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DISPERSIVE MICROEXTRACTION FOR
THE GC ANALYSIS OF SOME ENDOCRINE
DISRUPTORS

DISPERZIONA MIKROEKSTRAKCIJA U GC
ANALIZI IZVESNIH IZAZIVAČA
ENDOKRINIH POREMEĆAJA

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Ključne reči

Disperziona mikroekstrakcija,
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Abstract

The determination of microamounts of benzophenones in water and human urine samples using miniaturized dispersive liquid-liquid microextraction and GC-FID was described. The calibration curve for benzophenones is linear with correlation coefficient higher than 0.996 in the range 0,05–0,50 µg/mL. The average recoveries of benzophenones in human urine samples spiked with 0,1 and 0,2 µg/mL of key analytes are 85–104% (RSD: 2,7–12,3%) respectively. The detection limit and the quantification limit of benzophenones in human urine samples were 5-10 and 40–50 ng/mL, respectively.

INTRODUCTION

One of the most common preservatives and UV filters that are used in the cosmetic industry for the production of sunscreens, shampoos, gels, hair sprays, lipsticks and some perfume products are benzophenone's derivatives such as: benzophenone-3 (2-hydroxy-4-methoxybenzophenone), benzophenone-6 (2,2'-dihydroxy-4,4'-dimethoxybenzophenone), benzophenone-8 (2,2'-dihydroxy-4-methoxybenzophenone), etc.^[1-2]. Unsubstituted benzophenone can present in antiepileptic drug "phenytoin" (diphenylhydantoin) as an impurity arrived from active ingredient oxidation^[3].

As a result of using cosmetics or medicines, benzophenones can get into the body. It was found^[4] that benzophenones can be accumulated in living organisms and cause toxic effects on the endocrine system. In addition, some benzophenones can cause allergic reactions: skin redness, swelling of mucous membranes, runny nose, sore throat, etc.

Because of low content of benzophenones in the biological and environmental samples and the complexity of the matrix, they have to be separated and pre-concentrated prior the analysis. One of the modern and effective method of sample preparation is microextraction (ME). Microextraction has several advantages over the traditional liquid-liquid extraction. These are: miniaturization, better automatization, simplicity, low cost, capability of on-line coupling to chromatography^[5].

It is reported determination of benzophenones in urine by GC after their solid phase microextraction on C18 disks for the concentration range 10-1000 ng/mL, RSD - 7%^[6]. Solid-phase pre-concentration of benzophenones on PDMS-coated stirring bars been applied in urine analysis^[7]. Further GS/MS determination of benzhydrol, benzophenone-2, benzophenone-3 and benzophenone-10 after their thermal desorption gave the linearity of calibration curve in the range of 0.2-10 ng/ml, RSD 1,5-4,8%^[7].

Solid phase microextraction techniques are good alternative to the traditional liquid-liquid extraction. However, polymeric fiber materials and coated bars are expensive and also can give bad recoveries due to an unpredictability of coatings' properties from different manufacturers. Also stir bar ME does not allow to minimize volumes of solvents and complicates the analysis.

The aim of this study was a development an analytical method for the analysis of some benzophenones in water matrices using dispersive microextraction and GC/FID. Dispersive microextraction (DME) allows carrying out separation and preconcentration in one step and very small volumes of organic solvents are used. This method is simple, low cost and fast.

In this work the conditions of DME of benzophenone (BP), benzhydrol (BP-OH), 2-hydroxy-benzophenone (BP-2OH) and 2-hydroxy-4-methoxybenzophenone (BP-3) from aqueous samples were investigated and optimized for their GC/FID determination in water matrices.

METHODS AND MATERIALS

Benzophenone, 2-hydroxy-benzophenone, 2-hydroxy-4-methoxy-benzophenone, benzhydrol were p.a. purity (Sigma-Aldrich, 99%). Acetone, methanol, dichloromethane, acetonitrile, chloroform were p.a. purity, obtained from Merck. Sodium chloride, hydrochloric acid, potassium hydroxide was p.a. purity, obtained from company "Chemreagents", Ukraine.

Gas Chromatography

The carrier gas was helium, chromatographic purity. High purity hydrogen and compressed air was used for FID. Parameters of GC analysis were following: capillary column HP-530 m, 0.32 mm, 0.25 mm. Helium flow rate was 2 ml/min. Injector temperature: 270°C, temperature program was from 100-180°C/5°C min., 180-270°C/25°C min., splitless mode. Temperature of FID was 300°C.

Sample preparation

The trap water, Kyiv was taken before the analysis. The mineral water "BONAQUA" was bought from a nearest supermarket. The river water (Dnipro) was taken in Kyiv, filtered through cellulose membrane filter with a pore size of 0.45 µm and stored in a refrigerator at 4°C.

Fresh urine samples were collected from two healthy volunteers. All samples were filtered and stored at 4°C prior to use.

Preparation of solutions

Stock standard solution of benzophenones (1g/L) was prepared by dissolving of 10mg of each benzophenones: benzophenone, benzhydrol, 2-hydroxy-benzophenone and 2-hydroxy-4-methoxy-benzophenone in 10 ml of methanol. Standard solution of benzophenones has been kept in a refrigerator, the solutions with a lower concentration were prepared freshly. Working standard mixtures of benzophenones containing each compound at 10,0 µg/mL were prepared daily in water, and used to spike water and biological samples. A solution of NaCl, 30% was prepared by dissolving 7,5 g NaCl in 25 ml of distilled water.

Procedure of dispersive microextraction

To 5 ml of water samples with or without additives of benzophenones add 1,08 mL of dispersive mixture methanol:chloroform (1000:80 µL, v/v). After extraction (5 min.) solution was centrifuged at 3000 rpm for 2 min. Afterwards the acceptor phase (organic phase at the bottom of the solution) is formed and 1 µl of organic extract is injected into the GC system.

The analysis of urine was performed under the optimized conditions: 2,5 mL of urine sample, 500 µL of phosphate buffer solution (pH 7,0), spiked aqueous solutions of benzophenones, to which respective volumes of water were added in order to reach an final volume (5 mL). Then mixture of 500 µL of acetone and 100 µL of methylene chloride was added. The extraction was accomplished after 5 min and mixture was centrifuged for 2 min, and afterwards the remaining acceptor phase (1 µL) was injected into the GC system.

RESULTS AND DISCUSSION

The DME is based on the formation of a stable emulsion formed in water samples due to quick inject a mixture of an extraction and a disperser solvents [8]. The mixture includes: a high-density solvent - extractant (chloroform, dichloromethane, tetrachloride carbon etc.) and a water miscible, polar solvent - disperser (acetone, methanol, acetonitrile etc.) [9].

Microextraction occurs almost instantly due to the large contact area between two phases. For the separation of extract is used centrifugation.

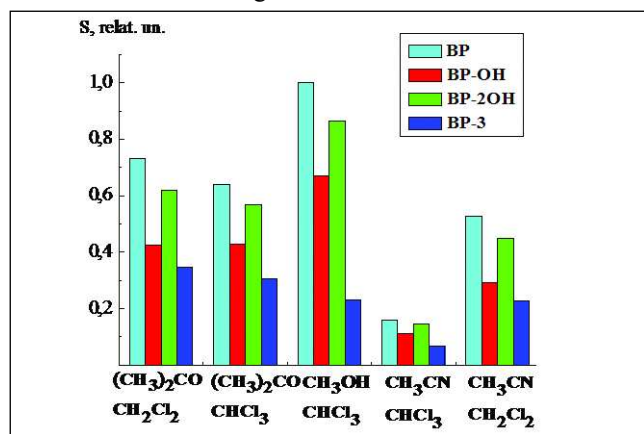


Fig.1. Effect of dispersive and extraction solvents on the DME of benzophenones.

The combination of the extraction solvent and the disperser solvent is a key issue in the DME. Dichloromethane and chloroform were evaluated as extraction solvent in this research. Methanol, acetonitrile and acetone were examined here as disperser solvents. As it is shown in the Fig.1, the best extraction of all studied benzophenones is observed when methanol and chloroform were used as solvents for DME.

The effect of the extraction solvent volume and of the disperser solvent volume was investigated further. When volume of the extraction solvent is increased, the amount of extracted analyte is slightly decreased probably due to the dilution effect, Fig. 2. It can be considered that volume of the extraction solvent has no influence on the analyte extraction degree, so better results can be obtained using small amount of the extraction solvent. However, the actual volume of extract cannot be less than 20-25 µl due to the difficulty of manual sampling, so 70 µl sampling was chosen for further experiments.

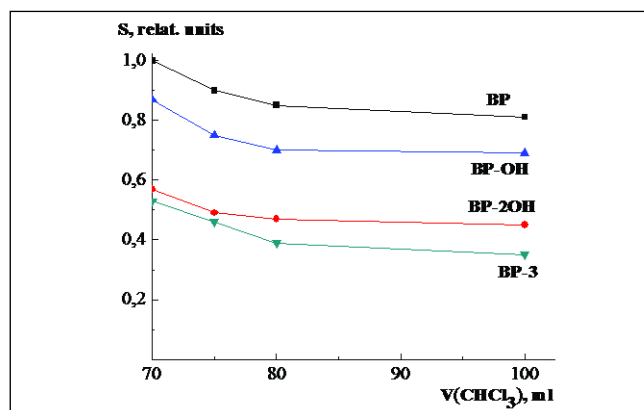


Fig.2. Effect of the volume of the extraction solvent on the DME of benzophenones.

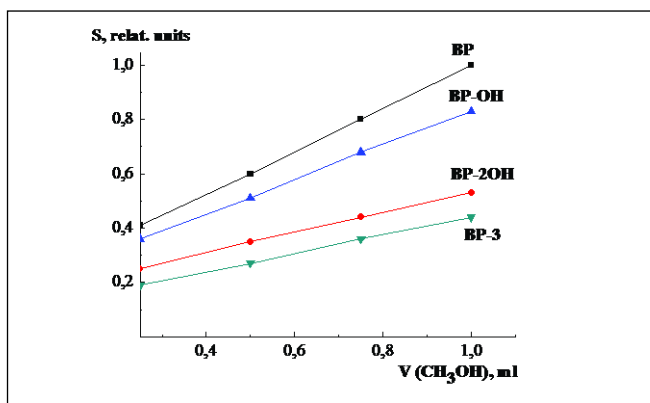


Fig. 3. Effect of the volume of the disperser solvent on the DME of benzophenones.

With increasing of the volume for the disperser solvent extraction efficiency is increased in the range of 250-1500 μ L, Fig.3. However, partial dissolving of extraction solvent in resulting suspension is possible for high loading of the disperser. This may result in changing of the extract volume and so in bad reproducibility of the method. In this research the volume of the dispersive solvent was chosen as 1000 μ L.

The extraction time is one of the most important parameters of microextraction. In DME the extraction time is defined as time interval between the injection of the binary mixture of disperser and extraction solvents to a sample and centrifugation.

As it is shown on figure 4, the optimal extraction time for DME is reached in 5 min for all benzophenones. This time was selected for all further experiments.

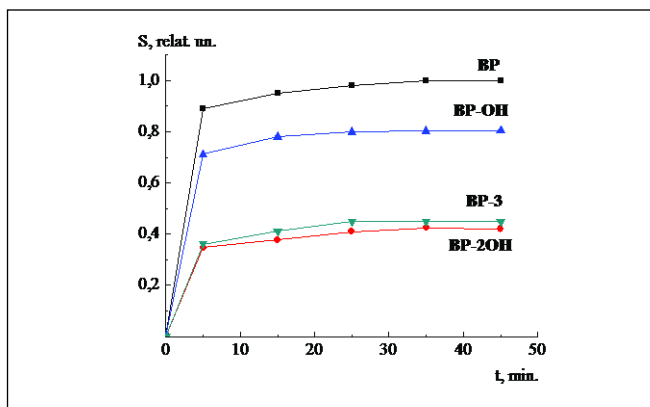


Fig. 4. Effect of the time on the DME of benzophenones.

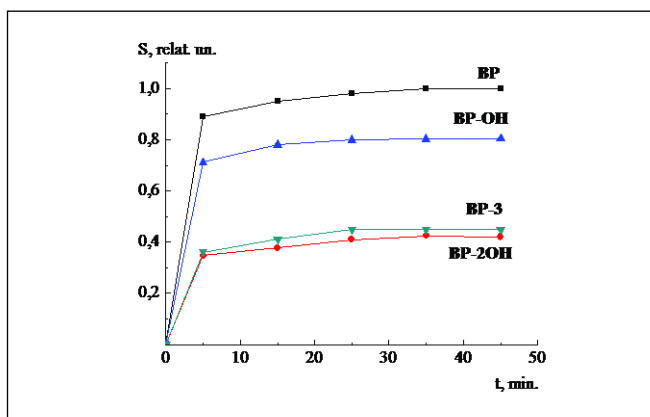


Fig. 5. Effect of pH on the DME of benzophenones.

The effect of pH on the DME of target analytes was also studied, Fig.5. It can be assumed that the acidity of the suspension should primarily effect on the extraction degree of polar benzophenones. Experimental data is fully supported this estimation: efficiency of BP extraction is higher than for the hydroxylated benzophenones in all studied pH range, Fig.5. Optimum pH for extraction of benzophenones was selected in the range of 5.0-7.0. It is agreed with values of dissociation constants for 2-hydroxybenzophenone and 2-hydroxy-4-methoxybenzophenone having pKa 8,1 and 7,6, respectively [10].

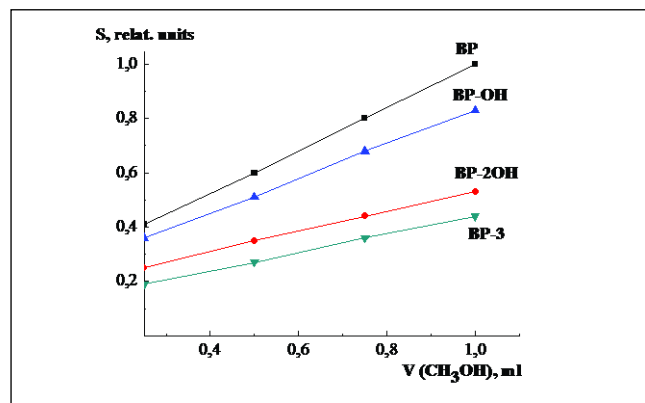


Fig. 6. Calibration curves for benzophenones (after DME).

The calibration curves of benzophenones were obtained under optimal conditions (Fig.6). The equations for the key compounds are:

- $y = 4,8 + 567,2 \cdot x$ - for BP;
- $y = 1,17 + 300,3 \cdot x$ - for BP-OH;
- $y = 453,2 \cdot x - 4,2$ - for BP-2OH;
- $y = 225,5 \cdot x - 6,8$; - for BP-3, where x- concentration, mg/L.

In Table 1 the concentration range, enrichment factors and limit of detection are presented. According to Table 1, enrichment factors of benzophenones after microextraction range present from 60 to 200. Good linearity range 0,05-1,0 μ g/mL was obtained.

Table 1 Enrichment factors, Linear range, Linearity, LOD for benzophenones

analyte	Enrichment factor	Linear range (mg L ⁻¹)	Linearity (R ²)	LOD, (mg L ⁻¹)
benzophenone	200	0,06-1,00	0,995	0,06
benzhydreole	93	0,05-1,00	0,999	0,03
2-hydroxy-benzophenone	150	0,05-1,00	0,999	0,05
benzophenone -3	90	0,06-1,00	0,998	0,06

The applicability of the proposed dispersive microextraction coupled with GC/FID was investigated by analyzing water samples with spiked benzophenones. Result are shown in Tables 2a and 2b. The method showed good precision and reproducibility with RSD of 3,2-5,2%.

Table 2a: The results of analysis of different types of water
($n=3$, $P=0,95$)

Sample	Concentration of BP, mg/L			Concentration of BP-OH, mg/L		
	Added	Found	RSD, %	Added	Found	RSD, %
Tap water, Kyiv	0.20	0.22±0.01	4.4	0.2	0.21±0.01	3.3
River water, Dnipro	0.20	0.21±0.01	3.3	0.2	0.23±0.01	3.2
Mineral water "BONAQUA"	0.20	0.22±0.01	3.2	0.2	0.22±0.01	4.3

Table 2b: The results of analysis of different types of water
($n=3$, $P=0,95$)

Sample	Concentration of BP-20H, mg/L			Concentration of BP-3, mg/L		
	Added	Found	RSD, %	Added	Found	RSD, %
Tap water, Kyiv	0.20	0.21±0.01	3.4	0.2	0.26±0.02	3.2
River water, Dnipro	0.20	0.16±0.02	5.1	0.2	0.17±0.01	4.3
Mineral water "BONAQUA"	0.20	0.15±0.02	4.6	0.2	0.17±0.01	5.2

Sample	Concentration of BP, µg/mL			Concentration of BP-OH, µg/mL		
	Added	Found	RSD, %	Added	Found	RSD, %
Urine	0,100	0,094±0,027	11,1	0,100	0,078±0,017	8,9
Urine	0,200	0,216±0,019	5,3	0,200	0,202±0,010	2,7

Sample	Concentration of BP-20H, µg/mL			Concentration of BP-3, µg/mL		
	Added	Found	RSD, %	Added	Found	RSD, %
Urine	0,100	0,095±0,028	12,1	0,100	0,101±0,027	10,9
Urine	0,200	0,208±0,040	10,0	0,200	0,206±0,040	12,3

Table 3: The results of analysis of urine ($n=3$, $P=0,95$)

The urine samples with additives of benzophenones were analyzed. Under the dispersive microextraction of benzophenones from urine into chloroform: acetone mixture solidification of the organic extract occurs. It becomes impossible to take samples. Perhaps this occurs due to the extraction of certain non-polar organic compounds that are soluble in chloroform from urine into the organic phase. Therefore dispersive mixture chloroform: methanol was substituted by methylene chloride: acetone, which is also quite effective for microextraction of target analytes (Fig.1).

The results of urine analysis and precision of the method are summarized in Table 3.

The determination of microamounts of benzophenones in water and human urine samples using miniaturized dispersive liquid-liquid microextraction and GC-FID was described. The calibration curve for benzophenones is linear with correlation coefficient higher than 0.996 in the range 0,05–0,50 µg/mL. The average recoveries of benzophenones in human urine samples spiked with 0,1 and 0,2 µg/mL of key analytes are 85–104% (RSD: 2,7–12.3%) respectively. The detection limit and the quantification limit of benzophenones in human urine samples were 5-10 and 40–50 ng/mL, respectively.

So, the dispersion microextraction is a promising method of sample preparation in the analysis of benzophenones in aqueous matrices and can be used for the analysis of different types of water (for example, tap water) and biological samples such as urine, serum with subsequent GC/FID detection.

Apstrakt

U radu je prikazano određivanje mikrokoličina benzofenona u vodi i uzorcima urina korišćenjem disperzione tečno-tečne mikroekstrakcije i gasne hromatografije sa plamenojonizacionom detekcijom (GC-FID). Kalibraciona kriva za benzofenone je linearna sa koeficijentom korelacije većim od 0,996 u opsegu od 0,05 do 0,50 µg/mL. Srednji prinos ekstrakcije benzofenona u urinu opterećenom sa 0,1 i 0,2 µg/mL iznosi 85–104% (RSD: 2,7–12.3%). Limit detekcije i limit kvantifikacije za benzofenone u uzorcima urina iznosili su 5-10 and 40–50 ng/mL

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