.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Askew K (2017) Glyphosate gets five-year respite as license renewed. In: FoodNavigator. https://www.foodnavigator.com/Article/2017/11/28/Glyphosate-gets-five-year-respite-as-license-renewed. Accessed 19 May 2022
2. Bhadoria P, Nagar M, Bharihoke V, Bhadoria AS (2018) Ethephon, an

organophosphorous, a Fruit and Vegetable Ripener: Has potential hepatotoxic effects? Family Med Prim Care 7:179-183.

3. European Food Safety Authority (2008) Conclusion regarding the peer review of the pesticide risk assessment of the active substance ethephon. https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2006.174r. Accessed 19 May 2022
4. European Food Safety Authority (2015) Glyphosate: EFSA updates toxicological profile. https://www.efsa.europa.eu/en/press/news/151112. Accessed 19 May 2022
5. European Medicines Agency (2012) Guideline on bioanalytical method validation. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf. Accessed 19 May 2022
6. George J, Prasad S, Mahmood Z, Shukla Y (2010) Studies on glyphosate-induced carcinogenicity in mouse skin: A proteomic approach. J Proteomics 73:951-964. doi:10.10161j.jpro1.2009.12.008
7. International Agency for Research on Cancer (2015) IARC Monographs Volume 112: evaluation of five organophosphate insecticides and herbicides. https://www.iarc.fr/wp-content/uploads/2018/07/MonographVolume112-1.pdf. Accessed 19 May 2022
8. Myers JP, Antoniou MN, Blumberg B, Carroll L, Colborn T, Everett LG, Hansen M, Landrigan PJ, Lanphear BP, Mesnage R, Vandenberg LN, vom Saal FS, Welshons WV, Benbroo CM (2016) Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement. Env Health 15:19. doi: 10.1186/s12940-016-0117-0
9. Nandula VK (2010) Glyphosate: Discovery, Development, Applications, And Properties. In: Glyphosate Resistance in Crops and Weeds: History, Development, and Management. Wiley, Hoboken, NJ, pp 1–34
10. Pollegioni L, Schonbrunn E, Siehl D (2011) Molecular basis of glyphosate resistance: Different approaches through protein engineering. FEBS J 278(16): 2753–2766. doi:10.1111/j.1742-4658.2011.08214.x.
11. Rendón-von Osten J, Dzul-Caamal R (2017) Glyphosate Residues in Groundwater, Drinking Water and Urine of Subsistence Farmers from Intensive Agriculture Localities: A Survey in Hopelchén, Campeche, Mexico. Int. J Environ Res Public Health 14:595. doi:10.3390/ijerph1406059
12. Schreiber A, Jin W (2016) LC-MS/MS Quantitation of Underivatized Glyphosate and Other Polar Pesticides. https://sciex.com/content/dam/SCIEX/pdf/tech-notes/all/glyphosate_analysis.pdf. Accessed 19 May 2022
13. Schuette J (1998) Environmental Fate Of Glyphosate. In: Environmental Monitoring & Pest Management. Department of Pesticide Regulation, Sacramento, CA, pp 1-13
14. Shimelis O (2019) LC-MS/MS Method for Determination of Glyphosate, AMPA, and Glufosinate in Cereals. In: Chromatography Today https://www.chromatographytoday.com/article/lc-ms/48/milliporesigma/lc-msms-method-for-determination-of-glyphosate-ampa-and-glufosinate-in-cereals/2556/download. Accessed 19 May 2022
15. Suárez-Larios K, Salazar-Martínez AM, Montero-Montoya R (2017) Screening of Pesticides with the Potential of Inducing

DSB and Successive Recombinational Repair. J Toxicol 2017:3574840. doi:10.1155/2017/3574840
16. Székács A, Darvas B (2018) Re-registration Challenges of Glyphosate in the European Union. Front Environ Sci 6:78. doi: 10.3389/fenvs.2018.00078
17. Trass M, Misa A, Turner J (2014) Glyphosate Analysis by LC/MS/MS using FMOC Derivitization or Underivitized Method. In: Phenomenex Applications. https://phenomenex.blob.core.windows.net/documents/f3c8f206-d287-4607-a459-3b48e13fd589.pdf. Accessed 19 May 2022
18. Ulrich JC, Ferguson PL (2021) Development of a sensitive direct injection LC-MS/MS method for the detection of glyphosate and aminomethylphosphonic acid (AMPA) in hard waters. Anal Bioanal Chem 413(14): 3763–3774. doi:10.1007/s00216-021-03324-5
19. United States Environmental Protection Agency (1995) Reregistration Eligibility Decision - Ethephon. https://www3.epa.gov/pesticides/chem_search/reg_actions/reregistration/fs_PC-099801_1-Apr-95.pdf. Accessed 19 May 2022
20. United States Environmental Protection Agency (2020) Ingredients Used in Pesticide Products. https://www.epa.gov/ingredients-used-pesticide-products/glyphosate. Accessed 19 May 2022
21. United States Patent Office (1964) Patent No. 3,160,632.