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N.T. Tran Thi, Nicolas Mazzella, François Delmas

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Development of purification procedures and HPLC-MS/MS method for the determination of glyphosate and AMPA in environmental water

Nhu-Trang TRAN-THI*, Nicolas MAZZELLA and François DELMAS
Cemagref, UR REBX, 50 avenue Verdun, 33610 Cestas (France)

* Corresponding author: nhutrang.tranthi@cemagref.fr

INTRODUCTION

Glyphosate [*N*-(phosphonomethyl)glycine] is one of the most widely applied herbicides in the world. This compound came on the market in 1970s under the trade name Roundup.

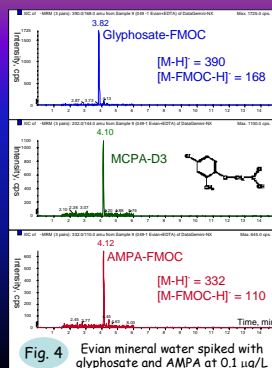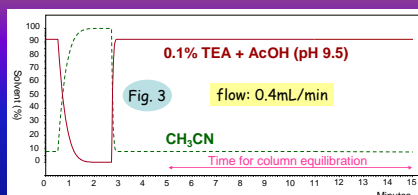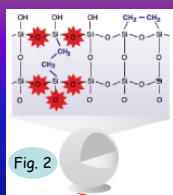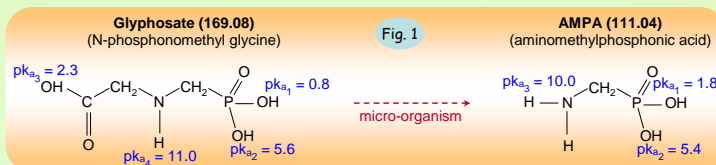It is used for weed control in agriculture, forestry and gardens, and also on railway embankments.

Glyphosate is rapidly adsorbed in soil and degraded to aminomethylphosphonic acid (AMPA) (fig. 1) – its main metabolite and then transported to groundwater and surface water.

The high polarity and water-solubility of glyphosate and AMPA has, until recently, made their analysis in water samples problematic, especially at trace levels.

Because of the lack of adequate chemical groups (e.g. chromophores), glyphosate and AMPA are derivatized with 9-fluorenylmethylchloroformate (FMOC-Cl) to form fluorescent derivatives. This derivatization reduces also the polar character of the analytes and facilitates the chromatographic retention.
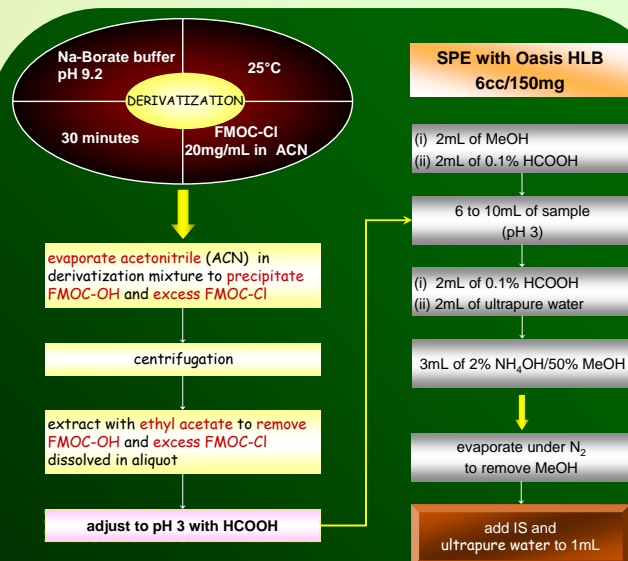
The aim of this study is to improve both extraction and purification protocol, and to develop an efficient and robust HPLC-ESI-MS/MS method for determining glyphosate and AMPA in environmental water.

According to **EU Directive 98/83/EC**, the permissible level for **glyphosate and AMPA in drinking water** is **0.1 µg/L**

Glyphosate (169.08)
(N-phosphonomethyl glycine)

Fig. 1

AMPA (111.04)
(aminomethylphosphonic acid)

$pk_{a_3} = 2.3$ $pk_{a_1} = 0.8$ $pk_{a_3} = 10.0$ $pk_{a_1} = 1.8$

micro-organism

$pk_{a_4} = 11.0$ $pk_{a_2} = 5.6$ $pk_{a_2} = 5.4$

Fig. 2

0.1% TEA + AcOH (pH 9.5)

Fig. 3   flow: 0.4mL/min

CH_3CN

Time for column equilibration

Fig. 4

3.82   Glyphosate-FMOC
$[M-H]^- = 390$
$[M-FMOC-H]^- = 168$

4.10   MCPA-D3

4.12   AMPA-FMOC
$[M-H]^- = 332$
$[M-FMOC-H]^- = 110$

Evian mineral water spiked with glyphosate and AMPA at 0.1 µg/L

The novel columns with Ethylene Bridged Hybrid particles (fig. 2) – XBridge (2.1x50mm, 3.5µm) or Gemini-NX (2x50mm, 3µm) – were used for the application at a high pH mobile phase. The best separation and sensibility were further obtained with 0.1% triethyl amine (TEA) adjusted to pH 9.5 with acetic acid (fig. 3).

MCPA-D3 was used as internal standard (IS) (fig. 4) and quadratic calibration curves were obtained for both analytes.

Procedure (flow diagram)

DERIVATIZATION
- Na-Borate buffer pH 9.2
- 25°C
- 30 minutes
- FMOC-Cl 20mg/mL in ACN

↓

evaporate acetonitrile (ACN) in derivatization mixture to precipitate FMOC-OH and excess FMOC-Cl

↓

centrifugation

↓

extract with ethyl acetate to remove FMOC-OH and excess FMOC-Cl dissolved in aliquot

↓

adjust to pH 3 with HCOOH

SPE with Oasis HLB 6cc/150mg

(i) 2mL of MeOH
(ii) 2mL of 0.1% HCOOH

↓

6 to 10mL of sample (pH 3)

↓

(i) 2mL of 0.1% HCOOH
(ii) 2mL of ultrapure water

↓

3mL of 2% NH_4OH/50% MeOH

↓

evaporate under N_2 to remove MeOH

↓

add IS and ultrapure water to 1mL

Recovery of Glyphosate: 93%
Recovery of AMPA: 70%

CONCLUSION

The new chromatographic method based on the application of C_{18} bonded hybrid particle column and basic mobile phase gave reproducible retention times and very sharp peaks.

The combination of liquid-liquid extraction with ethyl acetate and solid-phase extraction (SPE) on Oasis HLB improved the elimination of the impurities and the pre-concentration of the analytes. We obtained high recoveries (93% for glyphosate and 70% for AMPA) with ultrapure and mineral waters spiked at 0.1 µg/L.

In order to improve the recoveries, ongoing research concerns a preliminary sample treatment with EDTA for removing Ca^{2+}, Mg^{2+} and others bivalent cations that form strong complexes with analytes.