Determination of glyphosate in soil/sludge by high performance liquid chromatography

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A B S T R A C T
In order to evaluate the pollution caused due to glyphosate (Glyp) in soils and sludge, it is important to establish a set of standard determination techniques. In this work, the previously reported HPLC analytical method for determination of Glyp has been improved in order to be applied for all kinds of soils/sludge. The soil/sludge samples were extracted using sodium phosphate and trisodium citrate aqueous solutions. The extract was adjusted to pH 9 and contaminations were removed by washing with n-hexane. The method developed in this work further involves derivatization with 9-fluorenylmethylchloroformate (FMOCl) followed by high performance liquid chromatography (HPLC) coupled with fluorescence detection. The method was validated in three soil (red soil, black soil and paddy soil) and two sludge samples (lake and river sludge) from China and verified in six laboratories. A good linear relationship (correlation coefficients >0.999) was observed within the range of 0.005–0.5 mg/L. The limit of detection (LOD) and the limit of quantitation (LOQ) were determined to be 0.01 mg/kg and 0.04 mg/kg, respectively. The precision and accuracy were satisfactory with the relative standard deviation (RSD) lower than 15% and the mean recovery values ranging from 75% to 110% (n = 6), that spiked at three levels (0.1, 0.5 and 1.0 mg/kg).

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1. Introduction

Glyphosate (Glyp, N-(phosphonomethyl)glycine), is extensively applied in agriculture to control the growth of various annual and perennial weeds as a broad spectrum organic phosphorus herbicide [1]. As one of the most effective herbicides, Glyp has made great contributions to agricultural development. A number of studies have been published in recent years on the environmental hazards of using Glyp in response to its potentially toxic effects on the ecological environment and human health [2–5]. Due to its extensive use, Glyp residues have been found in the soil, surface water, streams, and groundwater [6]. In a revised draft of the Environment Quality Standards for Soils in China (GB 15618–2008) [7], Glyp was added as one of the organic pollutants in soil. Therefore, it is necessary to establish an effective method analysing the pollution caused by Glyp in soil/sludge.

Analysis of Glyp is challenging due to its physico-chemical properties, including amphoteric character, water solubility, and high polarity [8–10]. Furthermore, its chemical structure lacks the necessary functional groups that might be helpful for direct detection [11]. In addition, the extraction of Glyp from soil/sludge is also tricky. Previous reports have described the use of potassium hydroxide and sodium bicarbonate solutions for the extraction of this herbicide, but the results have not been satisfactory for soil/sludge samples due to the interaction between Glyp and other substances, such as organic matter, humic acid or metal oxides present in them [12–15].

Although several studies have been published in this research area, only rare examples of analytical methods for determining the content of Glyp in soil/sludge samples, can be found in literature. The Environmental Protection Agency (EPA443285-06-S) [16] has published one such method that involves analysis of Glyp by using gas chromatography combined with mass spectrometry.
Na$_2$B$_4$O$_7$ in deionized water (0.05 mol/L) and FMOC dissolved in acetonitrile (1.0 g/L) were used for the derivatization step.

### 2.4. Instrument operational parameters

The HPLC system was coupled with a fluorescence detector for analysis. The chromatographic column was AR–C18, 5 μm particle size, L × I.D. 250mm × 4.6 mm, and the column temperature was 35 °C ± 5 °C. The flow rate was 1.0 mL/min and the mobile phase was time-programmed using acetonitrile (A) and 0.2% v/v phosphoric acid aqueous solution (B). The percentage of organic mobile phase (B) was changed linearly as follows: 0 min,35%; 10 min, 25%; 15 min, 80%; 20 min, 35%. The injection volume was 20 μL. The fluorescence detector was set to an excitation wavelength of 254 nm and emission wavelength of 301 nm.

### 2.5. Sample pretreatment method

#### 2.5.1. Extraction method

##### 2.5.1.1. Soil sample

10.0 g of the fresh soil sample was weighed accurately and extracted by using 50 mL of a mixture of sodium phosphate (0.03 mol/L) and trisodium citrate (0.01 mol/L) assisted by an ultrasonic sonicator for 30 min. The solution was then subjected to centrifugation at 10000 rpm for 5 min to obtain the supernatant.

##### 2.5.1.2. Sediment

Interstitial water in the sediment was removed by centrifugation at 10000 rpm for 10 min. 10.0 g of the sediment sample was weighed accurately and the subsequent process of extraction was the same as that for the soil sample.

#### 2.5.2. Purification method

For purification, pH of the extraction solution was first adjusted to 9 by HCl (1.0 mol/L). The solution was then allowed to stand for 10 min before filtration. Subsequently, 50 mL n-hexane was added, and the mixture was vigorously shaken. After letting the mixture stand for a certain period of time, the organic phase was discarded. These were repeated for a second time and finally 1.0 mL of the as-prepared solution was treated with the reagents for the derivatisation reaction.

#### 2.5.3. Derivatisation reaction

The FMOC derivative of Glyph reaction was prepared by reacting 1 mL of the purified extract with 0.12 mL of Na$_2$B$_4$O$_7$ (0.05 mol/L) and 0.2 mL of FMOC–Cl (1.0 mg/mL) at room temperature for 4 h. The progress of the reaction was monitored by analysing the aliquots obtained after filtration over a membrane (0.22 μm) using liquid chromatography coupled to fluorescence detection.

#### 2.5.4. Preparation of a blank sample

A blank sample was prepared by weighing 10.0 g of quartz sand and following the same steps for analysis as those for the soil samples.

### 2.6. Validation study

Linearity of the developed method was evaluated by analysing a series of standard solutions, having concentrations in the range of 0.005 mg/L–0.5 mg/L. Standard curves were plotted by assigning the concentration of standard solution as x-axis and the peak area as y-axis. Linearity was considered appropriate when the linear correlation coefficient was higher than 0.99.

The limits of detection (LOD) and the limit of quantitation (LOQ) of the method were evaluated by analysing quartz sand samples spiked at the lowest concentration (0.05 mg/kg). According to the “Environmental Monitoring–Technical Guidelines on Drawing and
Revising Analytical Method Standards’ (HJ 168) [19] requirements, LOD and LOQ were computed by the following formulas. Furthermore, the value of LOQ should be lower than the detection limit value (0.5 mg/kg) proposed in the Environmental Quality Standard for Soils [7].

$$\text{LOD} = t_{(n-0.98)} \times S(\text{mg/kg}); \text{LOQ} = 4 \times \text{LOD (mg/kg)}$$

Where: $n$— parallel determination of samples;

- $t$— the $t$ distribution when the degrees of freedom is $(n-1)$ and the confidence level is 99%;

- $S$— standard deviation of $n$ parallel determination.

Precision (in terms of RSD) and accuracy (percentage recoveries) were computed by recovery experiments in three soils and two sludge samples, spiked at three levels (0.1, 0.5 and 1.0 mg/kg), placed one night in the ventilation chamber and measured six times in parallel. Recoveries between 70%–120% with RSD lower than 20% were considered reasonable.

3. Results and discussion

3.1. Selection of derivatization reaction conditions

Due to a lack of necessary functional groups, Glyp cannot be identified by the fluorescence detector. Therefore, a derivatisation step was required to improve the detection sensitivity. In this study, 9-fluorenyl-methyl-chloroformate (FMOC-Cl) was chosen as derivatisation reagent, as it reacts with both primary and secondary amines. In addition, the derivatisation reaction can be controlled and is not affected by the mobile phase.

3.1.1. Dosage of derivatization reagent (FMOC-Cl)

As reported by Baez et al. [20], FMOC-Cl is soluble in ACN and insoluble in water, while Glyp is soluble in water and insoluble in organic solvents. Therefore, it is necessary to choose an appropriate solvent system in order to keep both compounds in solution. The standard solutions of Glyp with concentrations of 0.1 mg/L and 0.5 mg/L was used in the test. 0.12 mL of borax solution (0.05 mol/L) was added 1.0 mL of Glyp standard solution. The derivatization reaction was executed by adding the FMOC-Cl solutions of different concentrations, (1 g/L, 0.1, 0.2, 0.5 mL) and (10 g/L, 0.1, 0.2, 0.5 mL). The mixed solutions dissolved during the course of reaction. The results are shown in Fig. 1. The response values reached a maximum value when 0.2 mL of FMOC-Cl (1 g/L) was added. Due to a higher reactivity of derivatization reagent FMOC-Cl, when the dosage of FMOC-Cl was too large, the residual part could react with water and transform into FMOC-OH, which would interfere with the determination of target compounds. Therefore, 0.2 mg (0.2 mL, 1 g/L FMOC-Cl) was the optimum amount of FMOC-Cl.

3.1.2. Derived time

The standard solutions of Glyp with concentrations of 0.1 mg/L and 0.5 mg/L were mixed with 0.2 mL of FMOC-Cl (1 g/L) at room temperature for different reaction times (1, 2, 4, 8 h). The results showed that the response value increased with increasing the reaction time. As the reaction time exceeded 4 h, the response value became stable, and therefore, the optimal time was 4 h.

3.2. Extraction method

3.2.1. Type of extract solution

Although Glyp is insoluble in common organic solvents, its salts are easily soluble in water. Therefore, alkaline solutions have usually been chosen for the extraction solution of Glyp. Various alkaline solutions were tried in this study, such as KOH (0.6 mol/L), NaOH (0.6 mol/L), NaHCO3 (0.6 mol/L), Na3PO4 (30 mmol/L), and KH2PO4 (0.1 mol/L). The paddy soil spiked with 1.0 and 5.0 mg/kg of Glyp was chosen for this experiment due to its higher organic matter content. Na3PO4 (30 mmol/L) solution was the best choice with a highest recovery rate. Due to the similar mechanism of adsorption of Glyp and other phosphates in the

![Fig. 1. Effect of FMOC addition on the response value of glyphosate at two concentration level (n = 3).](image1)

![Fig. 2. Effect of Na3PO4 concentration in the extracting solution on the recovery of Glyphosate from paddy soil at two concentration level (n = 3).](image2)
soil, these compounds compete for adsorption sites. This generally reduces the adsorption of Glyphosate and improves the recovery rate [21,22].

3.2.2. Extract concentration

Na₃PO₄ aqueous solutions with different concentrations were used for the extraction process. The recovery rates of Glyphosate in soil samples spiked at 0.5 and 2.0 mg/kg are shown in Fig. 2. Recovery rate was found to increase at the beginning and decrease afterwards with an increase in the Na₃PO₄ concentration. The highest extraction recovery was obtained with 50 mM. However, the peak shape varied with the increase of the Na₃PO₄ concentration. On optimisation, 30 mmol/L of Na₃PO₄ solution was selected as the topgallant concentration.

3.2.3. The addition of metal ions complexing agent

Typically, soil contains a wide variety of ions, such as Ca²⁺, Mn²⁺, Zn²⁺, Mg²⁺, Fe³⁺, Fe²⁺, and Al³⁺ that can react with Glyphosate and reduce its recovery rate. In order to eliminate the interference from these ions, a proper complexing agent is required to improve the recovery rate. Three types of metal ion complexing agents were tried and compared. In terms of the separation of chromatagram peaks, the consequences of using Seignette salt and trisodium citrate were better than that of disodium EDTA. As shown in Fig. 3, of trisodium citrate (0.01 mol/L) showed a better extraction effect and higher recovery rate in the two selected typical soil and sludge samples that were spiked at 1.0 mg/kg.

3.3. Purification method

The detection of Glyphosate can also be affected by humus and a variety of other organic substances. Therefore, it is necessary to select the appropriate purification method to remove the interference of impurities. Liquid–liquid extraction and solid phase extraction are two common methods of purification. Liquid phase extraction is more convenient, fast, and cheaper than solid phase extraction purification. Therefore, liquid–liquid extraction method was used for purifying. The recovery rate of several kinds of organic reagents was shown in Fig. 4. According to the recovery rate, hexane was the best purification reagent for the five representative soils and sediments.

3.4. Validation study

Standard solutions with concentrations ranging from 0.005 mg/L to 0.5 mg/L were prepared by diluting the stock standard solution with an appropriate amount of deionized water. Standard curves are plotted by the concentration of standard solution as x-axis and peak area as y-axis. The regression equation was regarded to fit with a good linear relationship (correlation coefficients ≥0.999). This range was equivalent to 0.025–2.5 mg/kg in the sample.

The LOD and the LOQ of the method were evaluated by analysing quartz sand samples spiked at 0.05 mg/kg (n = 7). The relative standard deviation (RSD) of the seven experiments was 5.93% and the LOD and LOQ values were calculated to be 0.01 mg/kg and 0.04 mg/kg, respectively. This data is summarized in Table 1. Thus, this method met the requirements of quality standards in China.

The method was validated on the basis of analyses of five representative samples consisting of three kinds of soils (red soil, black soil and paddy soil) and two sludge samples (lake sludge and river sludge), and validated in six laboratories in China. Precision (in terms of RSD) and accuracy (percentage recoveries) were computed by means of recovery experiments in these samples, which were spiked at three levels (0.1, 0.5 and 1.0 mg/kg) and determined six times in parallel. The relative standard deviation (RSD) and recovery rate are given in Table 2. The method was validated as the RSD.
was found to be lower than 15% and the recovery percentages lay between 75 and 110%. Due to the different physical and chemical properties, the recovery from soil and sludge were different. The recovery percentages for sludge were lower compared to that for soils, and the recovery from red soil was highest of all samples.

### Table 1
The LOD and LOQ of the method in quartz sand samples (n = 7).

<table>
<thead>
<tr>
<th>parallel sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>concentration (mg/kg)</td>
<td>0.048</td>
<td>0.049</td>
<td>0.052</td>
<td>0.048</td>
<td>0.046</td>
<td>0.052</td>
<td>0.055</td>
</tr>
<tr>
<td>average concentration (mg/kg)</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
</tr>
<tr>
<td>standard deviation S (mg/kg)</td>
<td>0.003</td>
<td>0.003</td>
<td>0.003</td>
<td>0.003</td>
<td>0.003</td>
<td>0.003</td>
<td>0.003</td>
</tr>
<tr>
<td>$t_{(n-1,0.99)}$</td>
<td>3.143</td>
<td>3.143</td>
<td>3.143</td>
<td>3.143</td>
<td>3.143</td>
<td>3.143</td>
<td>3.143</td>
</tr>
<tr>
<td>LOD (mg/kg)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>LOQ (mg/kg)</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
</tbody>
</table>

$t_{(n-1,0.99)}$: the t distribution when the degrees of freedom is (n - 1) and the confidence level is 99%.

### Table 2
Precision and accuracy of the method in five soil/sludge samples (n = 6).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Recoveries (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low level</td>
<td>Medium level</td>
</tr>
<tr>
<td>red soil</td>
<td>101.15</td>
<td>100.67</td>
</tr>
<tr>
<td>black soil</td>
<td>83.66</td>
<td>80.71</td>
</tr>
<tr>
<td>paddy soil</td>
<td>88.79</td>
<td>86.86</td>
</tr>
<tr>
<td>lake sludge</td>
<td>80.62</td>
<td>75.58</td>
</tr>
<tr>
<td>raver sludge</td>
<td>82.03</td>
<td>79.99</td>
</tr>
</tbody>
</table>

### Fig. 5
a) Sample of red soil; b) Sample of black soil; c) Sample of paddy soil; d) Sample of river sludge; e) Sample of late sludge.

3.5. **Analyses of soil/sludge samples**

The validated method was applied to the analysis of samples from a few representative regions in China. Soil samples from the Hunan province (red soil), Northeast Region (black soil), and the area around Tai Lake (paddy soil) were collected, where the
pesticide Glyp is widely used in the fields. Two kinds of sludge samples were obtained from the Qinhuaui River (river sludge) and the Qin Lake (lake sludge). The target compound was found in all the tested soil samples in concentrations of 0.15 mg/kg in red soil, 0.19 mg/kg in black soil and 0.11 mg/kg in paddy soil. The concentration of detection was much lower than the detection limit value (0.5 mg/kg) reported in the Environmental Quality Standard for Soils. However, no target compound was detected in the sludge samples because Glyp can undergo rapid degradation in the water-sediment system. The chromatograms of Glyp in soil/sludge samples are shown in Fig. 5a–e.

4. Conclusions

This work describes a convenient and simple protocol for the determination of glyphosate (Glyp) in soil/sludge. In contrast to techniques such as LC–MS/MS and GC–MS/MS that have been reported before, this method involves pre-column derivatization and uses an HPLC coupled with a fluorescence detector. Using the procedure developed in this study, it is possible to analyse all types of soil and sludge samples that possess different physical and chemical properties. This novel technique requires the use of sodium phosphate solution for the extraction of Glyp present in soil/sludge. Furthermore, due to possible interference from some metal ions, humus, and other organic substances in the soil, an ioncomplexing agent is added and extraction purification is implemented with hexane. The method demonstrates a good linear relationship (correlation coefficient = 0.999) in the Glyp concentration range of 0.005–0.5 mg/L, and is also fairly sensitive (LOD: 0.01 mg/kg, LOQ: 0.04 mg/kg) and accurate (recovery: 75% to 110% with the RSD lower than 15%). The parameters of this methodology meet the required limits of agricultural soil environmental quality standard (0.5 mg/kg). And as this analysis protocol is cheap, simple, and sensitive, it can be widely applied.

Conflicts of interest

All authors declare no actual or potential competing financial interest.

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References


