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Determination of glyphosate and its metabolite aminomethylphosphonic acid in fruit juices using supported-liquid membrane preconcentration method with high-performance liquid chromatography and UV detection after derivatization with *p*-toluenesulphonyl chloride

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Abstract

The application of supported-liquid membrane (SLM) technique for effective extraction of *N*-(phosphonomethyl)glycine (glyphosate) and its primary metabolite aminomethylphosphonic acid (AMPA) from juices (orange, grapefruit, apple and blackcurrant) in combination with HPLC-UV detection after derivatization with *p*-toluenesulphonyl chloride (TsCl) is presented. The influence of various parameters such as the composition of acceptor phase, flow-rate, concentration of analytes, on the performance of extraction procedure, was studied. It was shown that by appropriate manipulation of SLM parameters the level of detection could be significantly improved. The influence of SLM conditions on extraction efficiency of studied compounds was also discussed. Selection of the optimal conditions enable detection of glyphosate and AMPA in juices at concentrations as low as 0.025 mg/l. The calculated recoveries for glyphosate were—71.1, 72.1, 93.6, and 102.7% and for AMPA—64.1, 64.6, 81.7, and 89.2%, for orange, grapefruit, apple and blackcurrant juices, respectively. The results suggest that the application of SLM extraction as a method for glyphosate and AMPA enrichment from complicated liquid matrices may be useful mean of routine analysis.

Keywords: Glyphosate; Aminomethylphosphonic acid; Supported-liquid membrane extraction; HPLC; Fruit juice; Pesticides

1. Introduction

Glyphosate (*N*-(phosphonomethyl)glycine) is a nonselective, post-emergence herbicide used for the control of a wide range of weeds [1]. It can be used on non-crop land as well as in a great variety of crops. Glyphosate is the active ingredient in the commercial herbicide Roundup®, marketed by Monsanto, and Touchdown, marketed by Zeneca Ag Products. It is an acid, but usually used in a salt form, most commonly the isopropylamine salt. Because of its relatively low toxicity to mammals, it has become one of the most widely used herbicides in the world. This widespread application generates problems with the contamination of the environment with this substance and therefore reliable methods are required for monitoring of this herbicide in crops, fruits and vegetables.

A great variety of analytical methods have been applied for determination of glyphosate. Both gas chromatography (GC) and liquid chromatography (LC) are used with various detection systems. GC analysis is performed after a derivatization procedure that converts glyphosate to a sufficiently volatile and thermally stable derivative [2–5]. In LC methods derivatization procedures, producing fluorescent derivatives, are often employed to enhance the sensitivity and selectivity of detection [6–9]. In many cases derivatization procedures are quite complicated and require special equipment. In recent years capillary electrophoresis (CE) has become a technique utilized for glyphosate determination more and more frequently [10–12]. While GC, HPLC and CE are well-developed methods for glyphosate analyses, enzyme-linked

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immunosorbent assay (ELISA) has become an alternative method [13–15].

In order to analyze low concentrations of glyphosate methods for enrichment and purification of analytes are required. They include such well-established techniques as: liquid-liquid extraction, solid-phase extraction as well as ion-exchange chromatography. Supported-liquid membrane extraction technique (SLM) can be considered as alternative for pretreatment of liquid samples containing herbicides.

SLM is a porous polymeric hydrophobic membrane with organic solvent immobilized in its pores. This membrane separates the aqueous (donor) phase and the receiving aqueous (acceptor) phase. During the extraction three simultaneous processes take place: extraction of the compound into the organic phase, its transport through the membrane and re-extraction into the acceptor phase. One of the main advantages of SLM is simultaneous extraction and clean-up of the compound of interest. Enrichment factors and the limits of detection obtained after SLM application are comparable to other extraction techniques, moreover, in many cases samples are much more cleaner.

SLM extraction has been successfully applied for separation and enrichment of various types of herbicides, such as, triazines [16,17], chlorophenoxyalkanoic acids [18], and chlorinated phenols [19]. There are two ways of operating the SLM system, which depend on the charge of the extracted analyte. In the case of acidic and basic compounds the enrichment is achieved by adjusting the pH of the donor and acceptor phases to appropriate values [20]. In order to extract multicharged compounds it is necessary to use a carrier incorporated into the membrane organic phase [21,22]. This carrier should bear a functional group with a charge opposite to the charge of the transported molecule. Such a carrier facilitates passage of the analyte through the liquid membrane by formation of neutral, organic phase soluble ion-pair complexes.

In comparison to all herbicides used in agriculture glyphosate is one of the most difficult to analyse. These difficulties originate from its chemical properties, namely high water solubility and polar nature, which limit the options for application of standard preparation methods, like solvent–solvent extraction. Its similarity to naturally occurring amino acids and small amino sugars contributes to the difficulty in determining residues of the compound in food samples. Many studies describing analysis of water samples for glyphosate presence have been published, but the number of publication where food samples have been analyzed is quite limited [2,4,23].

Our previous experiments showed that supported-liquid membrane extraction may be a very effective technique for simultaneous extraction and purification of herbicides of various structure from fruit juices [16]. There are reports in the literature describing extraction of glyphosate with SLM from water [24,25], but this technique has not been directly applied as preparation step for analysis of food samples.

The main goal of this work was a development of a simple analytical procedure involving SLM technique for the extraction and purification of glyphosate and AMPA from fruit juices followed by their determination with HPLC-UV after a simple derivatization procedure. It was also important that the detection limits reaches the maximum residue limits for the herbicide in food established in European Union, which is set at the level 0.1 mg/l (ppm) [26]. Various parameters affecting the analyte extraction, namely: flow-rate of phases, composition of acceptor phase and concentration of the studied compounds were examined and optimized.

2. Experimental

2.1. Chemicals

Aliquat 336-methyltrioctylammonium chloride was obtained from Janssen (Beerse, Belgium). Di-*n*-hexyl ether (DHE) used as the liquid membrane, and *N*-(phosphomethyl)glycine (glyphosate) was obtained from Sigma (St. Louis, MO, USA); aminomehtylphosphonic acid (AMPA) was obtained form ICN (Warsaw, Poland); the derivatization reagent, *p*-toluenesulphonyl chloride (TsCl) was from BDH (Poole, UK); acetonitrile for HPLC was from Chempur (Piekary Śląskie, Poland). Inorganic salts: KH_2PO_4, KOH, NaOH were purchased from POCh S.A. (Gliwice, Poland). All chemicals were of analytical grade. Water was purified with a Milli-Q-RO4 system (Millipore, Bedford, MA, USA).

All fruit juices are commercially available and are produced by Hortex Holding (Poland).

2.2. Membrane equipment

The membrane unit is composed of two circular PTFE blocks (120 mm diameter and 8 mm thickness) with grooves arranged as an Archimedes' spiral (0.25 mm depth, 1.5 mm width and 2.5 m length, with total volume of ca. 0.95 ml). To stabilize the whole construction aluminum blocks of 6 mm thickness were used on both sides of the PTFE blocks. A porous PTFE membrane with polyethylene backing (0.2 μm pore size, 175 μm total thickness with 115 μm backing and porosity 0.70 (Millipore FG, Millipore) was impregnated with 20% Aliquat 336 solution in DHE for 30 min. The membrane was then placed between two PTFE blocks and the whole construction was clamped tightly with six screws. After installation of the membrane, excess of organic solution on the surface was eliminated by pumping ca. 10 ml of water through both channels.

The water solutions and juices used in experiments were pumped with a peristaltic pump (Minipuls 3, Gilson Medical Electronics, Viliers-le-Bel, France) using acid resistance tubing (Acid Mainfold Tubing, Elkay Products, Shrewsbury, MA, USA) connected to the membrane unit with Altex screw