Solid-phase microextraction of benzophenones coupled with gas chromatography analysis

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Abstract. In this study, solid-phase microextraction method combines with gas chromatography-flame ionization detector. The proposed method is used for the preconcentration of some benzophenones. Influence of different factors on the efficiency of extraction is described in detail. The analytical procedure was optimized for fiber coating selection, extraction time, temperature, sample pH, ionic strength. For all benzophenones, the highest enrichment factors were achieved using carboxen/polydimethylsiloxane/divinylbenzene fibre immersed directly into the water samples, containing 100 mg/mL of sodium chloride, at room temperature. The optimum pH range is 5.0 – 7.0. The relative standard deviations (RSDs) were from 1.3 to 10.0 % (n = 3). The method was applied to the determination of benzophenone, benzophenone-3, 2-hydroxybenzophenone in the lake water and urine.

Keywords: benzophenones, solid phase microextraction, gas chromatography, water analysis, urine analysis

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Introduction

Benzophenone (BP) - type ultraviolet (UV) filters, including 2-hydroxy-4-methoxybenzophenone ((benzophenone-3, BP-3), benzophenone (BP) and 2-hydroxybenzophenone (benzophenone-2OH, BP-2OH) have the ability to absorb and dissipate ultraviolet light. Therefore, they are widely used for the protection of skin and hair from UV irradiation in cosmetics, sunscreens [1], many other personal care products (PCPs) such as hair shampoos, lipsticks and even in packaging materials to enhance their light stability [2–5]. Their maximum individual concentrations ranged up to 10 % in different cosmetic products.

UV filters are reaching the aquatic environment either directly, via wash-off water from the skin and clothes during water recreational activities, or indirectly, via discharges of sewage and swimming pool waters.

Several studies carried out *in vitro* or *in vivo* (using animals, e.g., fishes or rats) indicate

that some organic UV filters have significant estrogenic and/or antiandrogenic activity [6]. BPs are also suspected to cause pruritus and contact allergies. Their continuous input into the environment may lead to accumulative negative effect on ecospecies and human beings. Benzophenones and their metabolites were examined previously by using different extraction and separation procedure. Liquid–liquid extraction (LLE) [7,8] or solid-phase extraction (SPE) [9,12] and alternatively, micellar-mediated extraction (MME) [13], stir-bar sorptive extraction (SBSE) [14,15] and solid-phase microextraction (SPME) [16–17] have been proposed as pre-concentration approaches for the GC [18,19], HPLC [20] determination. Most of these methods are time-consuming and involve many steps. In contrast, SPME is fast, sensitive, solvent-free and simple analytical method requiring only one step for the extraction of compounds. Typically, a fused silica fiber coated with a stationary phase is immersed in aqueous solution. The organic analytes adsorb onto stationary phase until equilibrium has been reached. The analytes may be transferred directly into HPLC or GC instrument for the analysis.

In this work, the method based on SPME combined with GC-FID detection was developed for the determination of benzophenones in the water samples. Some factors included pH of a sample solution, extraction time and amount of salt addition, temperature were investigated. Under optimized conditions, the developed method was successfully applied to lake waters and urine samples.

EXPERIMENTAL

Reagents

Benzophenone, 2-hydroxy-4-methylbenzophenone, 2-hydroxybenzophenone, NaCl (analytical grade), methanol (HPLC grade) were purchased from Sigma-Aldrich (ALSI Ltd., Kiev, Ukraine).

The manual SPME holder and silica fused fibres coated with different polymers: a 100 μm film thickness polydimethylsiloxane (PDMS), a 75 μm film thickness carboxen/PDMS (CAR/PDMS), a 50/30 μm film thickness carboxen/PDMS/divinylbenzene (CAR/PDMS/DVB) were obtained from Supelco (ALSI Ltd., Kiev, Ukraine). Before the first use each fibre was conditioned following the supplier specifications.

Stock solutions containing 1 mg mL^{-1} of each benzophenone were prepared in methanol and refrigerated at +4 °C. Working standard solutions were prepared daily by diluting the stock solutions with distilled water to required concentrations.

Samples

The child’s urine sample was taken at the same day. The lake water sample was taken in

Kyiv, it filtered through cellulose membrane filter with a pore size of 0.45 μm and stored in a refrigerator at 4 °C.

SPME procedure

Under the final optimal conditions, the microextraction was accomplished in a 10 mL glass vials with screw caps containing 8.0 mL of the water or urine sample and magnetic stir bar. The pH was adjusted to 5 – 7, sodium chloride concenration was 50 mg/mL for CAR/PDMS/DVB and 100 mg/mL for PDMS fibre respectively. In the case of spiked samples, concentration of methanol in glass vial was limited up to 1%. Extraction was carried out in direct sampling mode at room temperature, using PDMS fibre and using CAR/PDMS/DVB fibre at 50 °C. After an exposition period of 60 min for CAR-PDMS-DVB fibre and 45 min for PDMS fibre, the SPME fibre was retracted into the needle of SPME holder syringe and it was exposed to GC evaporator set. The desorption time was 10 min at 270 °C.

GC analysis

Gas chromatography was carried out using Agilent Technologies 6890N (Agilent Technologies, USA) gas chromatograph equipped with a flame ionization detector. Separation was performed on a 30 m × 0.32 mm, 0.25 μm HP-5 capillary column (Agilent Technologies). The injector temperature was 270 °C and the detector temperature 300 °C. The oven temperature was programmed, i. e. initially set at 100 °C, then gradually ramped to 180 °C (5 °C min^{-1}) and then gradually ramped to 270 °C (25 °C min^{-1}). The following gas flow rates were used: carrier (helium) 2.5, make-up gas (helium) 20, hydrogen 30 and air 300 mL min^{-1}.

RESULTS AND DISCUSSION

Coating selectivity. First of all the selectivity of coating was examined. Selection of an appropriate coating is one of the most important stages in development of SPME method. The extraction efficiency of three different coated fibres (PDMS, CAR/PDMS and CAR/DVB/PDMS) was compared using direct extraction during 30 min at room temperature. The CAR/PDMS fibre exhibited the lowest extraction efficiency for target analytes, the PDMS coating better adsorbed benzophenones. The CAR/DVB/PDMS coating provided the most efficient extraction for benzophenones (Fig. 1). The PDMS and CAR/DVB/PDMS fibres were used for the further experiments.

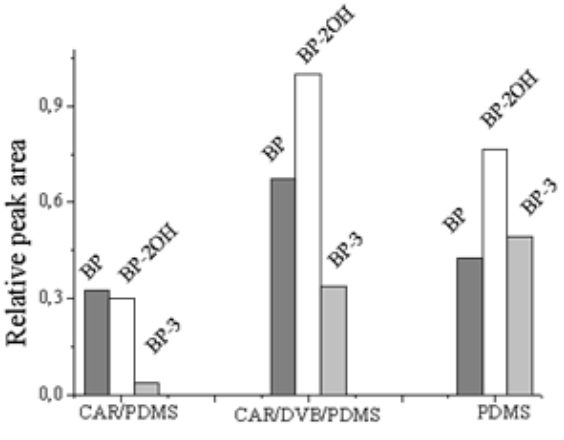

Fig. 1. Comparison of SPME efficiency for PDMS, CAR/PDMS and CAR/DVB/PDMS fibres. Direct extraction at room temperature for 30 min.

Extraction time. Fig. 2 and 3 depicts the responses obtained for each compound as function of the extraction time. In the case of benzophenones the equilibrium between concentrations in the fibre and in the sample was achieved after 45 min of exposition for PDMS fiber (Fig. 2). The CAR/DVB/PDMS fiber showed slower extraction kinetics. In order to maintain the duration of the sample preparation stage in reasonable values, the enrichment step was limited to 60 min (Fig. 3).

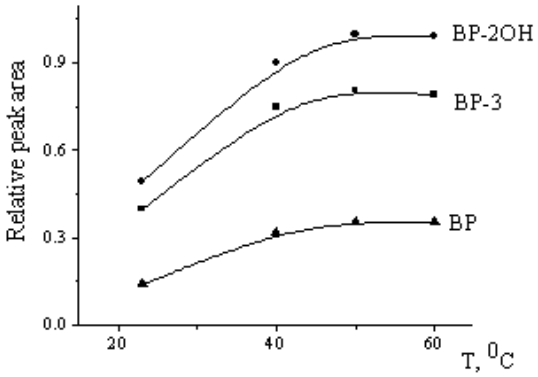

Fig. 2. Effect of extraction time on the peak area of benzophenones for PDMS fibre. Direct SPME at room temperature; desorption at 250 ºC for 10 min.

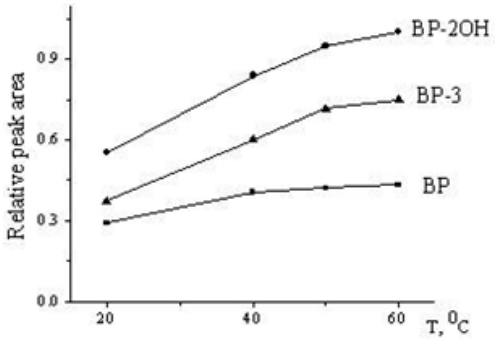

Fig. 3. Effect of extraction time on the peak area of benzophenones CAR/DVB/PDMS fibre. Direct SPME at 50 ºC; desorption at 270 ºC for 10 min.

Benzophenone desorption conditions. The injection port temperature is an important factor in the fibre desorption. The data sheet of SUPELCO SPME fiber assemblies showed that the coating can be operated without any damage up to the 250 ºC temperature for PDMS and 270 ºC CAR / DVB / PDMS, so, this desorption temperature was selected for the further experiments.

The desorption time was investigated from 5 to 20 min. The results showed that all the analytes were quantitatively desorbed from fibre coating after 10 min and no carry-over effect was observed in blank injections. Therefore, ten-minutes desorption time was used for the further work. Fibres were additionally heated for 15 min at 280 ◦C in the injection port of chromatograph before SPME of next sample to reduce the risk of cross contamination between samples.

Effect of sample pH. Sample pH plays an important role in the extraction procedure because pH value determines the existing form of analytes, and also sample pH affects the extraction efficiency. The effect of pH on the SPME process was investigated in the range of pH 2.0 – 10.0 that is below the p*K*a of analytes (from 7.5 to 8.1). Figures 4 - 5 show that the peak area of benzophenones was small in acidic and basic solutions and the uptakes enhanced gradually with increase of pH. The maximum peak area for analytes appeared at pH 5.0 – 7.0 using CAR/DVB/PDMS and PDMS fibers when benzophenones are in molecular form that improves their extraction.

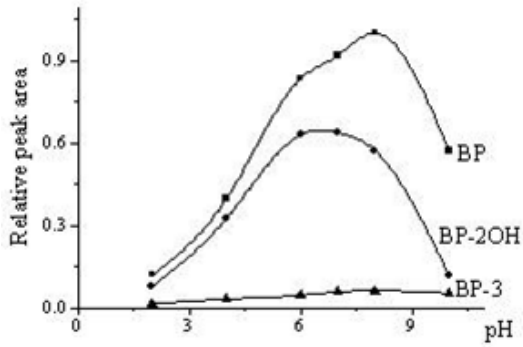

Fig. 4. Effect of sample pH. Conditions: CAR/DVB/PDMS fiber, equilibrium time, 60 min; NaCl, 5 %; desorption time, 10 min.

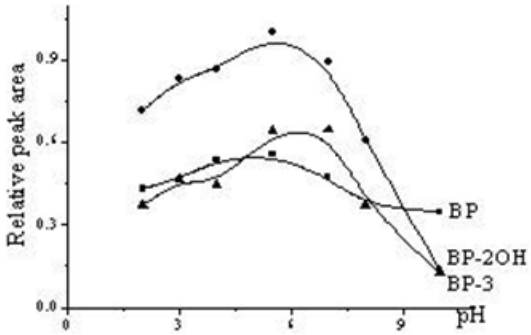

Fig. 5. Effect of sample pH. Conditions: PDMS fiber, equilibrium time, 45 min; NaCl, 10 %; desorption time, 10 min.

Effect of the salt addition. The **e**ffect of salt addition is also an important factor in the enrichment procedure. The salt addition in SPME method could improve the sensitivity of analytes determination because of increased ionic strength and salting-out effect. In order to increase the ionic strength sodium chloride was added. In these experiments, the salt concentration was optimized in the range of 0 – 15%, and the results are shown in Fig. 6-7. It can be seen (Fig. 6), the peak area of benzophenones increase with change in NaCl concentration up to 10% for PDMS fiber. The peak area of benzophenone-3 and benzophenone-2OH decreased slightly with the increase of NaCl concentration from 10 to 15 %. As shown in Fig. 7 adding of NaCl enhances the extraction efficiency slightly for CAR/PDMS/DVB fiber. In further experiments, 5 % of NaCl was added.

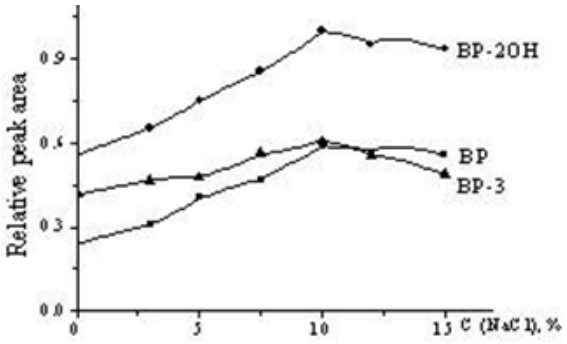

Fig. 6. Effect of the salt addition. Conditions: PDMS fiber, equilibrium time, 60 min; desorption time, 10 min.

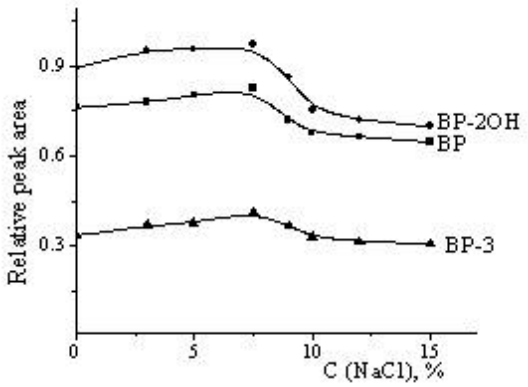

Fig. 7. Effect of salt addition. Conditions: CAR/DVB/PDMS fiber, equilibrium time, 45 min; desorption time, 10 min.

Determination of benzophenones in real samples

In this study, the lake (Kyiv, Ukraine) water and urine samples were analyzed for benzophenones using the present method. Fig. 8 presents a chromatogram of the lake water, spiked with 0.1 mg/L of each compound for CAR / DVB / PDMS fiber. The results showed that the water

and urine samples analyzed were free of benzophenones (Table. 1). **).** The water and urine samples were free of benzophenones because the quantity of benzophenones added are equal to the quantity determined.

The relative standard deviation show that repeatability of the method is satisfactory (Table. 1).

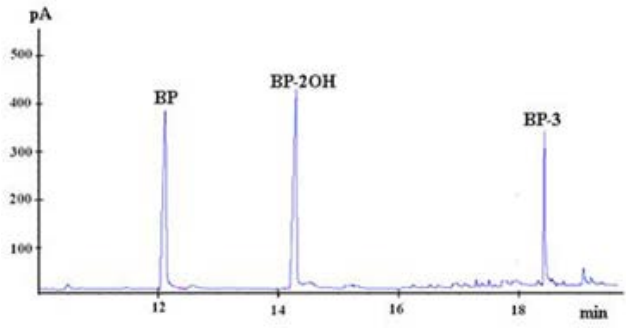

Fig. 8. Typical chromatogram of lake water samples, spiked with 0.1mg/L for each compound. Conditions: CAR / DVB / PDMS fiber; NaCl, 5%; pH, 6; equilibrium time, 45 min; desorption time, 10 min.

Table 1. Analytical results for the determination of benzophenones in lake water and urine samples, n = 4 replicates.

Analyte	CAR / DVB / PDMS fiber			PDMS fiber		
	Spiked Level, mg/L	Found, mg/L	RSD, %	Spiked Level, mg/L	Found, mg/L	RSD, %
Lake water						
BP	0.05 0.10	0.05 ± 0,02 0.09 ± 0,03	5.9 4.5	0.2 0.4	0.20 ± 0.01 0.38 ± 0.13	6.9 4.6
BP-2OH	0.05 0.10	0.07 ± 0.02 0.10 ± 0.02	3.4 2.3	0.2 0.4	0.18 ± 0.05 0.38 ± 0.08	3.5 2.8
BP-3	0.05 0.10	0.05 ± 0.03 0.10 ± 0.01	8.8 9.3	0.2 0.4	0.20 ± 0.01 0.38 ± 0.09	6.9 3.1
Urine						
BP	0.10 0.20	0.10 ± 0.01 0.20 ± 0.2	4.0 4.0	0.10 0.20	0.08 ± 0.01 0.18 ± 0.03	1.8 2.1
BP -2OH	0.10 0.20	0.13 ± 0.01 0.21 ± 0.02	3.1 3.8	0.10 0.20	0.07 ± 0.02 0.17 ± 0.04	3.1 3.6
BP-3	0.10 0.20	0.09 ± 0.02 0.14 ± 0.02	2.3 5.7	0.10 0.20	0.08 ± 0.02 0.16 ± 0.03	2.3 2.7

The quality parameters of the suggested method such as limits of detection, enrichment factors were calculated under the optimal extraction conditions. The results are presented in *Table 2*. The developed SPME method showed the high enrichment factors for CAR / DVB / PDMS and PDMS fibers ranged from 8700 to 16800, which ensured the accuracy of the amount of benzophenones detected in the spiked samples.

Table 2. Enrichment factors and detection limits.

Analyte	CAR / DVB / PDMS fiber		PDMS fiber	
	Enrichment factor, $*10^4$	LOD, mg/L, $*10^4$	Enrichment factor, $*10^4$	LOD, mg/L, $*10^4$
BP	1.68	0.008	0.96	0.025
BP-2OH	1.60	0.005	0.87	0.010
BP-3	0.10	0.08	0.10	0.030

Conclusion

The method of solid-phase microextraction combined with gas chromatographic detection of benzophenones has been developed. For higher extraction efficiency, various parameters, such as extraction temperature, extraction time, and desorption time, effect of the salt addition and pH have been optimized. The established method is simple, rapid, inexpensive, and solvent-free with good extraction efficiency and excellent selectivity. The performance SPME CAR / DVB / PDMS fiber is better than SPME PDMS fiber and CAR/PDMS fiber. The analytical results indicate that the proposed SPME method is a good prospect for selective extraction and determination of benzophenones from water samples by GC-FID.

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References

1. Giokas D.L., Salvador A., Chisvert A. UV filters: From sunscreens to human body and the environment // Trends Anal. Chem. – 2007. –. P. 26– 60.

2. Zenker A., Schmutz H., Fent K. Simultaneous trace determination of nine organic UV-absorbing compounds (UV filters) in environmental samples. J. Chromatogr. A. 2008; 1202: 64–74.

3. Rodil R., Quintana J.B., López-Mahía P., Muniategui-Lorenzo S., Prada-Rodríguez D. Multiclass Determination of Sunscreen Chemicals in Water Samples by Liquid Chromatography-Tandem Mass Spectrometry. Anal. Chem. 2008; 80: 1301–1315.

4. Ro K.W., Choi J.B., Lee M.H., Kim J.W. Determination of salicylate- and benzophenone-type sunscreen agents in cosmetic products by gas chromatography-mass spectrometry. J. Chromatogr. A. 1994; 688: 375.

5. Jeon H.K., Chung Y., Ryu J.C. Simultaneous determination of benzophenone-type UV filters in water and soil by gas chromatography-mass spectrometry. J Chromatogr. A. 2002; 1131: 192–202.

6. Negreira N. et al. Sensitive Determination of salicylate and benzophenone type UV filters in water samples using solid-phase microextraction, derivatization and gas chromatography tandem mass spectrometry. Analytica Chimica Acta. 2009; 638: 36–44.

7. Kupper T., Plagellat C., Braendli R.C., de Alencastro L.F., Grandjean D., Tarradellas J. Fate and removal of polycyclic musks, UV filters and biocides during wastewater treatment. Water Res. 2006; 40: 2603–2612.

8. Balmer M.E., Buser H.R., Müller M.D., Poiger T. Occurrence of some organic UV filters in wastewater, in surface waters, and in fish from Swiss Lakes. Environ. SciTechnol. 2005; 39: 953–962.

9. Poiger T., Buser H.R., Balmer M.E., Bergqvist P.A., Müller M.D. Occurance of UV filter compounds from sunscreens in surface waters: regional mass balance in two Swiss lakes. Chemosphere. 2004; 55: 951–963.

10. Giokas D.L., Sakkas V.A., Albanis T.A. Determination of residues of UV filters in natural waters by solid-phase extraction coupled to liquid chromatography–photodiode array detection and gas chromatography–mass spectrometry. J. Chromatogr. A. 2004; 1026: 289–293.

11. Kawaguchi M., Ito R., Endo N., Sakui N., Okanouchi N., Saito K., Sato N., Shiozaki T., Nakazawa H. Stir bar sorptive extraction and thermal desorption-gas chromatography-mass spectrometry for trace analysis of benzophenone and its derivatives in water sample. Anal. Chim. Acta. 2006; 557: 272–277.

12. Rodil R., Moeder M. Development of a method for the determination of UV filters in water samples using stir bar sorptive extraction and thermal desorption–gas chromatography–mass spectrometry. J Chromatogr A. 2002; 1179: 81–86.

13. Giokas D.L., Sakkas V.A., Albanis T.A., Lambropoulou D.A. Determination of UV-filter residues in bathing water. Jchromatgr. A. 2005; 1077: 19–27.

14. Cuderman P., Heath E. Determination of UV filters and antimicrobial agents in environmental water samples. Anal. Bioanal. Chem. 2007; 387: 1343–1350.

15. Lambropoulou D.A., Giokas D.L., Sakkas V.A., Albanis T.A., Karayannis M.I. "Gas Chromatographic Determination of 2-hydroxy-4methoxybenzophenone and Octyl Dimethyl-p-aminobenzoic acid Sunscreen Agents in Swimming Pool and Bathing Waters by Solid-Phase Microextraction. J. Chromatogr A. 2002; 967: 243–253.

16. Sakkas V.A., Giokas D.L., Lambropoulou D.A., Albanis T.A. Aqueous photolysis of the sunscreen agent octyl-dimethyl-p-amino-benzoic acid (ODPABA). Formation of disinfection byproducts in chlorinated swimming pool water. J.Chromatogr A. 2002; 1016: 211–222.

17. Felix T., Hall B.J., Brodbelt J.S. Determination of benzophenone-3 and metabolites in water and human urine by solid-phase microextraction and quadrupole ion trap GC-MS. Anal. Chim. Acta. 1998; 371: 195–203.

18. Jeon H.K., Chung Y., Ryu J.C. Simultaneous determination of benzophenone-type UV filters in water and soil by gas chromatography-mass spectrometry. J. Chromatogr. A. 2006; 1131–192.

19. Chisvert A., Canals A., Salvador A. Sensitive determination of free benzophenone-3 in human urine samples based on an ionic liquid as extractant phase in single-drop microextraction prior to liquid chromatography analysis // Journal of Chromatography A. 2007; 1174: 95 – 103.

20. León Z., Chisvert A., Tarazona I., Salvador A. Solid-phase extraction liquid chromatography–tandem mass spectrometry analytical method for the determination of 2-hydroxy-4methoxybenzophenone and its metabolites in both human urine and semen. Anal. Bioanal. Chem. 2010; 398: 831–843.