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Phenyltin compounds: dispersive liquid–liquid microextraction and gas chromatographic–mass spectrometric determination

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Dispersive liquid–liquid microextraction in combination with gas chromatographic–mass spectrometric determination is suggested for phenyltin compound analysis in aqueous solutions. The derivatization of the analytes with sodium tetraethylborate was carried out prior to the extraction. The effects of extraction and disperser solvent type, volume and extraction time on the extraction efficiency were investigated. Tetrachloromethane was used as an extraction solvent, ethanol was used as a disperser solvent, and hexachloroethane was used as an internal standard. The calibration graphs were linear from 46 ng l^{-1} (monophenyltin), 161 ng l^{-1} (diphenyltin) and 152 ng l^{-1} (triphenyltin) up to 1 mg l^{-1} (for all the analytes), correlation coefficients were 0.996–0.999, limits of detection were 14, 58 and 46 ng l^{-1} for monophenyltin, diphenyltin and triphenyltin, respectively. Repeatabilities of the results were 4.6–17.3%. A possibility to apply the proposed method for phenyltin compound determination in river water was demonstrated.

Key words: phenyltin compounds, derivatization, microextraction, gas chromatography–mass spectrometry, river water

INTRODUCTION

Phenyltins are among the most often used organotin compounds in commercial applications. Triphenyltin (TPhT) compounds are used in agriculture as fungicides, antihelmintics, herbicides, pesticides, as biocides in antifouling paints to prevent growth of fouling organisms on boats and ships. Mono- and disubstituted phenyltins are effective heat and sunlight stabilizers in polyvinyl chloride plastics such as rigid pipes, panels and soft wall-coverings, furnishing, floorings, toys and containers for food [1–5]. Because of their widespread use, phenyltin compounds can be found in different ecosystems. In the environment phenyltin degradation is thought to be via di- and monophenyltin intermediates and finally to inorganic tin, Sn(IV) [4, 6].

Toxicity of tin compounds strongly depends on their species [4]. Inorganic tin is considered to be harmless, while TPhT is very toxic and can promote harmful effects on aquatic organisms, even at low ng l^{-1} level [6, 7]. Recent studies showed that phenyltin compounds interfere with heme metabolism as well as the cardiovascular system, cause a fall of blood pressure resulting from a depression of the vascular smooth muscle, alert blood composition and result in a decrease in the organ [1], decrease human natural killer cell cytotoxic function, thus increasing the risk of exposed individuals to cancer and / or viral infections [3].

Since toxicity is strongly dependent on the species, speciation is of major interest in phenyltin analysis. For this purpose, gas chromatography is the most common approach. It offers excellent resolution, and several detectors, such as flame photometric [8–10], atomic emission spectrometric [2, 11], mass spectrometric detectors [4, 11–14], inductively coupled plasma mass spectrometry [7, 15] well suited to phenyltins.

As a rule, phenyltin compounds present in the environment are ionized, thus before gas chromatographic analysis they should be derivatized to obtain their volatile and thermostabile forms. The most commonly used derivatization strategies are formation of hydrides by sodium borohydride and alkylation by alkylborates or by Grignard reagents [16]. Grignard derivatization is tedious, time-consuming and requires dry conditions. Derivatization products obtained using sodium borohydride suffer from the lack of stability. Contrarily, alkylborates obtained by a derivatization procedure using sodium tetraalkylborate are stable in the water and the derivatization step can be accomplished in the aqueous phase [17].

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Thus, sodium tetraethylborate is the most frequently used derivatization reagent for organotins.

Different extraction techniques have been used to isolate and concentrate phenyltin compounds from the matrix [18]. Liquid–liquid extraction and solid phase extraction are the most widespread techniques, however, they are slow, labour intensive, consume large volumes of toxic organic solvents. In recent years, a preconcentration using microextraction techniques is gaining a growing interest. For phenyltins extraction, a miniaturized version of solid phase extraction – solid phase microextraction – is quite popular [5, 12, 19, 20]. Tributyltin and triphenyltin were also extracted by stir bar sorptive extraction [15]. Liquid phase microextraction (LPME) techniques have been developed as a miniaturised version of liquid–liquid extraction. Only few articles deal with liquid phase microextraction of phenyltins. In [14] single drop microextraction of butyltins and phenyltins is followed by gas chromatographic analysis, in [21] butyl- and phenyltins are extracted into a single drop of an ionic liquid and analyzed by HPLC.

Dispersive liquid–liquid microextraction (DLLME) was introduced in 2006 [22]. For DLLME, a mixture of water-immiscible extraction solvent and water-miscible disperser solvent is prepared. The mixture is injected rapidly into the aqueous sample solution. The cloudy solution formed consists of fine droplets of the extraction solvent that are dispersed into the aqueous phase. Due to a considerably large surface area of the finely dispersed extraction solvent, the extraction of the analytes is achieved rapidly. The extraction solvent containing the analytes is separated by centrifugation. Till now only one article has been published on phenyltin compounds determination using dispersive liquid–liquid microextraction [8]. The extracted analytes were determined by gas chromatography–flame photometric detection.

This paper reports the results of the optimization of dispersive liquid–liquid microextraction and gas chromatographic–mass spectrometric determination for the speciation analysis of phenyltin compounds in aqueous solutions.

EXPERIMENTAL

Reagents and solutions

All the reagents were of analytical grade. Monophenyltin trichloride (MPhT) (98%), diphenyltin dichloride (DPhT) (96%), triphenyltin chloride (TPhT) (97%), sodium tetraethylborate ($NaBEt_4$) (97%), acetone (99.9%), methanol (99.95%), acetonitrile (99.9%), tetrachloromethane (99.5%), chlorobenzene (99%), 1,2-dichlorobenzene (99%), hexachloroethane (99%) were obtained from Sigma-Aldrich (Germany). Sodium chloride (99.5%) was obtained from Reachim (Ukraine). Ethanol (96%) was purchased from Merk (Germany).

The stock solution of phenyltin compounds (1 mg ml^{-1}) was prepared in methanol. The working solutions of phenyltin compounds were prepared by dilution of the stock solution with distilled water. All solutions were stored in the dark at 4 °C.

The sodium tetraethylborate ($NaBEt_4$) solution in water (4%) was prepared immediately before use.

The buffer solution was prepared by dissolving the necessary amount of sodium acetate in distilled water to get 0.1 M concentration and then adding acetic acid to adjust pH to 4.5.

Instrumentation

The chromatographic analysis was performed on a Perkin-Elmer Clarus 580 series gas chromatograph (GC) equipped with a programmable temperature vaporizer injector and coupled to a PerkinElmer Clarus 560 S mass spectrometer (MS) (PerkinElmer, Shelton, USA). The GC system was equipped with the Elite-5MS capillary column (30 m × 0.25 mm id, 0.25 μm film thickness) coated with methylpolysiloxane (5% phenyl). Centrifugation was carried out with a Boeco S-8 centrifuge (Germany).

GC-MS conditions

Helium was employed as a carrier gas with a constant flow of 1 ml min^{-1}. The injector temperature was held at 250 °C. Injection was performed in the pulsed splitless mode (pulsed to 4 ml min^{-1} until 1.5 min, split (50:1) open at 1.55 min).

The oven temperature was programmed as follows: 60 °C for 1 min, from 60 to 250 °C at 30 °C min^{-1} and held at 250 °C for 6 min. The capillary column was connected to the ion source of the mass spectrometer by means of the transfer line maintained at 280 °C. The electron ionization ion source conditions were the following: electron energy 70 eV and temperature 180 °C.

GC-MS in the full scan mode was used for the optimization of the DLLME method. The analyses were carried out with a filament multiplier delay of 2 min and the acquisition was performed in the range of m/z 35–400. In order to improve sensitivity and reduce interferences, the selected ion monitoring (SIM) mode was used for the quantitative analysis. The ions with the high abundance which was different to the ions of fragments of column bleed were chosen. The quantification ions (m/z values) were the following: 197 and 255 for MPhT, 197 and 303 for DPhT, 197 and 351 for TPhT, 119 and 201 for internal standard hexachlorethane.

Derivatization and DLLME procedure

The optimized derivatization and DLLME procedure was the following: to a 10 ml centrifuge tube with a conic bottom 8 ml of phenyltin compounds aqueous solution adjusted to pH 4.5, and 80 μl of 4% of $NaBEt_4$ (derivatization reagent) were placed. The solution was left for 10 min for ethylation of phenyltin compounds. Then 500 μl of the mixture, 480 μl of ethanol (as a disperser solvent) and 20 μl of tetrachloromethane (as an extraction solvent) containing hexachlorethane as an internal standard (1 μg ml^{-1}) were rapidly injected to the solution using a 1 ml syringe. The cloudy solution formed was centrifuged for 3 min at 5 000 rpm. The carbon tetrachloride phase with the analytes was sedimented in the bottom of the tube. One μl of the extraction phase was injected into GC-MS.

RESULTS AND DISCUSSION

Derivatization conditions

In phenyltin analysis, one of the most important steps is derivatization. The variables involved in the derivatization reaction, such as solution pH, reaction time, $NaBEt_4$ concentration, were optimized.

For derivatization conditions investigation experiments, liquid–liquid extraction was carried out prior to the GC-MS analysis: to 25 ml of 10 μg l^{-1} aqueous phenyltin solution, 250 μl of 4% $NaBEt_4$ solution were added and after 15 min the solution was vigorously extracted with 1 ml of *n*-hexane for 2 min. The extract was transferred into the sampling vial and automatically injected into the GC injection port.

The pH value is a critical parameter in aqueous phase derivatization. The organotins act as weak acids that favour the reaction with $NaBEt_4$. Thus, pH values should be as low as possible. However, at pH ≤ 2, $NaBEt_4$ is rapidly decomposed to BEt_3 and ethane [18].

In this work, derivatization efficiency was studied in the pH range 4–6 using acetate buffer solutions. The maximum peak areas were obtained at pH 4.5 (Fig. 1).

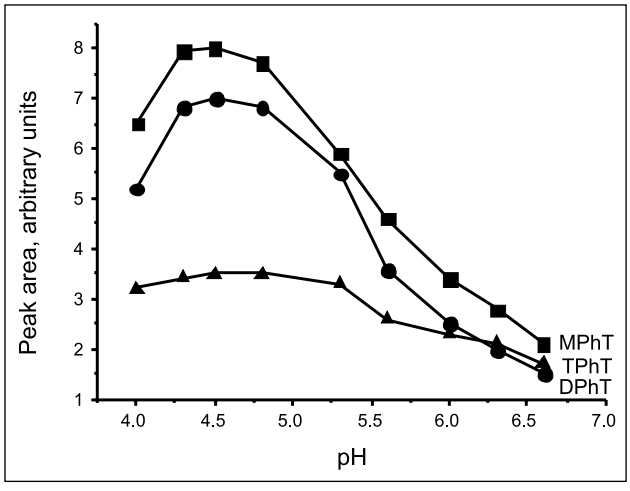

Fig. 1. Effect of pH on the derivatization efficiency. Sample volume 25 ml, concentration of phenyltins 10 μg l^{-1}, derivatization with 0.04% $NaBEt_4$, derivatization time 15 min, extraction with 1 ml of *n*-hexane for 2 min

The derivatization time was studied between 5 and 40 min. The extraction efficiency increased up to 15 min and then remained constant. Thus, for the further work 15 min derivatization time was chosen.

DLLME conditions

Selection of an appropriate extraction solvent plays the main role for DLLME efficiency. An extraction solvent for traditional DLLME should have a higher density than water, should demonstrate a good extraction capability of the compounds of interest and its solubility in water should be low. Tetrachloromethane, chlorobenzene and 1,2-dichlorobenzene were examined for the extraction of derivatized phenyltins. A mixture containing 500 μl of acetone and 50 μl of the extraction solvent was rapidly injected to 8 ml of the aqueous solution of derivatized phenyltins. A cloudy solution formed was centrifuged at 5 000 rpm and 1 μl of the sedimented organic phase was manually injected into the GC injection port.

Tetrachloromethane showed the highest extraction efficiency (Fig. 2), thus it was selected as an extraction solvent.

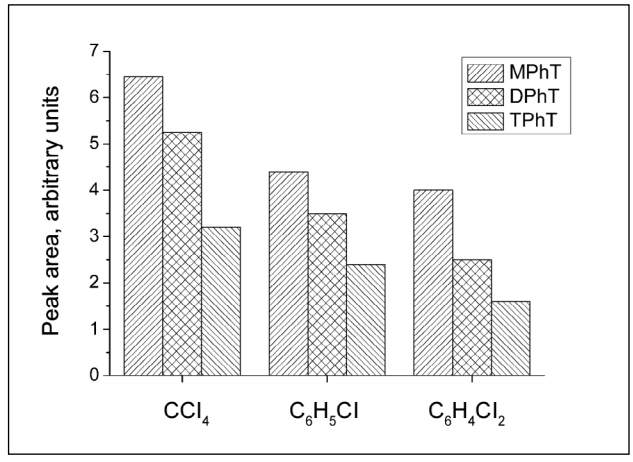

Fig. 2. Effect of the extraction solvent type on the DLLME efficiency. Sample volume 8 ml, concentration of phenyltins 10 μg l^{-1}, derivatization with 0.04% $NaBEt_4$ for 15 min, centrifugation for 3 min at 5 000 rpm

The main requirement for the disperser solvent is its miscibility with an extraction solvent and an aqueous phase. Only few solvents, namely acetone, acetonitrile, methanol and ethanol, fulfil this requirement and were studied. The mixture, containing 500 μl of the disperser solvent and 50 μl of CCl_4, was used. Ethanol was selected as a disperser solvent because the extraction efficiency using ethanol was higher than using acetone, methanol or acetonitrile (Fig. 3).

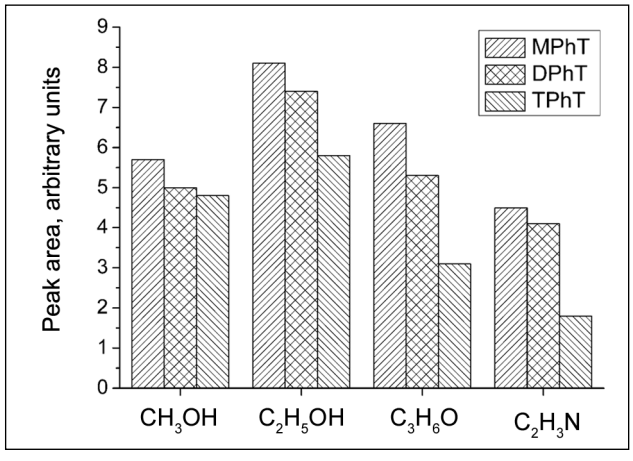

Fig. 3. Effect of the disperser solvent type on the DLLME efficiency. Sample volume 8 ml, concentration of phenyltins 10 μg l^{-1}, derivatization with 0.04% $NaBEt_4$ for 15 min, extraction solvent CCl_4 (20 μl), centrifugation for 3 min at 5 000 rpm

To investigate the effect of the extraction solvent volume, a solution containing 500 µl of ethanol and 15–50 µl of CCl_4 was used. With the increase in the extraction solvent volume, the peak areas initially increased and reached the maximum at 20 µl (Fig. 4). Thus, for the further work 20 µl of CCl_4 were used.

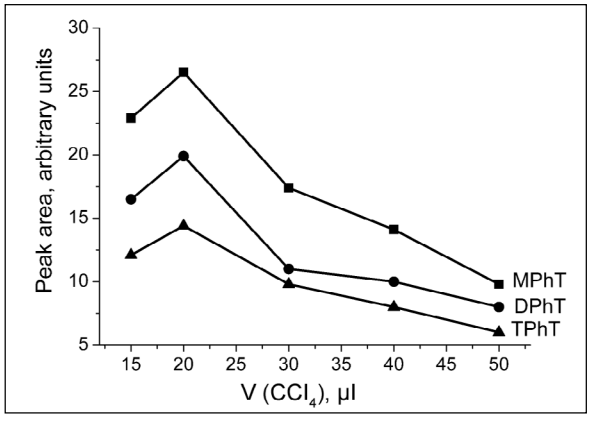

Fig. 4. Effect of the extraction solvent (CCl_4) volume on the DLLME efficiency. Sample volume 8 ml, concentration of phenyltins 10 µg l^{-1}, derivatization with 0.04% $NaBEt_4$ for 15 min, disperser solvent ethanol (500 µl), centrifugation for 3 min at 5 000 rpm

To investigate the effect of the disperser solvent volume, different ethanol volumes (0.1–0.7 ml) and 20 µl of the extracting solvent were used. As it is demonstrated in Fig. 5, the highest extraction efficiency was achieved using 0.4–0.6 ml of ethanol. For the further work, in order to have a convenient ethanol–tetrachloromethane mixture volume for the injection (0.5 ml) and considering that the optimum CCl_4 volume is 20 µl, 0.48 ml of ethanol was selected.

At the optimized extraction conditions, a concentration of the $NaBEt_4$ was additionally assayed in the range 0.0025–0.05%. For all the phenyltins, peak areas increased with the increase of $NaBEt_4$ concentration up to 0.03–0.04% (Fig. 6). Based on the results, the 0.04% concentration of $NaBEt_4$ was selected.

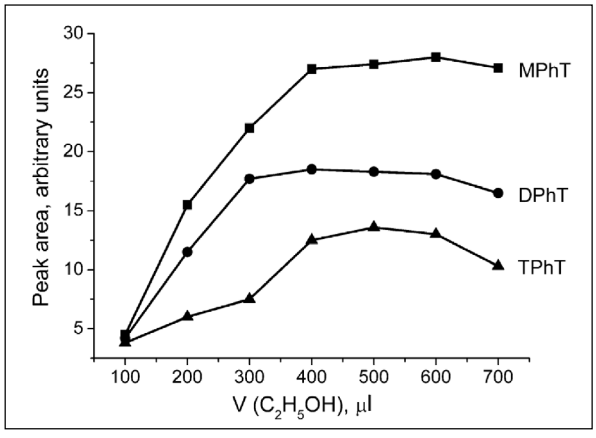

Fig. 5. Effect of the disperser solvent (ethanol) volume on the DLLME efficiency. Sample volume 8 ml, concentration of phenyltins 10 µg l^{-1}, derivatization with 0.04% $NaBEt_4$ for 15 min, extraction solvent CCl_4 (20 µl), centrifugation for 3 min at 5 000 rpm

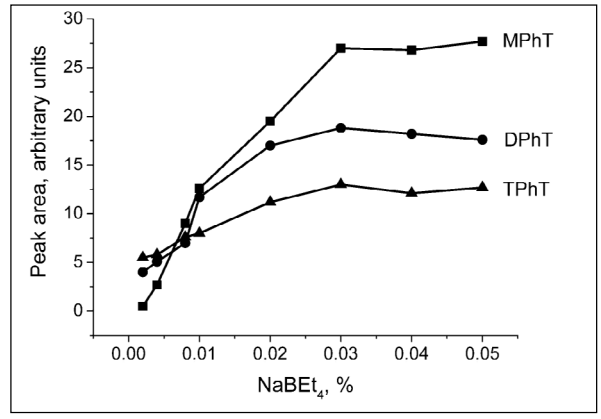

Fig. 6. Effect of $NaBEt_4$ quantity on the derivatization efficiency. Sample volume 8 ml, concentration of phenyltins 10 µg l^{-1}, derivatization with 0.04% $NaBEt_4$ for 15 min, extraction solvent CCl_4 (20 µl), disperser solvent ethanol (480 µl), centrifugation for 3 min at 5 000 rpm

Addition of salt to an aqueous sample solution generally causes a decrease in the solubility of organic compounds in water. This feature has been widely used to enhance the extraction of the analytes. In our case the aqueous solution already contained salts used for the buffer preparation and derivatization. Further increase of the salt concentration was accomplished by addition of NaCl which is commonly used for this purpose. The addition of up to 0.005 g ml^{-1} of NaCl slightly promoted the transport of the analytes to the extracting drop. However, with the further increase of NaCl, the density of the organic phase resulted in lower than that of the aqueous phase. Because of that, the organic phase formed the upper phase in the two-phase system and did not sediment any more. In order to avoid this, in further experiments NaCl was not added to the samples. A chromatogram of phenyltins obtained at the optimized extraction conditions is presented in Fig. 7.

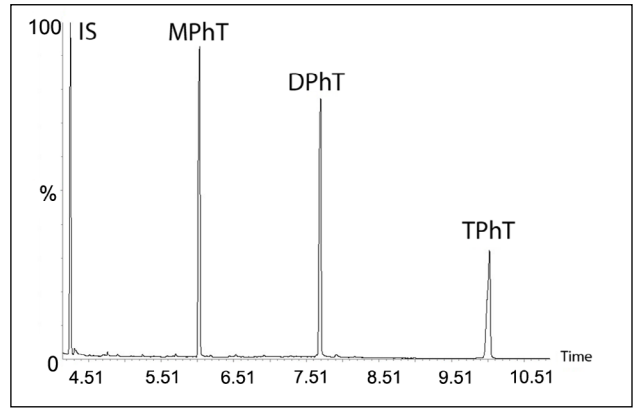

Fig. 7. Chromatogram of ethylated standard mixture of MPhT, DPhT and TPhT (10 µg l^{-1}) and internal standard hexachloroethane after DLLME. The oven temperature was programmed as follows: 60 °C for 1 min, to 250 °C at 30 °C min^{-1} and held for 6 min

Validation of the method

The quality parameters of the suggested method such as linearity, limits of detection and repeatabilities were determined under the optimized extraction conditions. In order to alleviate the injected extract volume error, hexachloroethane (1 μg ml^{-1}) was added to the extraction solvent as an internal standard. For the determination of quality parameters GC-MS in SIM mode was used. The calibration curves were drawn with 8 calibration points with three replicate injections of the extracts obtained after applying a DLLME procedure. The linear ranges were from 46, 191 and 152 ng l^{-1} for MPhT, DPhT and TPhT, respectively, up to 1 mg l^{-1} for all the analytes. The correlation coefficients were 0.996–0.999. The repeatabilities were determined by five repetitions analysis for 1 and 10 μg l^{-1} of phenyltin compounds. The relative standard deviations were 4.6–17.3%. The limits of detection were defined as three times of base-line noise and were 14–58 ng l^{-1} (Table).

Table. **Limits of detection and repeatabilities**

Analyte	LOD, ng l^{-1}	RSD, %	
		c = 1 μg l^{-1}	c = 10 μg l^{-1}
MPhT	14	10.1	4.6
DPhT	58	17.3	11.2
TPhT	46	13.5	10.8

Application

The proposed method was applied for the determination of phenyltins in river water samples. Samples from three rivers in Lithuania, namely Nemunas near Kaunas, Venta near Kuršėnai, and Akmena in the estuary, were taken for the analysis. The derivatization, extraction and GC-MS analysis procedures were as described above. In all the four samples the studied phenyltin compounds were not detected. In order to assess the matrix effect, the water samples were spiked with 10 μg l^{-1} of the studied phenyltin compounds. The obtained results were compared with those obtained from spiked distilled water samples. Relative recoveries were determined as the ratio of the concentrations found in real and distilled water samples spiked at the same analyte concentration and were between 88 and 109% indicating that the river water matrix has little effect on the extraction efficiency.

CONCLUSIONS

This work demonstrated that dispersive liquid–liquid microextraction coupled to gas chromatographic–mass spectrometric determination can be successfully applied for the speciation analysis of phenyltin compounds in aqueous solutions. Before the extraction a derivatization should be applied in order to convert the analytes to more volatile compounds. The proposed technique is fast, reliable and environment-friendly as it consumes only 20 μl of the extraction solvent tetrachloromethane. Ethylation using sodium tetraethylborate allows the derivatization of the analytes directly in the aqueous phase. It was demonstrated that the suggested method can be applied for river water analysis.

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FENILALAVO JUNGINIAI: DISPERSINĖ SKYSČIŲ-SKYSČIŲ MIKROEKSTRAKCIJA IR DUJŲ CHROMATOGRAFINIS-MASIŲ SPEKTROMETRINIS NUSTATYMAS

S a n t r a u k a

Pasiūlytas fenilalavo junginių dispersinės skysčių-skysčių mikroekstrakcijos ir dujų chromatografinio-masių spektrometrinio nustatymo metodas. Prieš ekstrakciją analitės buvo derivatizuojamos natrio tetraetilboratu. Ištirta ekstrahuojančio ir disperguojančio tirpiklių prigimties ir tūrio, ekstrakcijos trukmės ir tirpalo joninės jėgos įtaka ekstrakcijos efektyvumui. Ekstrahentu pasirinktas tetrachlormetanas, disperguojančiuoju tirpikliu – etanolis, vidiniu standartu – heksachloretanas.

Kalibracinės kreivės tiesinės nuo 46 ng l^{-1} (monofenolalavo), 161 ng l^{-1} (difenilalavo) ir 152 ng l^{-1} (trifenilalavo) iki 1 mg l^{-1} (visų analičių) koncentracijos, koreliacijos koeficientai 0,996–0,999, aptikimo ribos 14 ng l^{-1} (monofenolalavo), 58 ng l^{-1} (difenilalavo) ir 46 ng l^{-1} (trifenilalavo). Santykiniai standartiniai nuokrypiai – 4,6–17,3 %. Parodyta galimybė pritaikyti paruoštą metodą fenilalavo junginiams nustatyti upės vandenyje.