Monitoring of Glyphosate Residues in Environmental Groundwater Samples by ELISA and LC-MS/MS

Lina Kantiani[1], Josep A. Sanchis[1], Marta Llorca[1], Fernando Rubio[2], Marinella Farré[1,*], and Damià Barceló[1,3]
[1] Environmental Chemistry Department, IDAEA-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain
[2] Abraxis LLC, 54 Steamwhistle Drive, Warminster, PA 18974, USA
[3] Catalan Institute for Water Research ICRA, Parc Científic i Tecnològic de la Universitat de Girona, Pic de Peguera, 15, 17003, Girona, Spain
* mfuqam@cid.csic.es

1. INTRODUCTION

Glyphosate is a herbicide widely used in order to control various types of weeds, as well as in genetically-engineered crops like soy and maize. Although it is considered to be relatively safe compared to some organochlorine pesticides, various studies in the last decade have shown possible toxicological effects linked to its use (slightly toxic to invertebrate species, possibly toxic to amphibian species, increased neurologic developmental effects in exposed children, loss of fertility). Existing analytical methods for the detection of glyphosate in waters and other matrices are based on chromatographic techniques, usually coupled to mass spectrometric detection systems. Generally, pre-derivatization of the sample is required both in gas chromatography in order to convert the polar glyphosate to a less polar more volatile derivative and in liquid chromatography, making the analysis of this compound quite challenging.

The present work demonstrates a rapid and efficient combined methodology for the analysis of water samples for glyphosate residues, based on a previously validated commercially available ELISA kit (Abraxis), based on polyclonal glyphosate antibodies covalently bound to paramagnetic particles, and the competitive reaction between the present glyphosate and the horseradish peroxidise (HRP) enzyme labeled glyphosate analog (conjugate). The confirmatory method with fully automated pre-concentration via the Symbiosis Pico (Spark Holland, The Netherlands) on-line with LC-MS/MS permits the rapid analysis using small sample volume. This combined procedure has been applied to the monitoring study of 46 groundwater samples from Catalonia (NE of Spain), where groundwater constitutes 35% of the total resources used towards drinking water supplies to the industry and agriculture. The samples were collected during a sampling campaign carried out in 2010 and the results obtained showed good correlation between the two methods. Results indicated the presence of glyphosate in 57% of the analysed groundwater samples, with levels ranging from 0.08 to 0.94 µg/L (mean value of 0.353 µg/L), and a single occurrence at 2.57 µg/L.

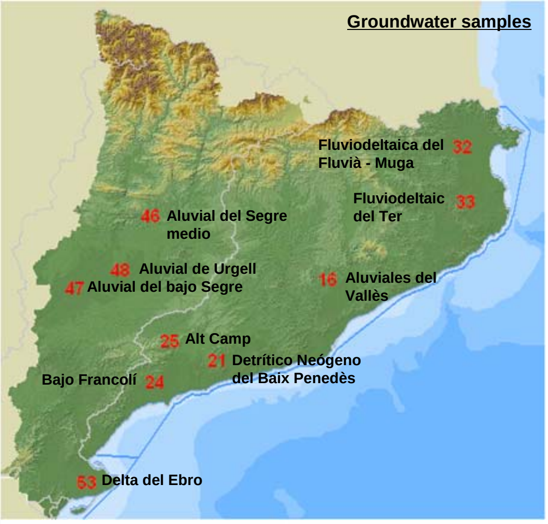


Figure 1: Geographic location of the groundwater masses sampled during the three sampling campaigns between 2007 and 2009. The numbers in bold red colour correspond to specific water body zones and are given by the ACA.

2. METHOD OF MEASUREMENT

Glyphosate ELISA Kit (Magnetic Particle format, 120T) PN 500081

- Specific antibody for Glyphosate. No cross-reaction with other non-related agricultural compounds.
- Sensitivity: 0.05 ppb
- Linearity range: 0.075 - 4.0 ppb
- Rapid sample extraction (filtration, derivatization)
- Total measurement time < 90 minutes
- Cross-reactivity:

Compound	LDD (ppb)	50% B/Bo (ppb)
Glyphosate	0.10	2.40
Glyphosine	50	3,000
Glufosinate	2,000	70,000
AMPA	35,000	>1,000,000
Glycine	>10,000	>1,000,000

The following compounds demonstrated no reactivity in the Glyphosate Assay when tested at concentrations up to 1,000 ppb: aldicarb, aldicarb sulfoxide, aldicarb sulfone, acetochlor, alachlor, atrazine, ametryn benomyl, butachlor, butylate, captan, carbaryl, carbendazim, carbofuran, 2,4-D, 1,3-dichloropropene, dinoseb, metribuzin, MCPA, metolachlor, PCP, picloram, propazine, simazine, terbufos, thiabendazole, thiophanate-methyl

3. CONFIRMATION METHOD

Derivatization procedure

- Sample volume: 10 ml
- Acidification at pH 1 with 6M HCl
- Shaking for 1 hour → Glyphosate decomplexation.
- Neutralization to pH 7 with 6M KOH
- Derivatization: 8 ml of sample + 1 ml of buffer* + 1 ml of FMOC**
- Reaction stops after 2 hrs reaching pH 3 (with ~10 µl of conc. FA)
- Addition of 0.5 ml of EDTA***

* 40 mM sodium tetraborate in HPLC water
** 6,5 mM fluorenylmethyloxycarbonyl chloride in ACN
*** 0,4 g/ml ethylenediaminetetraacetic acid, in HPLC water

Online extraction parameters

Cartridge: C18-EC
Injection volume: 2500 µl
Activation: 2 ml MeOH, 2 ml/min
Equilibration: 2 ml ACN (0,1% FA), 2 ml/min
Loading: 1 ml ACN (0.1% FA), 2 ml/min
Washing: 0.5 ml water (pH 7)

LC parameters

Column: Synergy 4µ Hydro-RP
(Phenomenex, 50 x 2,0 mm x 4 µm)
Mobile phase: 2.5 mM AcNH4 in water (A) and ACN (B)

Gradient program

t (min)	A (%)	B (%)
0	90	10
1	90	10
2	10	90
3	10	90
4	90	10
10	90	10

MS/MS parameters

Compound	SRMs	DP (V)	CE (V)	EP (a.u.)	CXP (a.u.)
Gly-FMOC (1)	390>168	-40	-15	12	8
Gly-FMOC (2)	390>150	-40	-18	12	8
Free Gly (1)	168>150	-40	-30	12	8
Free Gly (2)	168>24	-40	-20	12	8

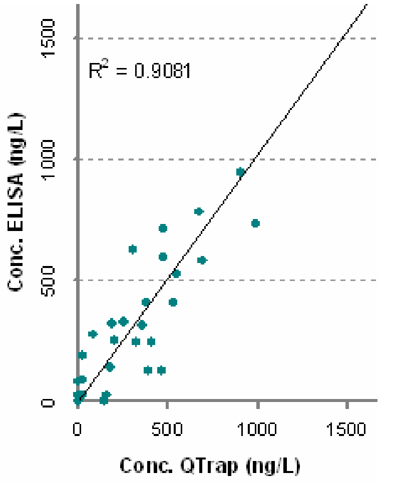
MS/MS/MS parameters

Transition: 390>168>150
DP: -40 V
CE: -18 V
AF2 (excitation RF amplitude): 35
CES (collision energy spread): 0

4. RESULTS

Sample	Water body	Location	GLY conc. (µg/L)
08088-0025	16	Al·luvials del Vallès	0,590
08106-0041	16	Al·luvials del Vallès	0,580
08086-0049	16	Al·luvials del Vallès	2,566
08181-0018	16	Al·luvials del Vallès	0,126
08135-0021	16	Al·luvials del Vallès	BDL
08108-0009	16	Al·luvials del Vallès	0,154
08086-0049	16	Al·luvials del Vallès	BDL
08219-0025	18	Maresme	BDL
08029-0031	18	Maresme	BDL
08029-0029	18	Maresme	0,322
08197-0048	18	Maresme	0,086
08197-0033	18	Maresme	0,186
08121-0117	18	Maresme	0,524
08009-0060	18	Maresme	0,313
08065-0003	21	Detritic neògen del Baix Penedès	ND
43140-0056	21	Detritic neògen del Baix Penedès	ND
43020-0074	21	Detritic neògen del Baix Penedès	0,624
43074-0056	21	Detritic neògen del Baix Penedès	ND
43020-0056	21	Detritic neògen del Baix Penedès	BDL
43047-0032	24	Baix Francoli	ND
43047-0018	24	Baix Francoli	ND
43095-0011	24	Baix Francoli	0,249
43047-0033	24	Baix Francoli	0,273
43119-0016	25	Alt Camp	ND
43034-0002	25	Alt Camp	ND
43005-0083	25	Alt Camp	BDL
43161-0159	25	Alt Camp	0,458
43161-0148	25	Alt Camp	ND
43161-0138	25	Alt Camp	ND

Sample	Water body	Location	GLY conc. (µg/L)
17067-0001	33	Fluviodeltaic del Ter	0,404
17199-0031	33	Fluviodeltaic del Ter	0,176
17191-0021	33	Fluviodeltaic del Ter	BDL
17199-0044	33	Fluviodeltaic del Ter	0,375
17205-0012	33	Fluviodeltaic del Ter	ND
17070-0049	33	Fluviodeltaic del Ter	BDL
17022-0027	33	Fluviodeltaic del Ter	0,134
17018-0013	33	Fluviodeltaic del Ter	0,126
17211-0025	33	Fluviodeltaic del Ter	ND
25172-0005	46	Al·luvial del Segre mig	0,107
25102-0001	47	Al·luvial del baix Segre	0,243
25120-0020	47	Al·luvial del baix Segre	BDL
25211-0001	47	Al·luvial del baix Segre	0,729
25254-0004	47	Al·luvial del baix Segre	0,407
25231-0003	47	Al·luvial del baix Segre	BDL
25096-0008	48	Al·luvial d'Urgell	0,087
25225-0003	48	Al·luvial d'Urgell	0,252
25168-0001	48	Al·luvial d'Urgell	BDL
25248-0002	48	Al·luvial d'Urgell	0,711
25168-0003	48	Al·luvial d'Urgell	0,242
25168-0004	48	Al·luvial d'Urgell	BDL
25158-0004	48	Al·luvial d'Urgell	0,320
25048-0003	48	Al·luvial d'Urgell	0,939
25050-0006	48	Al·luvial d'Urgell	0,135
43155-0056	?	Al·luvial de Tortosa	0,781
43155-0923	?	Al·luvial de Tortosa	0,080



5. CONCLUSIONS

Detected concentrations of glyphosate ranged between 0.08 and 0.94 µg/L with 58% (33 out of 57 samples) of the total sample number showing quantifiable amounts of this herbicide. The most contaminated sites were showed to be "Al.luvials de Vallés (16)" followed by "Detrític neògen del Baix Penedès (21)". MDL and MQL for the developed LC-MS/MS method were 3.2 and 10.8 ng/L, respectively. Comparison of results obtained by ELISA to those by LC-MS/MS showed good correlation.

6. ACKNOWLEDGEMENTS

This study was funded by the Catalan Water Agency (Agència Catalana de l'Aigua) and by the Spanish Ministry of Science and Innovation [CGL2007-64551/HID] and reflects only the author's views, and the EU is not liable for any use that may be made of the information contained therein. Lina Kantiani acknowledges the AGAUR (Generalitat de Catalunya, Spain) for economical support through the FI pre-doctoral grant and Alexander S. Onassis public benefit foundation (F-ZD 029/2007-2008). Abraxis (USA) is gratefully acknowledged for the gift of ELISA immunoreagents.