Residue determination of glyphosate and AMPA in soils, after derivatization, by liquid chromatography coupled to tandem mass spectrometry.

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INTRODUCTION

Glyphosate (N-phosphonomethylglycine) is a broad spectrum, non-selective, post-emergence herbicide that control weeds by inhibiting their ability to synthesise amino acids. Aminomethylphosphonic acid (AMPA) is the major metabolite of glyphosate, found in plants, water and soil. Glyphosate is strongly adsorbed to most soils and thus, does not leach or run off appreciably. Then soil microorganisms break it down. The estimated half life of glyphosate in soil is approximately 60 days. After 360 days, residue levels were from 6 to 15% of the initial applied dose. Precipitation, soil composition, presence and absence of a soil constricting layer and drainage type may influence the leaching of glyphosate from soil. Glyphosate is an aminomethylphosphonic analogue of the natural amino acid glycine.

The lack of adequate chemical groups in glyphosate molecule (e.g. chromophores or fluorophores) hampers its measurements by conventional detectors. Moreover, due to itsionic character, low volatility and low mobility, GC is not the required for residue analysis is very difficult. Even more difficult can result the residue determination in soil at low concentration level (e.g. below 0.1 mg/kg), due to complexity of this matrix sample. Because of the highly polar nature of this compound, extraction from environmental matrices with organic solvents is almost impossible. This has made its determination a difficult challenge.

An analytical method has been developed for the rapid and robust determination at low concentrations of glyphosate and its major metabolite AMPA in soil by LC-MS/MS.

The method fulfilled the requirements of excellent sensitivity and unequivocal confirmation of the residues detected by using two MS/MS transitions for each compound.

EXPERIMENTAL

Analytical standards of glyphosate (99%) and AMPA (97.5%) were purchased from Dr. Ehrenstorfer. Analytical reagent-grade dimethyl tetrabutyl decylamide was obtained from Lachner, C. fluoromethylthiorforamid (FMOC)-Cl was purchased from Alfa Aesar. Reagent-grade hydrochloric acid (HCl) and potassium hydroxide (KOH) were obtained from Merck, whereas acetic acid (HAc) and ammonium acetate were purchased from Panreac. LC-MS-grade water and acetonitrile were obtained from Fischer Scientific.

Standard stock solutions of glyphosate and AMPA (1000 mg/L) were prepared in water and mixed standard solutions at different concentrations (1–0.01 μg/mL) were prepared in methanol. Soil samples were allowed to dry at room temperature in the dark, sieved and frozen at -20 °C till extraction. The extraction procedure is described in Figure 1.

To perform the chromatographic separation the mobile phase consisted of solvent A (water: ammonium acetate, acetic acid (pH 4.6)) and solvent B (acetonitril) in the gradient presented in table 1. The injection volume was 5 μL and the flow rate 0.2 mL/min.

According to the literature glyphosate and AMPAs have been recorded in negative and in positive ion mode. In our study, both compounds were recorded in positive ion mode as sensitivity was found to be much higher.

Table 1: Gradient for LC-separation

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>A%</th>
<th>B%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 0.66</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>0.66 - 1</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>1 - 2</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>2 - 3</td>
<td>0</td>
<td>100</td>
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<tr>
<td>4 - 5</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>5 - 6</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Method validation

The method developed for glyphosate and AMPA was validated, obtaining satisfactory recoveries and precision for both analytes. Method validation of the procedure was performed, included the study of: linearity, limit of detection and quantification, specificity, accuracy, precision, stability, matrix effects, robustness, and carry-over.

Calibration curves were obtained for both compounds in the range 0.01-1 μg/mL with correlation coefficients (R²) 0.9991 for glyphosate and 0.9995 for AMPA. The method was found to be precise as RSD% was found to be 12% for glyphosate at 0.1 μg/mL (lowest) fortification level, and RSD% was 9% for AMPA at the same fortification level. The accuracy of the method provided satisfactory recoveries between 65.5% - 98% for glyphosate and 67.6% - 118.8% for AMPA at 0.1 μg/mL fortification level. Limits of quantification (LOQ) were established as the lowest concentration assayed and validated which gave satisfactory recovery and precision as previously referred. The limits of detection (LOD) defined as the lowest concentration that the analytical process can reliably differentiate from background level, were obtained when the signal was three times the average of the background noise in the chromatogram at the lowest analyte concentration assayed.

Application

The procedure was applied to the analysis of soil (approximately 84 samples) from Kepaia, county of Viotia (Greece), an important agricultural area where glyphosate is widely used in cotton crop. The samples were collected in four different periods of time (February, May, July and September) in order to monitor the spatial and periodical variation of their concentration. In Figure 2 is illustrated the total concentration profile measured for soil samples from six different sampling sites. The occurrence of glyphosate was expected, due to the presence of a large number of herbicide per-kg application per spring season during the monitoring period.

CONCLUSIONS

The problem of soil pollution in the pilot area due to pesticide residues is focused on herbicides, mainly glyphosate. The analytical method described shows the rapid and simple-application of LC-MS/MS to the determination of glyphosate and its major metabolite AMPA. The concentration of glyphosate found depends on the type of soil, weather conditions and application practices used in the area.

Acknowledgments:

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References

3. ‘Validation, as well as in recovery tests’, California Environmental Protection Agency, December 1997.