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DETERMINATION OF GLYPHOSATE IN WATER SAMPLES WITH THE COMBINATION OF CATION-EXCHANGE CHROMATOGRAPHY AND CAPILLARY ELECTROPHORESIS

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ABSTRACT

An analytical method for determination of pesticide Glyphosate in water as a combination of cation-exchange chromatography and capillary electrophoresis is presented. Pure water was spiked with Glyphosate at concentrations 0.1, 0.25, 0.5 and 1 mM and percolated through a strong cation-exchange column packed with Dowex 50WX4-400 resin in its H^+ form. The extract was further analyzed by capillary electrophoresis in indirect detection mode. The calibration curve for the pesticide in the range 0.1–2.5 mM was linear and with high degree of reproducibility. The obtained recoveries for all the studied concentrations amount 85%. Afterwards, the possibility to determine Glyphosate at the concentration 0.001mM (0.17 μg/ml) was checked by percolation of 100 ml of water sample through a column. The calculated recovery was 97.7%.

Keywords: Glyphosate; Ion-exchange chromatography; Capillary electrophoresis; Pesticides

INTRODUCTION

Among different pesticides, *N*-(phosphonomethyl)glycine with the common name Glyphosate is one of the most intensively applied herbicides. Glyphosate gives the possibility to control a great variety of weeds. It has found its application in agriculture in pre-crop, post-weed emergence in a wide range of crops as well as in forestry, gardening and horticulture. This compound is resistant to hydrolytic and photolytic degradation. Typically, in plants Glyphosate only slightly metabolized to (aminomethyl)phosphonic

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acid (AMPA). In soils, Glyphosate is readily decomposed by microorganisms to AMPA, and further to carbon dioxide and phosphate. In animals, Glyphosate is excreted rapidly in the faeces and urine and undergoes very little metabolism.

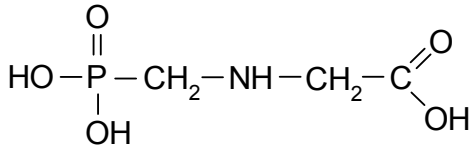

Fig. 1. Structure of Glyphosate.

The widespread appearance of pesticides in crops and other plant residues brings about several problems that concern possible environmental pollution and, in turn, the harmful influence on the human health. Therefore, several public institutions such as The Environmental Protection Agency and The European Union Commission set the strict limits for the concentration of pesticides in environmental and food samples. For example, in the case of a single active compound in water, the maximum concentration limit is 0.1 μg/ml, and for the content of all pesticides 0.5 μg/ml [1-3]. These prerequisites and the need to control the level of those pollutants enforced the rapid development in analytical methods with the appropriate limit of detection.

There is a significant number of measurement procedures for determinung of Glyphosate by such analytical methods as high-performance liquid chromatography (HPLC,) liquid chromatography-mass spectrometry (LC/MS), gas chromatography (GC), gas chromatography-mass spectrometry (GC/MS), capillary electrophoresis (CE) ([4] and references within). All these methods can be characterised by high sensitivity, low limit of detection and reproducibility. Nevertheless, in the trace analysis of Glyphosate, concentrations of the analyte are often so extremely low that no method is available for Glyphosate determination without pre-concentration and/or purification step. Additionally, in many cases interferences accompany the studied analyte in native matrixes. For this reason clean-up of a sample is necessary to eliminate compounds interfering the detection.

The approved and emerging methods of Glyphosate analysis still suffer from some drawbacks in the sample preparation procedures. The most frequently applied technique, such as GC, requires previous derivatization of Glyphosate to obtain a volatile and stable molecule. Different schemes and reagents are applied for derivatization, e.g. trifluoroacetic anhydride – heptafluoro-1-butanol (TFAA–HFB) [5], acetic acid – trimethyl orthoacetate (acetic acid–TMOA) [6], trifluoroacetic anhydride – trifluoroethanol (TFAA–TFE) [7] and others. Flame

photometric detection (FPD), nitrogen-phosphorus detection (NPD), electron-capture detection (ECD), as well as such extremely sensitive detection technique as mass spectrometry detection (MS) have been adapted in Glyphosate analysis. Some authors showed that when GC is used with an appropriate derivatization and detection system, it is possible to determine Glyphosate in water at concentrations varying from 0.05 μg/L [5] to 0.36 μg/L [7]. Another attractive technique for Glyphosate trace analysis is LC. The lack of chromophore or fluorophore necessitated derivatization for determination of Glyphosate by this technique. The required selectivity and sensitivity in the LC analysis are reached by derivatization using either pre- or post-column mode. For derivatization, some reagents such as 9-fluorenylmethylchloroformate (FMOC–Cl) [8], *o*-phthalaldehyde – 2-mercaptoethanol (OPA–ME) [9] found their application yielding highly fluorescent derivatives, which then can be determined by fluorescence (FL) detectors. Applying LC technique with the derivatization step, very low concentrations of Glyphosate in water samples, ranging from 0.1 μg/L [8] to 2 μg/L [9] can be determined. The analytical techniques mentioned above cover the limit of concentration of Glyphosate in water (0.1 μg/ml). The application of GC and LC equipment with selective detectors gives very reliable results but frequently, it is unaffordable in many laboratories. Therefore, some alternative techniques should be developed for this purpose for example, e.g. capillary electrophoresis (CE). This advantageous technique is characterized by low cost of analysis, minimal use of the sample and robustness.

In our study, we decided to combine the cation-exchange chromatography pre-concentration with capillary zone electrophoresis for measurement of Glyphosate content in water. The pesticide concentration was determined using UV indirect signal detection. This investigation has been performed to verify the recoveries of pesticide in various concentrations. As a result, this verification should provide a helpful information that can be used in elaboration of the method of Glyphosate determination in juice samples.

EXPERIMENTAL

Materials

Potassium hydrogen phthalate was obtained from Fluka (Poland). Tetradecyltrimethylammonium bromide (TTAB), Dowex 50WX4-400 ion-exchange resin and *N*-(phosphonomethyl)glycine were purchased from Sigma (Poland). Water was purified with MilliQ system (Millipore, Bedford, MA, USA). Methanol was obtained from POCh (Gliwice, Poland). All other chemicals were of analytical-reagent grade.

Cation-exchange (CAX) mobile phase solution was obtained by mixing 160 ml water, 40 ml methanol and 2.7 ml concentrated HCl. Acidic Modifier Solution (AMS) was obtained by mixing 160 ml water with 13.4 ml concentrated HCl.

SAMPLE EXTRACTION

Pure water was spiked with Glyphosate at 0.1, 0.25, 0.5 and 1 mM. To 4.5 ml of each solution 0.5 ml AMS was added. Subsequently, 100 ml water sample with the concentration of Glyphosate of 0.001mM (0.17 μg/ml) was prepared. To 90 ml of this solution 10 ml AMS was added. pH of the obtained solutions ranged from 0.7 to 0.8.

A disposable glass pipette was packed with Dowex 50WX4-400 ion-exchange resin so that the volume of the bed was 2 ml. Prior to every enrichment step, the column was washed with 10 ml 1 M HCl and 10 ml of water to obtain H^+ form of the resin. Subsequently, 1 ml water sample containing herbicide was percolated through the column. Then the column was washed twice by 0.75 ml CAX solution, and after that, the studied compound was eluted with 12 ml CAX. The eluted solution was evaporated under vacuum at 45ºC. The residuum was dissolved in 10 ml water and evaporation was repeated. Finally, the residuum was dissolved in 1 ml water and was analyzed by means of CE.

GLYPHOSATE DETERMINATION WITH CAPILLARY ELECTROPHORESIS

To determinate Glyphosate, capillary zone electrophoresis was used. The analyses were performed with a Beckman P/ACE 5000 system (Beckman, Palo Alto, CA, USA) with a fused-silica capillary of the total length 57 cm and the effective length 50 cm x 50μm I.D. The capillary was thermostated at 25ºC. The samples were loaded by pressure at 10 seconds (20 psi) and separated under reversed polarity using voltage of 27 kV. Peaks were detected at 254 nm by indirect method. As background electrolyte (BGE) a 10 mM potassium hydrogen phthalate buffer with 0.5 mM TTAB adjusted to pH 7.5 with NaOH was used. At the beginning of the day capillary was conditioned for approximately 1 h using a rinse cycle of 5 min water, 30 min NaOH (0.1 M), 15 min water and 10 min BGE. Prior to every injection the capillary was rinsed with 1min NaOH (0.1 M), 1 min water and 1 min BGE.

All Glyphosate containing solutions were kept in plastic bottles because Glyphosate molecules tend to adsorb on glass surface.

RESULTS AND DISCUSSION

Calibration of capillary electrophoresis analysis

Calibration measurements for Glyphosate were performed by injecting standard solutions over the concentration range 0.1–2.5 m*M*. Within this range and under the given conditions the calibration curve was found to be linear with the correlation factor 0.9991 (n=5).

Analysis

In the literature, many pre-concentration and clean-up methods for water samples containing Glyphosate are described. Stalikas [4] and Tadeo

[11] presented in their comprehensive reviews all available sampling procedures for Glyphosate. Additionally, our laboratory took part in collaborative study for Glyphosate determination in food samples with cation-exchange chromatography as a clean-up step, derivatisation and GC-MS determination. Therefore, we decided to use this method for juice samples but using CE-UV instead of GC-MS determination. The main purpose of such exchange was to simplify the analytical procedure and reduce time of sample preparation. However, in order to achieve it, it was necessary to verify this method by using of water samples containing Glyphosate.

The pH of Glyphosate water solution was about 2.2. Before passing the sample through the cation-exchange column, AMS was added to each of the studied water solution in order to reach pH 0.8. Maintaining such pH is important as it influencesthe content of ionic form of Glyphosate. As we chose cation-exchange chromatography as the pre-concentration step, the studied molecule must be in cationic form:

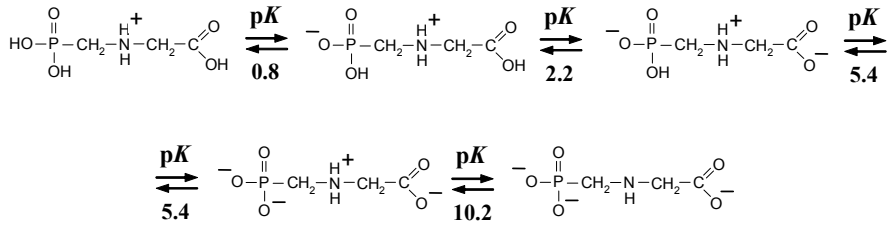

Fig. 2. Ionization processes for Glyphosate [10].

It has been noted before that the presence of acids as well as salts in sample directly interferes the performance of capillary zone electrophoresis and also such the effect was observed in our case. Therefore, in order to avoid it, after elution from the column solution was evaporated, residuum was dissolved in water and evaporation was repeated again. Double evaporation allows us to remove hydrochloric acid and to obtain more informative electropherogram.

Recovery studies

In Tab. 1, the recoveries after the cation-exchange chromatography extraction step are summarized.

As it can be seen from Tab. 1 the recoveries are acceptable and vary from 76% to 99%. It is worth pointing out that the interdependence between the concentration of the analyzed compound and the recovery values was observed. At lower concentrations, the recoveries are higher which is consistent with the purpose of this investigation, i.e. the determination Glyphosate at µg/ml level.

Tab. 1. Recovery factors for pre-concentration of 1 ml water sample containing Glyphosate

Concentration of Glyphosate in samples (mM)	Recovery (%)			
	1st analysis	2nd analysis	3rd analysis	mean
1	80.4	77.5	71.5	76.5
0.5	73.0	84.1	71.8	76.3
0.25	86.6	97.2	92.1	91.9
0.1	105	82.3	110.7	99.3

To check the possibility to determine lower concentration of Glyphosate we made an attempt to analize 100 ml water sample at concentration 0.001 mM (0.17 μg/ml). Solution was percolated through the cation-exchange column and pesticide concentration was determined by capillary electrophoresis with indirect UV detection. The typical electropherogram for this sample is presented on Figure 3.

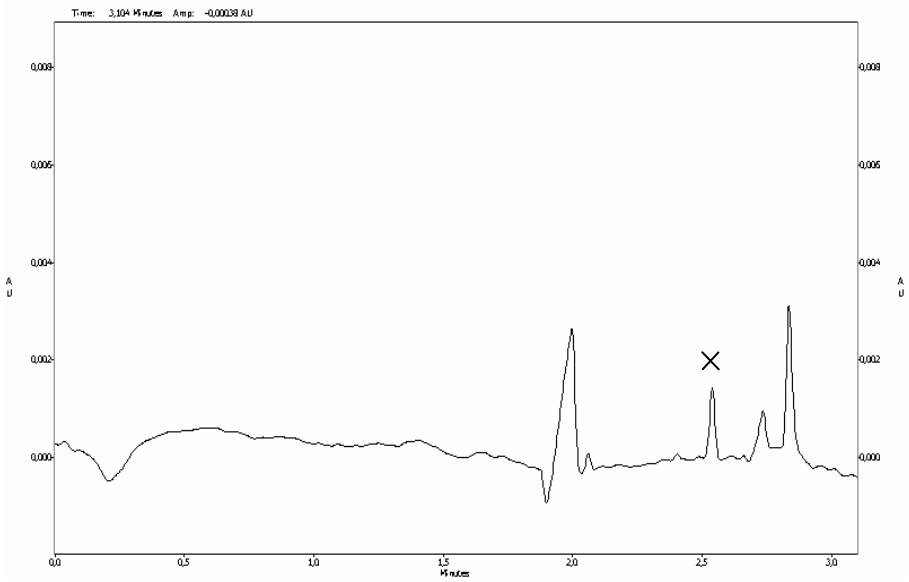

Fig. 3. Electropherogram of water sample containing Glyphosate at 0.001mM (0.17 μg/ml) after 100 fold concentration.

The calculated recovery was equal to 97.7%. Peak for Glyphosate corresponding to the concentration 0.001 mM (0.17 μg/ml) is high and sharp. In comparison with other developed techniques for Glyphosate analysis in water samples, for instance GC/MS and HPLC/FL by which very low concentrations of the studied pesticide can be determined (for GC/MS 0.05 μg/L [5], for HPLC/FL 0.1 μg/L [8]), the CE/UV technique is more or less at the same level of sensitivity. In the case of Glyphosate determination at the concentration 0.1 μg/ml it is possible to improve the sensitivity of the method through the appropriate optimization of the procedure. Because to CE analysis nanolitres of a sample are required, the residuum after evaporation can be dissolved in water of volume lower than 1 ml, for instance in 0.2 ml. In turn, contrary to GC and HPLC, the developed method does not require such a time-consuming step as derivatization, in which expensive and sometimes hazardous reagents are applied, or application of very selective detectors.

CONCLUSIONS

In the case of water samples, the most frequently applied pre-concentration method is evaporation of water with the following derivatization of Glyphosate. Another way is percolating the water sample containing the studied compound through the anion- or cation-exchange column. Our preliminary results show that when cation-exchange chromatography was used as a pre-concentration step, the obtained recoveries were about 85%. Comparing the recoveries obtained by other researches using the same pre-concentration method, our results are quite similar and acceptable.

The results of our investigation show that it is possible to apply a combination of cation-exchange chromatography to efficiently extract Glyphosate from water samples and detect the presence of this pesticide by the simple capillary electrophoresis method with indirect UV detection. Those results are very promising, however further optimization of the extraction procedure is required.

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