

Simultaneous Determination of Glyphosate, Glufosinate, and Aminomethyl phosphonic acid (AMPA) in different matrices by LC-ESI-MS/MS.

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INTRODUCTION

Glyphosate [*N*-(phosphonomethyl)glycine] and glufosinate [ammonium DL-homoalanin-4-(methyl) phosphinate] are broad spectrum, nonselective, post-emergence pesticides extensively used in various applications for weed control. AMPA [aminomethylphosphonic acid] is the mayor degradation product of glyphosate found in plants, water and soils. The determination of these herbicides at level less than $\mu\text{g/L}$ is difficult due to their ionic character, low volatility, low mass and lack of chemical groups that could facilitate their detection^[1]. For these reasons derivatization procedures have been applied.

OBJETIVE

The aim of this work was developed a rapid and robust method for the determination of low concentrations of glyphosate, its principal degradation product (AMPA), and glufosinate in different matrices as cereals and oily plants, soya's beverages and treated and natural waters by LC-ESI-MS/MS, that fulfill the requirements according to international regulations. In order to avoid reporting false positives we worked monitoring two MS/MS transition for satisfactory confirmation of the compound identity. We have used an internal standard to improve the precision and accuracy, in order to minimize the matrix effects.

EXPERIMENTAL

MS System: Micromass Quattro Ultima Pt triple quadrupole mass spectrometer, equipped with an electrospray interface operating in negative mode, using the multiple reaction monitoring (MRM).

HPLC: Waters Alliance 2695 HPLC system.
 Column: C18, 250 x 2.1 mm Hypersil Gold.
 Particle (μm): 5
 Size poro (Å): 175
 Oven temp: 35 °C.
 Solvent A: MeOH.
 Solvent B: Ammonium acetate 5 mM (pH 9)
 Flow: 0.2 mL/min.
 Vol. Iny. (μL): 30

Table 1: Gradient system

Time (min)	A	B	Flow	Curve
0.00	10.0	90.0	0.2	1
3.00	25.0	75.0	0.2	6
6.00	25.0	75.0	0.2	6
13.00	90.0	10.0	0.2	6
14.00	90.0	10.0	0.2	6
15.00	10.0	90.0	0.2	11
22.00	10.0	90.0	0.2	11

ANALYTICAL METHOD

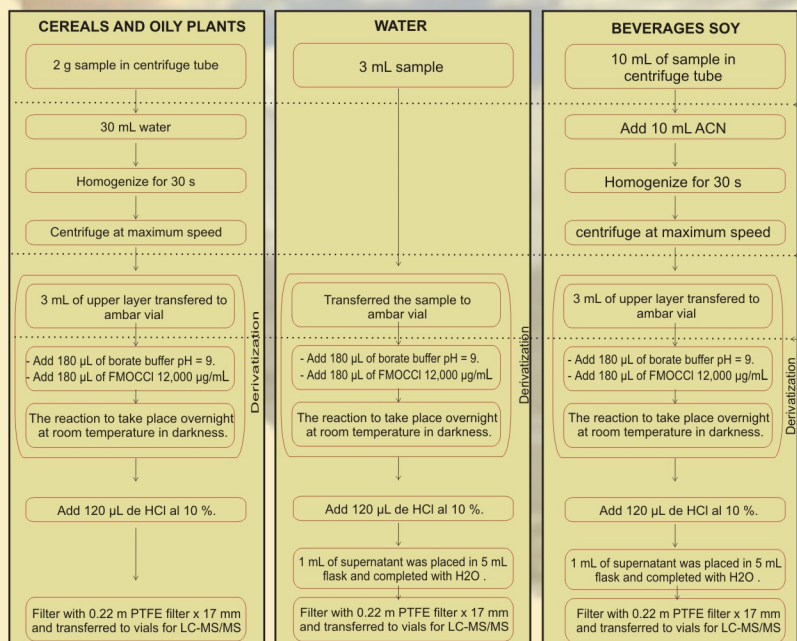


Fig. 1. The full analytical method is shown.

RESULTS

Table 2: Retention times, relative retention times, molecular and fragment ions for FMOCC-derivatized compounds of glyphosate, AMPA, glufosinate and internal standards with their collision energy

Compound	Retention time (minutes)	Relative retention time (minutes)	Molecular ion (m/z)	Product ion (m/z)	Collision energy (V)
Glyphosate-FMOCC	14.00	0.89	390.0	Q 167.8	10.00
				q 149.8	20.00
Glufosinate-FMOCC	15.81	1.01	402.0	Q 179.8	9.00
				q 205.9	14.00
Aminomethylphosphonic acid-FMOCC	16.59	1.06	332.0	Q 109.9	5.00
				q 135.9	13.00
Internal Standards					
Cysteic acid-FMOCC	15.60	--	390.0	193.8	14.00

[m/z: mass-to-charge ratio, FMOCC: 9-fluorenylmethylchloroformate; --: not applicable]

Q: Transition used for quantification; q: transition used for confirmation.

Table 3: Recoveries, RSD and LOD for different matrices

Compound	Cereals			Water			Soya's beverage		
	Recovery (%) ^a	RSD (%)	LOD (mg/kg)	Recovery (%) ^b	RSD (%)	LOD (mg/L)	Recovery (%) ^b	RSD (%)	LOD (mg/L)
Glyphosate	74.7	0.24	0.110	106.9	6.79	0.010	78.4	8.9	0.022
Glufosinate	91.5	6.72	0.145	110.2	2.29	0.005	80.2	8.1	0.028
AMPA	62	0.95	0.045	107	2.9	0.005	88.7	14	0.010

Three different samples, Spike at a: 0.230 mg/kg and b: 0.092 mg/L

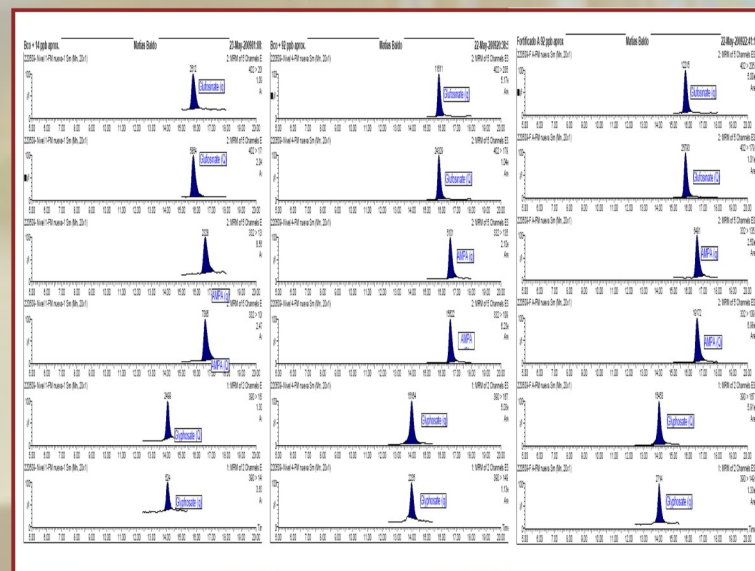


Fig. 2. LC-MS/MS chromatograms for (a) standard solution of glyphosate, glufosinate and AMPA at 15 $\mu\text{g/L}$, (b) standard solution at 90 $\mu\text{g/L}$, and (c) groundwater sample spiked at 90 $\mu\text{g/L}$. (Q) quantitative transition, (q) qualitative transition.

CONCLUSIONS

This method allows to determine the residues of Glyphosate, Glufosinate and AMPA in a single injection. It is applied to different vegetable products such as cereals, oilseeds, sub products and also environmental samples as water. The detection with LC-MS/MS, previous derivatization with FMOCC, is specific, reliable and rapid therefore it becomes a very good method for screening or routine analysis.

The LOD meet the international normative

The analytical curves are linear and the coefficient (r^2) result higher than 0.99 in all cases.

The recovery percentage (among 70 and 120 %) and the RSD (< 15%) are satisfactory for the 3 residues in the vegetables products tested.

To reduce the matrix effects the samples are diluted and quantified using internal standard obtaining good results.

To sum up, it is a reliable, easy and rapid multi residue method, doesn't require neither clean up nor any concentration method.

FUTURE PROJECTS

Currently JLA is working to develop a new and simple extraction to skip the derivatization.

Reference
 1-Ibañez M., Pozo O., Sancho J., López F. Hernández F. "Residue determination of glyphosate, glufosinate and aminomethylphosphonic acid in water and soil samples by liquid chromatography coupled to electrospray tandem mass spectrometry". Journal of Chromatography A, 1081 (2005) 145-155.

ACKNOWLEDGMENTS
 TO JLA GLOBAL AND ITS COMPANIES MEMBERS, PROVIDING THE TECHNOLOGICAL TOOLS TO PROVIDE QUALITY SERVICES, ACCORDING TO THE LATEST ANALYTICAL TECHNOLOGIES IN THE MARKET.