EXTRACTION AND IDENTIFICATION OF HERBICIDE GLYPHOSATE FROM NATURAL WATER WITH DIFFERENT CHARACTERISTICS

Ersilia ALEXA¹, A. LAZUREANU¹,
S. ALDA³, Monica NEGREA¹,
Alina BULMAGA¹
¹ Universitatea de Ştiinţe Agricole şi Medicină Veterinară a Banatului Timişoara,
Tel./Fax 0256/277263 , ersilia_alexa@yahoo.com

Determination of glyphosate in natural water with different characteristics involves the derivation with 9-fluorenylmethylchloroformiate (FMOCCL) in borate buffer and detection based on High Performance Liquid Chromatography (HPLC) coupled to fluorescent detector (LC-FL). The experimental results show the influence of physical-chemical water characteristics (conductivity, chloride, sulphate, Ca and Na content) on the derivatization rate of herbicide glyphosate from water samples. Depending on the sample composition the fluorimetric response was different for the same concentration of glyphosate and decreased in direct proportion to the increasing conductivity and ionic concentration in the sample.

Key words: glyphosate, water, conductivity, ionic content

Glyphosate (N-phosphonomethyl-glycine) is a systemic, broad spectrum herbicide effective against most plant species, including annual and perennial species and it is one of the world’s most widely used herbicide. 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 [5].

Literature studies indicates the glyphosate 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 [3].

In this work it was determined the influence of physical proprieties (conductivity) and chemical composition (chloride, sulphate, Ca and Na content) of water upon the derivatization capability of glyphosate with 9-fluorenylmethylchloroformiate (FMOCCL) in borate buffer.
MATERIAL AND METHOD

The extraction of glyphosate from the matrix of analysis and HPLC identification:

Water samples with different physical-chemical characteristics (table 1) was spiked simultaneous with 0.1 µg glyphosate. The analytical method used to detect glyphosate from water sample involve a derivatization step with 9-fluorenylemethylchloroformiate (FMOCCl) in borate buffer and detection of glyphosate based on liquid chromatography coupled to the fluorescent detector (LC-FL) [4].

<table>
<thead>
<tr>
<th>Water sample</th>
<th>Conductivity (mS/cm)</th>
<th>Ca (mg/l)</th>
<th>Na (mg/l)</th>
<th>Cloride (mg/l)</th>
<th>Sulphate (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>532</td>
<td>73.15</td>
<td>29.00</td>
<td>13.5</td>
<td>6.4</td>
</tr>
<tr>
<td>2</td>
<td>727</td>
<td>82.2</td>
<td>38.00</td>
<td>31.2</td>
<td>35.5</td>
</tr>
<tr>
<td>3</td>
<td>1096</td>
<td>122.20</td>
<td>25.00</td>
<td>19.9</td>
<td>70.5</td>
</tr>
</tbody>
</table>

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

The method was validated in water samples, spiked at 0.02 – 0.0125 µg/l [1]. Three samples spiked with glyphosate at each level were processed by use of the entire method and each was injected twice. The mean recovery values ranged from 85-90%. The linear dynamic range of the detector response for derivatized glyphosate was checked. The minimum detection levels of glyphosate from water samples have been established based on signal to noise ratios of 3:1 and was 0.03 µg/l glyphosate in water sample. The average correlation coefficient was 0.994 for derivatized glyphosate and the relative standard deviation (RSD) was <15% [2].

RESULTS AND DISCUSSIONS

The derivatization rate was established on the signal obtained (peak area) from the derivatization products of glyphosate. Have been determinated the quantities of Glyphosate-FMOCCl obtained through derivatization of 0.1 µg glyphosate in the case of 3 different samples and have been calculated the derivatisation rate taking count of Glyphosate-FMOCCl theoretical quantity which should be obtained with the following formula :

\[
\text{Rate of Derivatization} (%) = \left( \frac{C_{\text{Gly-FMOCCl}} \times MG_{\text{y}}}{0.1 \times M_{\text{Gly-FMOCCl}}} \right) \times 100
\]

Where:
- \(C_{\text{Gly-FMOCCl}}\) - quantity obtained through derivatization
- 0.1 – glyphosate quantity introduced (µg)
- \(MG_{\text{y}}\) - Glyphosate Molar weight (g) = 169.08 g
- \(M_{\text{Gly-FMOCCl}}\) - Molar weight Glyphosate - FMOCCl (g) = 391.32 g
The chromatogram of water sample spiked with Glyphosate-FMOCCl and detect by FLD is presented in figure 1 and the derivatisation of glyphosate with FMOCCl in figure 2. The experimental results which were obtained concerning the derivatization capability of glyphosate residues from water samples, with different physical-chemical characteristics, are presented in table 2.

Figure 1 The HPLC chromatogramm of water sample

Figure 2 The derivatization of glyphosate with FMOCCl
The rate of derivatization (%)

<table>
<thead>
<tr>
<th>Water sample</th>
<th>Ct Gly-FMOCCI *</th>
<th>C Gly-FMOCCI **</th>
<th>Rate of Derivatization (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.231</td>
<td>0.085</td>
<td>36.79</td>
</tr>
<tr>
<td>2</td>
<td>0.231</td>
<td>0.075</td>
<td>32.47</td>
</tr>
<tr>
<td>3</td>
<td>0.231</td>
<td>0.053</td>
<td>22.94</td>
</tr>
</tbody>
</table>

Ct Gly-FMOCCI * - Gly-FMOCCI quantity theoretical obtained
C Gly-FMOCCI ** - Gly-FMOCCI quantity obtained through derivatization.

The derivatization capability of glyphosate is low and varies between 22.94-36.79% in natural water. The water sample with the strong ionic content and high conductivity (sample 3) has a low fluorimetric signal and the glyphosate derivatization rate is the smaller. The decreasing of signal with the ionic concentration of water sample results from consumption of derivatization agent by the ions responsible for the strong conductivity. The decreasing of the sample conductivity and of the ionic concentration determinates the increase of glyphosate derivatization capacity in natural water samples.

CONCLUSIONS

The physical-chemical characteristics of water (conductivity and ionic concentration) influence the derivatization rate of herbicide glyphosate from water samples.

The derivatization capability of glyphosate in natural water samples decreases with the increasing of conductivity and ionic concentration.

BIBLIOGRAPHY

1. Alexa, E., Poiana, M., Peev, C., Cozma, A., Dehelean, C., 2006 – Determinarea reziduurilor de glifosat si a metabolitului său acidul aminometilfosfonic (AMPA) in apa prin HPLC, Revue of Chemistry, 57(10), 997-1002