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Determination of glyphosate by high performance liquid chromatography (HPLC) without prior extraction

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ABSTRACT

The aim of this study was to design a glyphosate analysis method. This molecule is an organic pollutant from water and soil. We have developed a chromatographic method with phenylisothiocyanate. This molecule has allowed obtaining an intermediate molecule with the glyphosate being easily detectable in chromatography. The peak relating to this intermediate was identified using a comparison with several samples, including a blank. The tests determined the retention time (RT) of glyphosate at 1.6 min and the values of the percentage of accuracy and repeatability of the method.

Keywords: Glyphosate, phenylisothiocyanate, liquid chromatography, detection limit, Phénylthiocarbamoylglphosate, retention time.

INTRODUCTION

Glyphosate [N-(phosphonomethyl) glycine, $HOOC\text{-}CH_2\text{-}CH_2\text{-}NH\text{-}PO_3H_2$] is a non-selective amino-phosphonate herbicide. It was introduced in agriculture since 1974 by Monsanto Company (Baylis, 2000; James, 2006) and it is very much used (Woodbum, 2000). Glyphosate is known as an environmentally-friendly herbicide due to its biodegradation and adsorption in soil. The lower concentrations of glyphosate have been found in shallow aquifers, which challenge the common idea that glyphosate has limited mobility in soils (Barja et al., 2005; Vereecken, 2005). This herbicide is highly toxic after a threshold of 0.1 $\mu g.L^{-1}$ into the groundwater and has an effect on human placental cells (Richard et al., 2005) leading to spontaneous death or abortions (Savitz, 2000), therefore, it is important to remove these organic pollutants from the contaminated water and soil.

The aim of this study was to design a method of analysis of glyphosate in water, and to provide method of elimination. On the other hand, several other methods have already been developed but have shown their inefficiency. In fact, glyphosate can exist in different ionic forms depending on pH effects on its functional groups (carboxylic acid,

phosphonic acid and secondary amine), which makes it very soluble in water (12.0 $g.L^{-1}$ at 25 °C) (Tortensson, 2008). The conventional removal liquid-liquid methods cannot be applied to the analysis of glyphosate. In addition, this molecule is rapidly degraded to aminophosphonic acid (AMPA) as amine form. The 9-fluorenylmethylchloroformate (FMOC) is often used as intermediate products to determine the amount of glyphosate (Sancho et al., 1996; Tsui et al., 2008). However, we are inspired by the Pico-Tag method, described by Heinrikson and Meredith (1984). The technique was developed to improve the speed sensibility to amino-acid analysis with phénylisothiocyanate for the rapid separation of very small amounts of complex peptides and protein mixtures (Bidlingmeyer et al., 1987).

MATERIALS AND METHODS

Materials

The following materials were used: HPLC (PROMIN 20AT Shimadzu) equipped with two pumps (LC 20A); an automatic injector (SIL-20A); a hot column (CTO-20A); Interchrom C_{18} column (5 µm particle size, Length x I.D: 250 mm x 4.6 mm); Detector Sensitivity (1 AUFS) and a computer system to check solvent gradient.

Methods

Sample preparation

Glyphosate (77% purity, made in France by Arysta LifeScience) was used. Thus, the following solutions were used: solution S1 made of a mixture of ethanol, water, phenylisothiocyanate, triéthylamine; Solution S_2 made of a mixture of solution S_1 and glyphosate. The blank solution was a mixture of solution S_1 and water. We used also an industrial product (Tuherb 480 SL). Tuherb 480 SL was provided by Callivoire (food-processing industry in Ivory Coast). These samples were prepared for acquisition data according to the procedure below.

Preparation of the derivative glyphosate solution and the blank solution

A volume of 20.0 µL of different concentrations of glyphosate solution was added to 20.0 µL of S_1 solution. The mixture was vigorously shaken for twenty seconds by vortex equipment, and then left at room temperature for thirty minutes. The S_1 solution consists of a mixture of ethanol/triethylamine/ water/phenylisothiocyante (7:1:1:1).

Figure 1 shows the chemical reaction of the formation of a parent product from glyphosate. This molecule reacts with an excess of phenylisothiocyante to form phenythiocarbamoylglyphosate which is detected by UV absorbance.

The mixture thus formed is called S_2 solution. The blank solution was obtained with bidistilled water and S_1 solution. The mixture of water and the industrial product (Tuherb 480 SL) had also undergone same transformation like S_2 solution before analysis.

Procedure

Every five minutes, the various types of solution were evaporated under vacuum at 40 °C with Rotavapor BUCHI R-250. Each sample received again 1000 µL of eluent consisted of acetonitril/water (3:7) mixture and was shaken by vortex equipment. Lastly, the tube content was transferred into a very small bottle and injected into the chromatography system for analysis.

Conditions of chromatographic analysis

The flow of the eluent within the column was 1 $mL.min^{-1}$. The injection volume was 5 µL. The mobile phase was 30% acetonitril/70% water, the column temperature was 30 °C and the pressure was set at 13 MPa, and UV detector at a wavelength of 254 nm. The recording of peaks for samples with different surfaces were realized with a microprocessor-assisted software SHIMADZU.

RESULTS AND DISCUSSION

The various solutions of glyphosate, S_1, S_2 and blank solutions are shown by recorded chromatograms in Figure 2. The chromatograms show the various peaks associated with each sample. Figure 2a presents S_1 solution; Figure 2b shows peaks related to the glyphosate solution; Figure 2c shows the blank solution and S_2 solution is shown by Figure 2d. When comparing Figures 2b and 2d, it can be seen that S_2 solution shows two new peaks at 1.41 min and 4.13 min. This second peak at 4.13 min, also appears with S_1 solution (Figure 2a), but with significant area. In this case, it could be in large amounts in S_1 solution than in S_2 solution.

It could also be attributed to phenylisothiocyanate because this component appears in the molar reaction between glyphosate and the S_1 solution. Phenylisothiocyanate could be found in small quantity in S_2 solution. However, the high peak at 1.4 min in Figure 2d seems to be the peak attributed to phenylthiocarbamoyl-glyphosate which is the result of this same reaction; this enables us to follow the glyphosate content in the samples. We also observe that the peak of the blank solution (Figure 2c) is exactly the same as that of the S_1 solution (Figure 2a), with a decrease in area. This similarity seems to suggest that S_1 solution is diluted, because the blank solution was obtained by mixing bidistilled water and S_1 solution.

With the blank solution, any peak appears at 1.4 min, while it is the same method of preparation with S_2 solution, which means that the method has not created this new peak. From what is mentioned above, we can deduce that the peak corresponding to the glyphosate does not appear when it is analyzed alone (Figure 2). This peak could be seen during its reaction with S_1 solution used for the analysis. S_1 solution yielded a product more observable in chromatography and allowed us to quantify waters contaminated.

Figure 3 shows the peaks of S_2 solution samples (0.5 $mg.mL^{-1}$) and 480 SL Tuherb concentration (0.48 $mg.mL^{-1}$). Figure 3a relating to the S_2 solution (0.5 $mg.mL^{-1}$) gives identical peak at 1.5 min like the one in Figure 2d, but its area decreased. We deduce that the signal can be attributed to the peak observed for transformed glyphosate (phenylthio-carbamoylglyphosate). To confirm the attribution of the peak, several tests were done on samples from the company Callivoire (Ivory Coast) in order to determine the quantity of glyphosate.

All the peaks observed after glyphostate transformation have been summarized in Figure 3b. A peak at 1.6 min, and the area corresponding to the peak is always equivalent to the sample of transformed glyphosate of 0.46 $mg.mL^{-1}$, approximately equal to that of the sample provided by the company which is 0.48 $mg.mL^{-1}$.

All these observations show that glyphosate does not give any signal without being transformed and S_1 solution is well suited for analysis of glyphosate in water. In well-defined analytical conditions, the peak of glyphosate could occur between 1 and 2 minutes. Then, we have determined some parameters for our work to validate our method: the retention time, linearity, repeatability, accuracy, limit of detection and limit of quantification. Table 1 presents some of these parameters.

Several tests for different concentrations of S_2 solution allowed us to observe that the retention time was about 1.6 min and the surface of the individual peaks was proportional to concentrations. These figures seem to be consistent with the retention time of 1.6 min for glyphosate obtained for our samples with an accuracy of 5%. The calibration curve between concentration and peak area observed gives a

good correlation (R^2) of 0.99 with a slope of $5{,}95.10^{-5}$. These values are very close to those generally determined into clean water (10^{-6}) (Colin et al., 2010). This value does not exceed 280 $mg.L^{-1}$ of the quantity of glyphosate.

Beyond this value, the solution seems to be too high for the chromatography to give signal. As for the values of the accuracy and the repeatability, they are well below 6%, maximum average allowable for the validity of a method of analysis. The limits of detection and quantification were obtained using the bottom noise. For an average mass concentration of glyphosate of 270 $mg.L^{-1}$, the limits of detection and quantification calculated are respectively 270 $\mu g.L^{-1}$ and 810 $\mu g.L^{-1}$. These values are widely superior to the limits of detection and quantification evaluated by most researchers in the development of a measuring method of glyphosate in water (Apha, 1995).

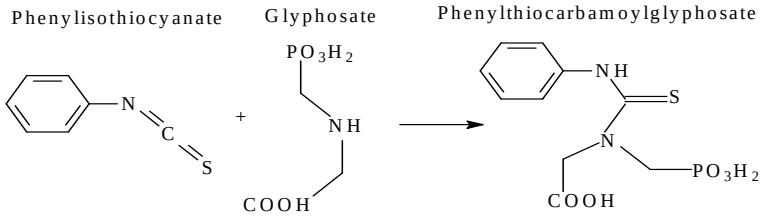
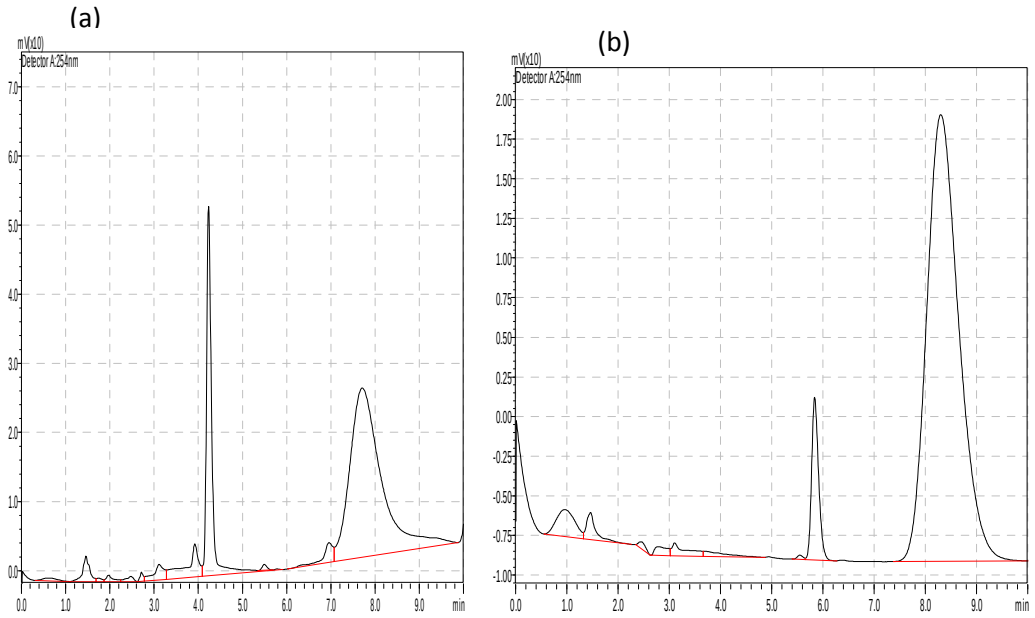


Figure 1: The glyphosate's transformation reaction.



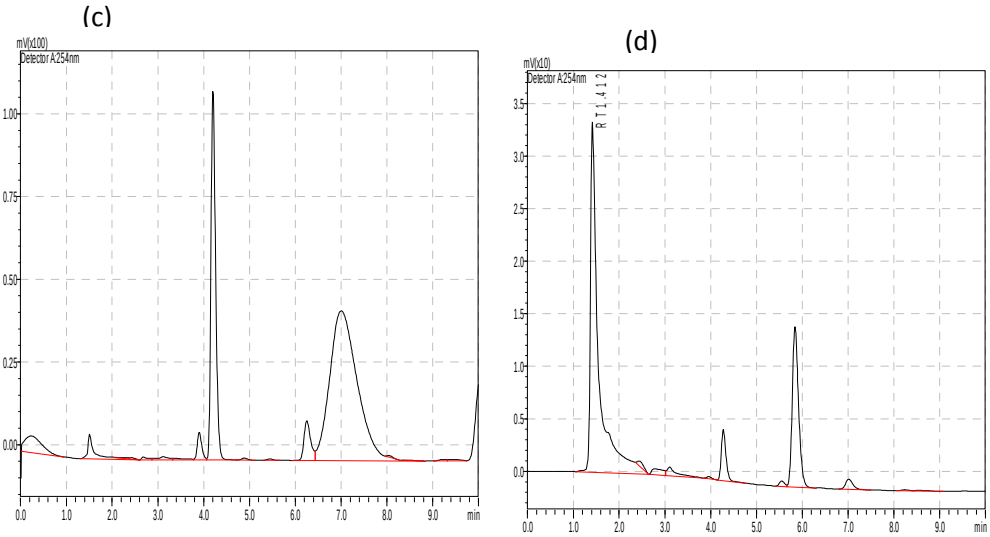


Figure 2: Chromatograms of samples: a) S_1 solution; b) glyphosate solution; c) blank solution; d) S_2 solution (1 mg.mL^{-1}).

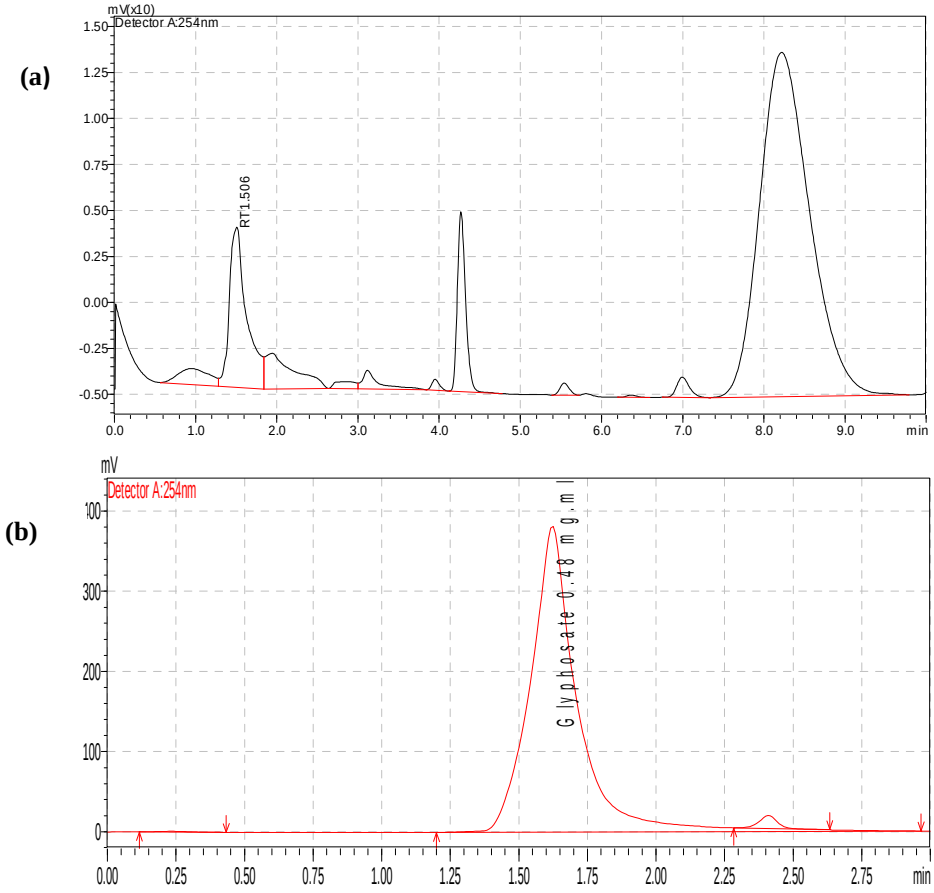


Figure 3: Chromatograms of samples: a) S_2 solution (0.5 mg.mL^{-1}) b) Tuherb 480 SL (0.48 mg.mL^{-1}).

Table 1: Validation settings.

Retention Time (RT) (min)	Coefficient of linear correlation R^2	Accuracy (%)	Repeatability (%)
1.63	0.998	0.29	0.47

This is the case of Colin et al. (2010) who, in their comparative study of FMOC-CI and NBD, found the limits of detection and quantification which were 0.04 $\mu g.L^{-1}$ and 0.06 $\mu g.L^{-1}$ respectively. This big difference between our values and those of the literature may be due to the choice of S_1 solution and especially fluorescent detector that some researchers used in their method. It is all the more verified that Pablo et al. (2008), who worked in the same operating conditions, almost reached the same limits (detection and quantification) when looking for amount of glyphosate in surface water and deposits in the North Argentina. Nevertheless, the research from the Center for Research, Development and Technology (ACER) transfer in maple syrup production, has detected up to 200 $\mu g.L^{-1}$ amino acid in the sap (Acer, 1992). These values are often closely related to the method and especially the equipment used.

Conclusion

This work shows that glyphosate alone virtually produces no signal. To quantify it, we must perform its transformation with phenylisothiocyanate (PITC). Our results are used at the Central Laboratory of Agrochemistry and Ecotoxicology (LCAE), a technical unity of National Laboratory of Support for Agricultural Development (LANADA) in Ivory Coast.

This method has been validated and tested extensively to analyze glyphosate at concentrations below 280 $mg.L^{-1}$ without any step of extraction and purification. This method is accurate to 0.29% with a repeatability of 0.47% with limits of detection and quantification of 270 $\mu g.L^{-1}$ and 810 $\mu g.L^{-1}$ respectively.

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