HPLC and GC Determination of Glyphosate and Aminomethylphosphonic Acid (AMPA) in Water Samples

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Abstract

This paper describes two methods for the sensitive and selective determination of glyphosate, and aminomethylphosphonic acid (AMPA) residues in water samples. First method involve a derivatisation step with 9-fluorenylmethylchloroformate (FMOC) in borate buffer and detection based on liquid chromatography coupled to fluorescent detector (LC-FL). The second method involve the extraction of glyphosate and AMPA from water sample with on cation-exchange column. Glyphosate and AMPA has been derivatised with Trifluorethanol (TFE) and Trifluoracethic anhidride (TFAA) and pesticide concentration was determined by gas-chromatography with ECD and NPD detector. Have been established the minimum detection levels and derivatisation capability of AMPA and glyphosate from water samples. The results show that the method which involve a derivatisation step with 9-fluorenylmethylchloroformate (FMOC) in borate buffer and detection based on liquid chromatography coupled to fluorescent detector (LC-FL) is the most sensitive method to detect glyphosate and AMPA from water sample, the minimum detection limit (LMD) is 0,03 μg/l glyphosate and AMPA in water sample.

Keywords: glyphosate, aminomethylphosphonic acid (AMPA), water, chromatography.

Introduction

Glyphosate (N-phosphonomethyl-glycine) is a systemic, broad spectrum herbicide effective against most plant species, including annual and perennial species and is one of the world’s most widely used herbicide. The US Environmental Protection Agency EPA recently estimated that glyphosate is used more than any other pesticide, with 350000 t per year and growing yearly with 41%, between 1999-2002 (Cox, 2004). Glyphosate is the most commonly used agricultural pesticide and the second most commonly used pesticide around and in homes and gardens (Tu et.al, 2001). Glyphosate use is currently growing at a rate around 20 percent per year. The Environmental Protection Agency EPA classified glyphosate in group E based on the evidence of toxicity. The toxicological studies demonstrated the ability of glyphosate and glyphosate containing product Roundup to cause genetic damage and National Institute for Occupational Safety and Health declared, in 2004, the glyphosate as mutagen and carcinogen (Kiely, 2004).

Literature studies indicates the glyphosphate concentration level in environment, after the herbicide application, as be between 0,09-1,7 mg/l in lacks, 0,02-1,237 mg/l in fluent water, 0,162-1 mg/l in surface water, and 0,11-2,82 mg/kg in sediments (Cox, 2004).

The identification of the glyphosate and his product of AMPA decomposition in traces has following problems: on one side the two substances have a high polarity, so they can not be extracted from analysed matrix easily using organic solvents, and on the another side the two substances has to be derivatised either to obtaining volatile and stable compounds that can be detected with GC, or for introduction of some chromophore or fluorophore groups for HPLC identification. Literature studies shows a few
chromatographic methods for glyphosate detection as: a high level liquid chromatography (HPLC), liquid chromatography coupled with the mass spectrometry (HPLC/MS), gas-chromatography (GC), gas-chromatography coupled with mass spectrometry (GC/MS), capillary electrophoreza (CE) (Babic et al., 2005; Bot et al., 2002, Corbera et al., 2006, Gauch et al., 1989). All these methods are characterized through high sensibility, reproductability and low detection limit.

Material and method
This paper describes two methods for the sensitive and selective determination of glyphosate, and aminomethylphosphonic acid (AMPA) residues in water samples.
The first method involve a derivatization step with 9-fluorenylmethylchloroformiate (FMOC) in borate buffer and detection based on liquid chromatography coupled to the fluorescent detector (LC-FL). (Gauch et. Al., 1989)
The second method involve the extraction of glyphosate and AMPA from water sample by the cation-exchange column. Glyphosate and AMPA have been derivatised with Trifluorethanol (TFE) and Trifluoracethic anhidride (TFAA) and pesticide concentration was determined by gas-chromatography with ECD and NPD detector (DIN 38407-22, 2002).

HPLC Conditions
Chromatographic column: NH2 (5μm) 200 mm x 4.6 mm, mobile phase: 85% water solution KH2PO4 with 1,5% NaOH 3M; 15% Acetonitril, for 16 min, debit: 0,5 ml/min, Fluorescence detection Ex. 265 nm, Em. 310 nm

GC Conditions
Hewlett-Packard Gaschromatograf 5840 A with NPD Detector (250 °C) and ECD Detector (300 °C). Column: HP-1 (25 m, 0,2 mm,:0,33μm). Temperature programm: 100 °C-280 °C; 4 °C/min, Initial Time 1,40 min, final time 15 min.

Results and discussions
25 ml water sample was spiked simultaneous with various amounts of glyphosate and AMPA and it was extracted and derivatised after methods described in the experimental methods. The minimum detection levels of AMPA and glyphosate from water samples have been established based on signal to noise ratios of 3:1.
The 9-fluorenylmethylchloroformiate (FMOC) is a derivation agent for the amino group and for fluorescent detection (ex.265 nm, em. 315 nm). The reaction of derivatisated AMPA and glyphosate are given in the figure 1. The calibration curve for the pesticides in range 0,00075 – 0,0125 μg/ml was linear and with high degree of reproducibility (figures 2-3). The minimum detection limit (LMD), in this case is 0,00075μg Glyphosat and AMPA /25ml water respectively 0,03 μg/l glyphosate and AMPA in water sample. These limits are lower than those stipulated in legislation on drinking water quality. The chromatogram of AMPA-FMOCCI and Glyphosate-FMOCCI with FLD detection is presented in figure 4. In the table 1 is presented the link between glyphosate and AMPA concentration and derivatised compounds, heeding of compounds molar weight.

In experimental results indicated in the table 2 it can be noticed that the derivatisation capability in AMPA case is between 68,33-73,66% and for Glyphosate is between 28,65-31,85%. This happend due to variant chimical structure of the two compounds. AMPA has one primary nitrogen atom and it will react easily with fluorescent compound FMOCI, thus the derivatisation capability will be higher than for Glyphosate, which has a secondary nitrogen atom and the interaction possibility of the fluorescent compound with higher molecular weight will be lower.

The reaction of glyphosate and AMPA with TFE and TFA and the GC chromatogram are given in figures 5-6. In the case of derivatised AMPA and glyphosate with TFAA and TFE and GC identification with ECD and NPD detector it can be seen from the table 3 that the ECD detector is more sensitive as NPD detector. That is
possible because the number of Halogen atoms is higher in derived product and the ECD signal is 10 time higher for AMPA-TFFA-TFE product and 14 time higher for glyphosate product. Also, the signal from Glyphosate-TFFA-TFE with ECD Detector is higher than AMPA-TFFA-TFE, because after the Glyphosate derivatisation contain 12 Halogen atoms and AMPA only 9 atoms. The minimum detection limit (LMD) is for Glyphosate derivatised with TFA-TFE and detected with ECD detector 0,0025 µg, respectively 0,1 µg/L glyphosate and 0,005 µg AMPA respectively 0,2 µg/L drinking water for AMPA. Using NPD detector the LMD is 0,025µg, respectively 1µg/L drinking water for AMPA and Glyphosate, higher that stipulated in legislation on drinking water quality.

Table 1. Molar weight of G-FMOCCL, AMPA-FMOCCL, GLYPHOSATE and AMPA

<table>
<thead>
<tr>
<th>Molar weight Glyphosate (g)</th>
<th>169.08</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molar weight Glyphosate-FMOCCL (g)</td>
<td>391.32</td>
</tr>
<tr>
<td>Molar weight AMPA (g)</td>
<td>111.04</td>
</tr>
<tr>
<td>Molar weight AMPA-FMOCCL (g)</td>
<td>333.28</td>
</tr>
</tbody>
</table>

Table 2. HPLC values and rate of derivatisation AMPA GLY-FMOCCL analysed in water by FLD

<table>
<thead>
<tr>
<th>Derivatised sample with</th>
<th>AMPA-FMOCCL</th>
<th>Glyphosate-FMOCCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>area weight µg</td>
<td>725.4</td>
<td>1</td>
</tr>
<tr>
<td>Rate of Derivatisation</td>
<td>28.69</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3. The GC results of AMPA and glyphosate

<table>
<thead>
<tr>
<th>Conc. µg</th>
<th>AMPA</th>
<th>Glyphosate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Retention time</td>
<td>Area</td>
</tr>
<tr>
<td>0.05</td>
<td>4.523</td>
<td>18868</td>
</tr>
<tr>
<td>0.1</td>
<td>4.519</td>
<td>28272</td>
</tr>
<tr>
<td>0.2</td>
<td>4.516</td>
<td>67057</td>
</tr>
<tr>
<td>0.5</td>
<td>4.517</td>
<td>59003</td>
</tr>
</tbody>
</table>

Figure 1. The derivatisation of glyphosate with FMOCCL
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![Curba de etalonare glyphosat](image1.png)

- Regression coefficient (R=0.99995)
- Standard deviation (SD=0.08477)

**Figure 2.** Calibration curve for Glyphosate-FMOC

![Curba de etalonare AMPA](image2.png)

- Regression coefficient (R=0.99949)
- Standard deviation (SD=0.1355835)

**Figure 3.** Calibration curve for AMPA-FMOC

**Figure 4.** The HPLC chromatogram of glyphosate and AMPA

![Derivatisation of glyphosate with TFAA-TFE](image3.png)

**Figure 5.**
The derivatisation of glyphosate with TFAA-TFE
Conclusions

1). The method which involve a derivatization step with 9-fluorenylmethylchloroformate (FMOC) in borate buffer and detection based on liquid chromatography coupled to fluorescent detector (LC-FL) is the most sensitive method to detect glyphosate and AMPA from water sample. The minimum detection limit (LMD), in this case is 0.03 μg/L glyphosate and AMPA in water sample. Derivatisation capability with fluorescence reagent of AMPA metabolit is better than the one obtained for glyphosate.

2). The method which involve the extraction of glyphosate and AMPA from water sample with on cation-exchange column and derivatisation of Glyphosate and AMPA with TFE and TFAA is less sensitive than first method. In the case of GC identification, the ECD detector is more sensitive as NPD detector.
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References


*** (2002), Deutsche Norm zur Wasser, Abwasser, Schlammuntersuchung,. DIN 38407-22

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