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Determination of glyphosate and aminomethylphosphonic acid for assessing the quality tap water using SPE and HPLC

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ABSTRACT. The use of pesticides in agriculture is one of the current problems that may result in contamination of both ground and surface water and groundwater. Considering the environmental importance and the increasing use of herbicides in Maringá region, in the present work methods for extraction and determination of glyphosate (GLYP) and aminomethylphosphonic acid (AMPA) using solid phase extraction (SPE) and high performance liquid chromatography (HPLC) were developed. For SPE, anion exchange resin was used and elution was done with hydrochloric acid 50.0 mmol L^{-1}, achieving recovery rates of 82.5-116.2% and 67.1-104.0% for AMPA and GLYP, respectively. For HPLC determination the analytes were derivatized and injected in the HPLC with a C18 column and using mobile phase consisting of phosphate buffer 0.20 mol L^{-1} at pH 3.0 and acetonitrile (85:15); the monitoring was done at 240 nm. The analysis was performed in 8 min with the same limit of detection and limit of quantification for AMPA and GLYP of 0.09 and 0.20 mg L^{-1}, respectively. The methods were applied to analysis of public water supply samples and concentrations from 2.1 up to 2.9 μg L^{-1} for AMPA and from 2.3 up to 3.3 μg L^{-1} for glyphosate were found.

Keywords: glyphosate, aminomethylphosphonic acid, SPE, HPLC, water quality.

Determinação de glifosato e ácido aminometilfosfônico para verificar a qualidade da água de abastecimento público utilizando EFS e CLAE

RESUMO. O uso de pesticidas na agricultura é um dos problemas atuais que podem resultar em contaminação do solo e de águas superficiais e subterrâneas. Levando em consideração a importância ambiental e o crescente uso de herbicidas na região de Maringá, foram desenvolvidos métodos de extração e determinação de glifosato (GLYP) e seu metabólito ácido aminometilfosfônico (AMPA) utilizando extração em fase sólida (EFS) e determinação por cromatografia líquida de alta eficiência (CLAE). Para EFS utilizou-se a resina trocadora aniônica e como eluente ácido clorídrico 50,0 mmol L^{-1}, atingindo-se taxas de recuperação na faixa de 82,5-116,2% e 67,1-104,0% para o AMPA e glifosato, respectivamente. Para determinação por CLAE, os analitos sofreram derivatização sendo injetados em cromatógrafo contendo coluna C18 e fase móvel constituída de solução tampão fosfato 0,20 mol L^{-1} em pH 3,0 e acetonitrila (85:15); o monitoramento foi efetuado em 240 nm. A análise foi realizada em 8 min com os mesmos limites de detecção e limites de quantificação para o AMPA e GLYP de 0,09 e 0,20 mg L^{-1}, respectivamente. Os métodos foram aplicados em amostras de água de abastecimento público e as concentrações encontradas estavam na faixa de 2,1-2,9 μg L^{-1} para AMPA e 2,3-3,3 μg L^{-1} para glifosato.

Palavras-chave: glifosato, ácido aminometilfosfônico, EFS, CLAE, qualidade da água.

Introduction

The use of pesticides in agriculture is one of the current problems that may result in contamination of both ground and surface water and groundwater. This contamination stems from the action of rainwater and irrigation water that can carry these compounds and take them to rivers and lakes that are used in cities for water supply (LIPOK et al., 2010). Thus, with this water uptake for human consumption it is necessary a stringent control to verify the potability of the water furnished to people and, the determination of contaminants as pesticides should be done to verify if them were eliminated during the water treatment step (DI BERNARDO; DANTAS, 2005). In Brazil, the legislation that treats about water quality and the accomplishment monitoring programs to levels contamination control can be found in government agencies like Health Department and CONAMA (BRASIL, 2005 and 2012).

Pesticides are chemical substances that can kill or control a particular organism undesirable, because have

the common property of blocking a process of vital metabolic target organisms which are toxic; the type of pesticide where the target is plants is called herbicide. It is employed to kill weeds without harming desirable vegetation, being used instead of human action and the mechanical weeding in developing and developed countries. (CHRISTOFFOLETI, 2008; OLIVEIRA JUNIOR et al., 2011).

Among the various types of herbicides, stand out from those of broad spectrum of action, such as the case of non-selective (AMARANTE JÚNIOR; SANTOS, 2002a). Glyphosate (GLYP, N-(phosphonomethyl-glicine) is classified as a non-selective, systemic and post-emergent herbicide, cited as the most sold around the world in different formulations and produced by different pesticides companies (WHO, 2003; BRITT et al., 2003). Your main degradation product is aminomethylphosphonic acid (AMPA). They have high polarity, water solubility, are insoluble in organic solvents (GALLI; MONTEZUMA, 2005; AMARANTE JÚNIOR; SANTOS, 2002a) and, they present pKa values of 0.78, 2.29, 5.96 and 10.98 for GLYP and 0.9, 5.6 and 10.2 to AMPA. These molecules show the lack of chromospheres groups, which makes the detection a problem to be solved (GARCÍA DE LLASERA et al., 2005).

There are some articles in the literature with the aim of extract and concentrate these herbicides from water samples to become possible their determination in low concentrations. Cowell et al. (1986) proposed a method using a biphasic aqueous-organic extraction to eliminate the sample matrix, followed by a posterior herbicides extraction from the water phase using a chelant and anion-exchange resins before HPLC determination, achieving recoveries around 80%.

Posterior studies extracted low polarity organic interfering with dichloromethane and the aqueous phase was treated with anionic resin resulting in recoveries of 98.3% and 86.3% for glyphosate and AMPA, respectively. With the same strategy, recoveries around 85% for both compounds by elution with citrate buffer at pH 5.0 were obtained (AMARANTE JÚNIOR; SANTOS, 2002b). In the last years more simply and directly procedures have been developed using strong (SAX) and weak (WAX) anionic resins for extraction of compounds in water samples with good percentages of recovery (CORBERA et al., 2005; CORBERA et al., 2006; JIANG; LUCY, 2007).

Due to the lack of chromophore groups in the GLYP and AMPA, many authors have been reported the use of derivatization of compounds in order to monitoring them at wavelengths higher than 200 nm. Compounds such as NBD-F (4-fluoro-7-nitrobenzofurazan), which presents good reactivity with primary and secondary amines (ZHOU, 2007) and FMOC-Cl (9-fluorenylmethylchloroformate) has been widely used for determination of GLYP and AMPA by LC/MS (PERUZZO et al., 2008; HANKE et al., 2008, GHANEM et al., 2007), whereas the derivatization with TsCl (p-toluenesulphonyl chloride), which reacts rapidly in alkaline medium with glyphosate, has been used to obtaining products from GLYP and AMPA that can be separated and detected using HPLC with UV-Vis detection (KAWAI et al., 1991; CORBERA, et al., 2005; KHROLENKO; WIECZOREK, 2005).

Considering the wide use of the herbicide glyphosate in the Maringá region it is necessary the constant monitoring of water samples to check if the currently water treatment employed in city has been efficient to eliminate GLYP and AMPA contamination. Thus, a solid phase extraction and chromatographic methods were developed to extracts, concentrates and determines GLYP and AMPA in water samples from Maringá. For the task, variables as sample pH, sample and eluent flow rates, sample and eluent volumes and nature and concentration of eluent were studied for the extraction process, as well as, the better conditions for analytes derivatization and HPLC determination were studied.

Material and methods

The solutions were prepared using deionized water purified by the Milli-Q system (Millipore Corporation) and filtered through a membrane of cellulose ester 0.45 μm (Millipore). Standard stock solutions of AMPA and GLYP (Sigma) 1000 mg L^{-1} were prepared by dissolving 0.0100 g of each compound in 10 mL of water. Working solutions of 0.010 up to 10.0 mg L^{-1} were prepared by appropriate dilutions of the stock solution with water.

100 mL of standard or samples were pumped through a cartridge containing 100 mg of anionic resin Dowex Ag1 X8-100 strongly basic at a flow rate of 5.0 mL $min.^{-1}$ using a peristaltic pump (Ismatec Model 78017-10 CP) to extracts and concentrates glyphosate and AMPA. Prior to use, the resin was conditioned with 5 mL of HCl 3.0 mol L^{-1} and 10 mL of water. Solutions of hydrochloric acid, sodium hydroxide, sodium nitrate at different concentrations were also tested to conditioning the resin and also to elution of the compound of interest. After extraction, the compounds were eluted with 1.0 mL of HCl 50.0 mmol L^{-1}, and the eluate was collected in a 5 mL vial for subsequent analysis. The resin was regenerated to the chloride form by using 10 mL of HCl 0.1 mol L^{-1} and 5 mL of Milli-Q water.

The samples were collected in Maringá water treatment station that receives water taken from the

Pirapó river. The first sample corresponds to water from the Pirapó river before any treatment; the second one was sampled after sanitation company treatment and the third sample corresponds to treated tap water that arrived at the 'Universidade Estadual de Maringá' analytical chemistry laboratory. The samples were collected in polypropylene container containing 1.0 mL of sodium thiosulfate, the pH was adjusted to 6.0 and the samples were filtered through a membrane of 0.45 μm and the extraction procedure was carried out.

For chromatographic separation and detection of GLYP and AMPA the methodology employed by Kawai et al. (1991) was adapted. The derivatization of the compounds was performed with 1.0 mL of the sample solution, adding 0.500 mL of phosphate buffer 0.40 mol L^{-1} at pH 11.0, adding 0.200 mL of p-toluenesulfonyl chloride (10 mg mL^{-1} in acetonitrile) and heating the mixture at 50.0°C in a thermostated bath for five minutes. After, an aliquot of 50 μL of the derivatized sample was injected in the HPLC system containing a C18 chromatography column (Kromasil 250 x 4.6 mm particle size 5 μm), photodiode array spectrophotometer monitoring at 240 nm and as mobile phase sodium phosphate buffer solution at 0.20 mol L^{-1} ant pH 3.0 and acetonitrile (85:15) at flow rate of 1.0 mL $min.^{-1}$ was used in a temperature of 25.0°C.

Results and discussion

The resin Dowex AG1X8-100 with a quaternary trimethylammonium exchange group and chloride as counter ion was selected for extraction and concentration of GLYP and AMPA and, the pH should be optimized to permits to maximum analytes retention as AMPA and GLYP may have positive and negative charge depending on pH.

The AMPA pKa's are 0.9, 5.6, and 10.2, therefore when the sample pH is higher than 5.6, the compound already has at least one negative net charge required to anion exchange to occur. GLYP molecule has the pKa's 0.78, 2.29, 5.96 and 10.98. Taking into account the pKa's values, the sample pH in the initial studies was adjusted to 6.0 and 8.0 using chloride ion as exchange group. When the pH was increased from 6.0 to 8.0 a slightly decreasing in analytes retention was observed; probably because at pH 8.0 the net negative charge of the molecules increases, decreasing the number of active sites available for the resin ion exchange occurs. A molecule with only one net negative charge tends to occupy only one active site while two negative charges it takes up two active sites, reducing availability for active sites and the retention of the analytes to the resin. Furthermore, when the analytes have more charge their elution is more difficult.

To evaluate the presence of other counter ion that could resulted in better efficiency in ion exchange, the clhoride was change by nitrate anion as exchange group when it was observed that the analytes retention decreased by 32% for AMPA in both pH values, whereas for GLYP the exchange group had no significant influence and the signal decreased of 3 and 0.3 % for pH 6.0 and 8.0, respectively. As chloride anion was a more effective anion in the retention process associated to a pH of 6.0, these conditions were used in the following tests.

The flow rate of sample and elution solution flow through the resin assume great importance in extraction efficiency as depending on the flow rate applied to the system more or less retention of the analytes in the resin can occur due to the time available for the establishment of the balance between resin and analyte. So, the flow rate was varied in the sample cartridge containing the resin (1.0, 3.0 and 5.0 mL $min.^{-1}$) and, it was obtained the highest percentage of retention for both analytes occurred when the sample flow rate was 1.0 mL $min.^{-1}$, but for the condition of 5.0 mL $min.^{-1}$ it was observed that the AMPA signal remained practically constant and the GLYP signal decreased 'Ca' 15% (Figure 1). As its take 100 min. for processing 100 mL of sample at a flow rate of 1.0 mL $min.^{-1}$, whereas only 20 min. is necessary when the sample flow flow rate was 5.0 mL $min.^{-1}$ and, considering that the retantion decreased at this flow rate to GLYP is not sufficent to justify the increase of 80 min. in the analisys time, the chosen flow rate was 5.0 mL $min.^{-1}$.

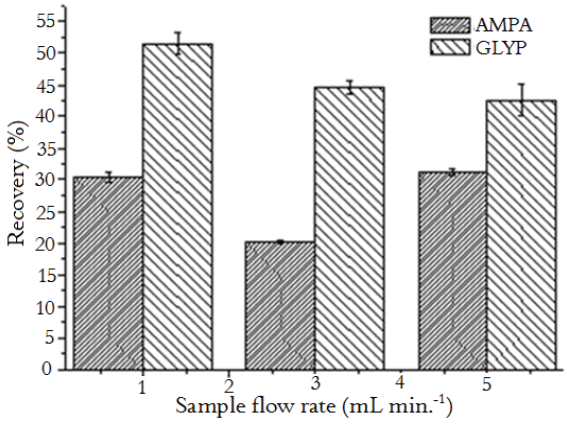

Figure 1. Effect of sample flow rate on AMPA and GLYP recoveries. Data obtained for 100 mL of AMPA and GLYP 0.50 mg L^{-1} at pH 6.00. Sample flow 1.0, 3.0 and 5.0 mL $min.^{-1}$ and HCl 10.0 mmol L^{-1} as eluent at flow rate 1.0 mL $min.^{-1}$.

After the establishment of the sample flow rate, the eluent flow rate was varied (0.5, 1.0 and 2.0 mL $min.^{-1}$)

and, it was not observed significant differences in the percentage of analyte eluted when the eluent flow rate varied from 0.5 to 1.0 mL min.$^{-1}$; thus, 1.0 mL was selected, which results in recoveries percentage of 30% and 40%for AMPA and GLYP, respectively (Figure 2).

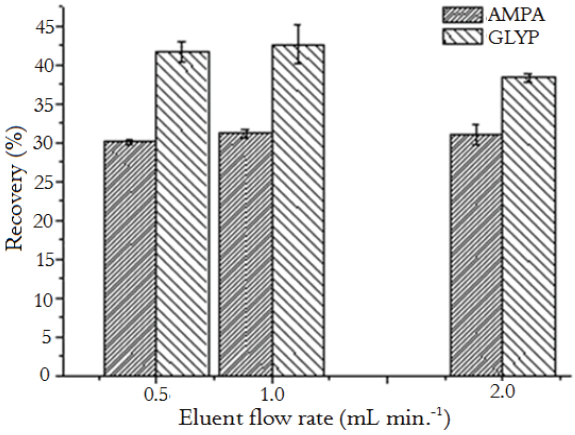

Figure 2. Influence of eluent flow rate in AMPA and GLYP recovery. Data obtained for 100 mL of AMPA and GLYP 0.50 mg L^{-1} at pH 6.00. Sample flow rate of 5.0 mL min.$^{-1}$ and HCl 10.0 mmol L^{-1} as eluent at flow rates of 0.5, 1.0 and 2.0 mL min.$^{-1}$.

Considering that analytes recoveries were low and it taking into account the hypotheses that the low yield was due to the saturation of the anionic resin because of high analyte concentration (0.50 mg L^{-1}), the analytes concentrations were decreased to 0.20, 0.10 and 0.010 mg L^{-1} and the recoveries increased considerably for both compounds to flow rates of 5.0 and 1.0 mL min.$^{-1}$ for sample and eluent, respectively (Figure 3).

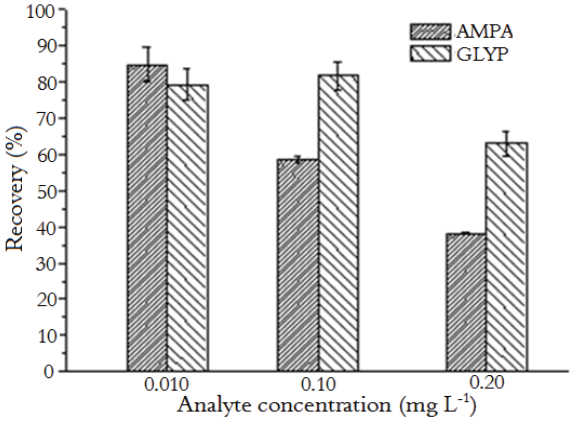

Figure 3. Effect of analyte concentration in the recovery of AMPA and GLYP. Data obtained for 100 mL of standard solutions of AMPA and GLYP at concentrations of 0.010, 0.10 and 0.20 mg L^{-1} at pH 6.00. Sample flow rate of 5.0 mL min.$^{-1}$ and HCl 10.0 mmol L^{-1} as eluent at flow rate 1.0 mL min.$^{-1}$.

It was also studied the sample volume which could be passed through the resin without being reached the maximum point of saturation. For this task, it was tested the sample volumes of 100, 150, 200, and 250 mL and the analyte concentration was kept as 0.010 mg L^{-1}. It was observed that for sample volumes of 150 and 250 mL low recoveries values were obtained for both analytes (Figure 4). These low recovery values may be related to the entrainment of analytes retained in the resin due to the excess of aqueous phase. When the sample volume was 100 mL it was obtained better analytes retention and better recoveries values (88.42 ± 1.50 and 76.16 ± 1.30% to GLYP and AMPA, respectively) (Figure 4).

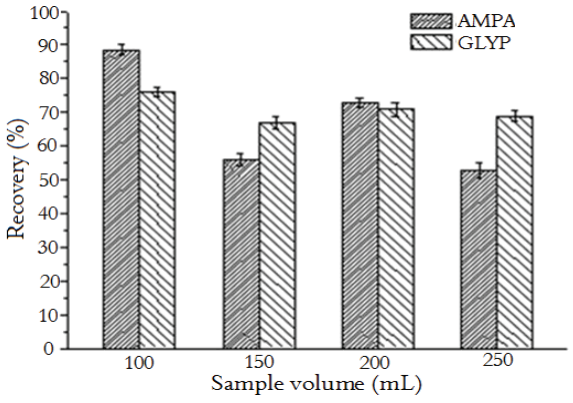

Figure 4. Effect of sample volume on the analytes recoveries. Data obtained for 100, 150, 200 and 250 mL of standard AMPA and GLYP solution 0.010 mg L^{-1} at pH 6.00. Sample flow rate of 5.0 mL min.$^{-1}$ and HCl 10.0 mmol L^{-1} as eluent at flow rate 1.0 mL min.$^{-1}$.

Finally, it was tested the eluent concentrations (10.0, 50.0, 75.0 and 100 mmol L^{-1} of HCl) and eluent volumes 1.0 and 5.0 mL (Figure 5). When it was used 1.0 mL of HCl 10.0 mmol L^{-1} the maximum analytes recoveries were 68.86 ± 1.37% for the AMPA and 46.37 ± 2.48% for GLYP (Figure 5A), indicating that part of analytes were still attached to the resin after the elution process. For the same concentration and for eluent volume of 5.0 mL (Figure 5B), there was a decrease in the AMPA recovery and slightly increase in GLYP recovery.

Increasing the eluent concentration to 50.0 mmol L^{-1} and using eluent volume of 1.0 mL there was a significant gain in the recovery percentage for both analytes, reaching values of 97 ± 3% for GLYP and 88 ± 10% for AMPA (Figure 5A). Maintaining eluent concentration and increasing the eluent volume to 5.0 mL the recovery to AMPA and GLYP decreased drastically to 39 and 6%, respectively. When the eluent concentration were increased to 75.0 and 100.0 mmol L^{-1} the analytes recoveries decrease sharply for both compounds. This may be related to the excess of HCl eluted with the analytes, which altered the ionic strength of the system, interfering in the subsequent analysis. Therefore, the selected eluent conditions were 1.0 mL at a flow rate of 1.0 mL min.$^{-1}$ and concentration of 50.0 mmol L^{-1}.

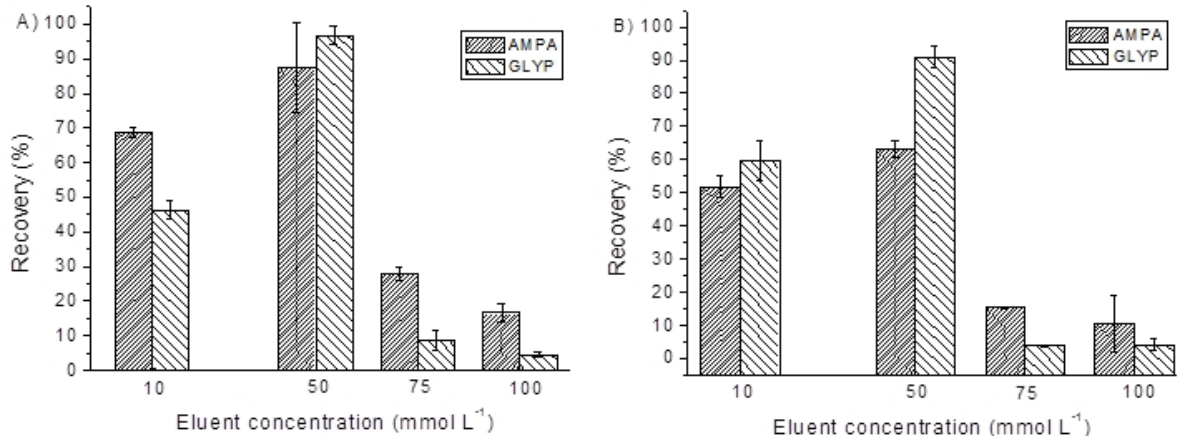

Figure 5. Effect of eluent concentration and volume on the analytes recoveries. Data obtained to 100 mL of standard solutions of AMPA and GLYP 0.010 mg L^{-1} at pH 6.0. Sample flow rate of 5.0 mL $min.^{-1}$ and HCl as eluent at flow rate 1.0 mL $min.^{-1}$ in different concentrations (10.0, 50.0, 75.0 and 100.0 mmol L^{-1}). A-1.0 mL of eluent B-5.0 mL of eluent.

The final condition for extraction of AMPA and GLYP are presented in Table 1; under these conditions it was possible to achieve analytes concentration factors of 90 and 97 times for GLYP and AMPA, respectively.

Table 1. Optimized conditions to extracts and concentrates AMPA and GLYP by SPE from water samples.

Solution	Volume (mL)	Flow rate (mL $min.^{-1}$)
HCl 0.10 mol L^{-1}	10.0	5.0
H_2O	5.0	5.0
Sample	100.0	5.0
HCl 50.0 mmol L^{-1}	1.0	1.0

The analytical curves were linear Area = -14499 + 360262 [AMPA], R^2= 0.9997 for AMPA and Area = -14146 + 373700 [GLYP], R^2= 0.99954 for GLYP, for the concentration range between 0.20 – 10.00 mg L^{-1} and the limits of detection (LOD) and quantification (LOQ) were equal for GLYP and AMPA (0.09 and 0.20 mg L^{-1}, respectively) to the injection volume of 50.0 μL.

When the proposed method was applied to determine AMPA and GLYP in water samples it is showed adequate, mainly because the sample enrichment factor was about one hundred times, which permitted to get the chromatographic determination of analytes in tap water samples (Table 2, Figure 6) that presents very low concentration of the analytes.

Table 2. Recovery assay (%) to AMPA and GLYP in river and tap water samples.

Water sample	Determined Concentration (mg L^{-1})		Added Concentration (mg L^{-1})	Recovery (%)	
	AMPA	GLYP		AMPA	GLYP
Pirapó river water	0.21 ± 0.03	0.33 ± 0.06	0.500	116.2 ± 12.4	104.0 ± 19.0
Filtered water	0.29 ± 0.10	0.29 ± 0.10	0.500	101.0 ± 5.0	67.1 ± 13.4
Tap water	0.26 ± 0.06	0.23 ± 0.03	0.500	82.5 ± 19.4	95.7 ± 4.4

The recovery test indicated that the recovery percentages ranging from 82.5 to 116.2% for AMPA and from 67.1 to 104.0% for GLYP (Table 2), which can be considered excellent for quantitative analysis for analytes with low concentration. Furthermore, the method presented good selectivity as it was not observed coelutions of interferants at the same analytes retention times (Figure 6).

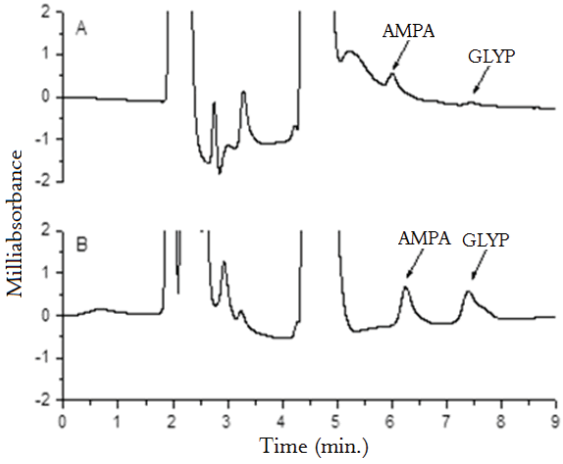

Figure 6. Chromatogram of river water sample before and after standard addition. Data obtained for 50 μL of extract of river water sample without (A) and with standard addition (B, 0.500 mg L^{-1}) of glyphosate and AMPA. The analytes were derivatized in the own mobile phase (sodium phosphate 0.20 mol L^{-1} at pH 3.00: acetonitrile (85:15) at a flow rate of 1.0 mL $min.^{-1}$) with p-toluenesulphonyl reagent 10 mg mL^{-1}.

Conclusion

The proposed method to extract and to determine AMPA and GLYP in water samples demonstrated the ability to extract and concentrate the analytes from tap and river water samples and it was able to do the chromatographic determination of the compounds after derivatization. The analysis was performed in 8 min. with the same limits of detection and

quantification to GLYP and AMPA (0.09 and 0.20 mg L^{-1}, respectively) and it was found analytes concentrations between 2.1 and 2.9 μg L^{-1} for AMPA and 2.3 and 3.3 μg L^{-1} for glyphosate, indicating that water treatment was not effective to removes the herbicide and its metabolite.

Acknowledgements

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