

Studies on preconcentration and isolation process of bezafibrate onto different types of sorbents by the SPE

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ABSTRACT

The performed investigations are concerned on the estimation of extraction properties of copolymer styrene-divinylbenzene (SDB-1) adsorbent and four chemically bonded silica gel materials: octadecyl (C₁₈), octyl (C₈), phenyl (C₆H₅), cyclohexyl (C₆H₁₁) for the solid phase extraction of bezafibrate (hypolipidaemic compound) from the model solutions and river water samples. Extraction conditions such as solvent selection, their volumes and water samples pH were found to have significant influence on extraction efficiency of the studied compound. The effect of water matrix on extraction efficiency was checked too. It was found that the best extraction efficiency of bezafibrate from water sample was obtained using bonded silica-octadecyl gel sorbents and polymer material. The presence of drug in elutes was detected by spectrophotometric (measurement of absorbance at 230 nm) and HPLC-UV methods. Under optimal conditions, recoveries of the pharmaceutical were higher than 80 %. The precision of the novel extraction procedures, calculated as coefficient variation (CV %), ranged from 0.008 to 0.018 % for the all tested sorbents.

INTRODUCTION

Bezafibrate(BZT) (Fig. 1) is one of the most popular compound of the fibrate hypolipidaemic class of drugs. Its pharmacological activity consists in reducing serum triglyceride (22 %) and low density lipoprotein cholesterol (LDL-C) (5 %) levels and significantly raising high density lipoprotein cholesterol (HDL-C) fraction. It is widely used in treatment of hypertriglyceridemia, which is now recognized as an independent risk factor of a coronary artery disease (CAD) (Duriez and Fruchart, 1999). About 95 % of orally administered bezafibrate is bonded by albumin and most of the absorbed drug is excreted with urine. It is known that approximately 50% of the administered dose is not completely metabolised and its unchanged form is excreted with urine within 24 h (Abshagen *et al.*, 1979). It is the reason why several performed recently studies in Europe, North and South Americas detected the presence of bezafibrate and other lipid-lowering drugs in wastewater and surface water (Ahrer and Buchberger, 2001; Ahrer *et al.*, 2001; Castiglioni *et al.*, 2005; Gros *et al.*, 2006; Lindqvist *et al.*, 2005; Metcalfe *et al.*, 2003a; Metcalfe *et al.*, 2003b; Pitarch *et al.*, 2004; Stolker, 2004; Stumpf *et al.*, 1999; Ternes, 1998). According to these data, the

determined concentration of bezafibrate in STP influents ranged from the 0.3 ng L⁻¹ to the 4.7 µg L⁻¹ and in STP effluents (0.01 µg·L⁻¹ to the 4.6 µg·L⁻¹). Their concentration in surface waters is mainly ranged between 1.9 ng L⁻¹ and 3.1 µg L⁻¹. The concentrations of bezafibrate in sewage treatment plants (SWT) and surface water have not been measured previously in Poland.

The mentioned above data, were obtained using several analytical methods mainly gas (GC) and liquid (LC) chromatography coupled to mass spectrometry or tandem mass spectrometry with electrospray (ESI) or atmospheric pressure chemical (APCI) ionisation modes. All of these methods, required intense sample preparation step included isolation, preconcentration and isolation of target analyte. Classic liquid-liquid extraction (LLE) or off-line solid phase extraction (SPE) are mainly applied for this purpose. The majority of the SPE sample pre-treatment for bezafibrate and other pharmaceutical residues in waters are performed on copolymer of divinylbenzene and vinylpyrrolidone, which has been commercialized under the trade name Oasis-HLB by Waters (Buchberger, 2007). The octadecyl sorbent is also commonly used. However, most SPE extraction methods on HLB and C₁₈ sorbents are used for multi-group analyses where bezafibrate is isolated in one fraction together with other compounds with similar physicochemical properties.

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It results in requirement of very sensitive and selective separation techniques for the final determination of target analyte, like GC-MS or LC-MS/MS. The goal of our study was to elaborate new solid phase extraction methods for isolation of BZF from river water samples using polymeric copolymer styrene-divinylbenzene (SDB-1) and four chemically bonded silica gel materials: octadecyl (C₁₈), octyl (C₈), phenyl (C₆H₅), cyclohexyl (C₆H₁₁) manufactured by Baker. The effect of various parameters have been investigated, in order to obtain a better understanding how they can influence on a recovery of bezafibrate and to gain the better selectivity of its further determination in water samples. For this reason the influence of the matrix constituents (humic acids, salts) on extraction efficiency were tested.

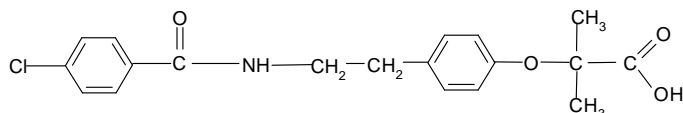


Fig. 1: Chemical structure of bezafibrate (BZF) 2[4-[2[(4-Chlorobenzoyl)amino]ethyl]phenoxy]-2-methylpropanoic acid.

EXPERIMENTAL

Reagents

Bezafibrate (BZF) was obtained from Sigma-Aldrich (Steinheim, Germany). Stock standard solutions of BZF (200 $\mu\text{g mL}^{-1}$) were prepared in methanol and stored in amber bottles at 4°C. Working solutions were prepared freshly every day before analysis by diluting standard solutions with double distilled water. Methanol at HPLC grade was purchased from Merck (Darmstadt, Germany). All other chemicals used were of analytical grade obtained from POCh (Gliwice, Poland).

Instruments and materials

Solid-phase extraction (SPE) was carried out on a J.T. Bakers SPE-12G System (Grosgerau, Germany) connected to vacuum pump from KNF LAB (Neuberger GmbH, Germany). 3 mL solid-phase extraction columns C₁₈, C₈, C₆H₁₁, C₆H₅, SDB-1 (200 mg, 40 μm , APD, 60Å) were purchased from J.T. Baker (Phillipsburg, NY, USA).

Hewlett-Packard HP-8452A diode-array spectrophotometer (Raleigh, CA, USA) was used for absorbance measurements. Chromatographic experiments were performed on the Thermo Separation System consisting 3D detector Spectra System UV 3000, the vacuum membrane degasser SCM Thermo Separation, and the Rheodyne loop injector (20 μL) (San Jose, CA, USA).

Procedures

UV-Spectrophotometric Determination of Bezafibrate

The calibration graph was prepared to control the extraction efficiency during every step of SPE procedures of BZF isolation from the water samples. For this purpose, the BZF standard solutions in the range of 25 – 1500 $\mu\text{g/mL}$ were placed into 10 mL calibrated flasks and diluted to the mark with

methanol. The absorbance measurements for every concentration of analyte were repeated three times at $\lambda_{\text{max}} = 230 \text{ nm}$. Subsequently, calibration curve was performed and the analytical parameters were established (Table 1).

Table 1: Characteristic of the UV-spectrophotometric procedure for the determination of BZF.

Compound	Calibration equation	R ²	Linearity $\mu\text{g mL}^{-1}$	CV [%]	Wavelength [nm]
BZF	$y = 0.0607x - 0.0018$	0.9999	0.5 – 30.0	1.97	230

Liquid Chromatography

HPLC assay of BZF was carried out using the reversed-phase analytical column, Lichrospher 100 RP-18 250 mm \times 4.6 mm (5 μm) (Merck, Germany). The mobile phase included acetonitrile:0.02 M H₃PO₄ (55:45, v/v) at flow rate of 1 mL min⁻¹ (Masnatta, 1996) was applied.

Solid-Phase extraction

At the beginning of the extraction process, all used sorbents (C₁₈, C₈, C₆H₅, C₆H₁₁, and SDB-1) were conditioned with 1.5 mL of methanol and 1 mL solution of HCl (pH = 4). Aqueous working solutions of bezafibrate were adjusted to pH = 4 and next a portion of 10 mL of its solution was passed slowly through the cartridges under mild vacuum (-200 mm Hg). Subsequently, the C₁₈, C₈ cartridges were washed with the 3 – 5 mL solutions of 0.1 mol L⁻¹ HCl (pH = 2) to wash out contaminants. The mixtures of methanol:water (30:70, v/v) were used in the case of phenyl, cyclohexyl and SDB-1 sorbents for the same purpose. Finally, bezafibrate was eluted with 2 mL of methanol from C₁₈, C₈, C₆H₅, C₆H₁₁ materials and with 4 mL of methanol in the case of SDB-1 sorbent.

All experiments were performed using 10 mL of aqueous standard solutions of bezafibrate at concentration 5 $\mu\text{g mL}^{-1}$ and 10 mL of river water samples spiked at concentration of 0.25 $\mu\text{g mL}^{-1}$ or 5 $\mu\text{g mL}^{-1}$ with standard solutions of the drug. Recoveries were calculated against the initial amount of analyte presented in the sample – aqueous standard or river water, using linear calibration curve based on standard solutions of bezafibrate.

RESULTS AND DISCUSSION

Optimization of the extraction procedures in aqueous standard samples

In order to find the most suitable sorbent for the preconcentration and isolation of bezafibrate in SPE procedures, two main categories of sorbents – the silica based C₁₈, C₈, C₆H₅, C₆H₁₁ and - the polymer based types SDB-1 were examined. A control of each step of extraction procedure was done spectrophotometrically by measurements the absorbance of eluents at 230 nm (the wavelength of the main absorbance peak of bezafibrate). At the beginning of optimisation of extraction process, it was stated that bezafibrate is retained by all used sorbents, so they all were used for further experiments.

Effect of the sample pH

To examine the effect of the pH sample on the extraction efficiency of tested compound, the samples contained BZF at concentration level $5 \mu\text{g mL}^{-1}$ were applied at different pH values within the range 2 – 5. The desired pH values were adjusted by addition of 0.1 mol L^{-1} solution of HCl. The influence of sample pH on recoveries of BZF are showed in Fig 2. As can be seen in Fig. 2, the efficiency of retention and extraction of analyte are the best at low pH of the sample for all studied bonded-phases (88 – 105 %). These results can be easily explained by the fact that BZF possess acidic character (pK_a value of 3.61). Thus it occurs at $\text{pH} < 5$ in uncharged form (no ionic functional groups are present). The complete retention of BZF onto C_{18} , C_8 , cyclohexyl and phenyl materials were observed, but the C_{18} cartridge - the most apolar among reversed-phase sorbents, gave the best results of extraction recovery (99 – 101 %). So it was used for further studies. BZF is adsorbed onto all phases mainly via Van der Waals forces, between analyte and apolar groups of silica modified surface, but the weak hydrophobic and dipole-dipole interactions may also play a role (Franke, De Zeeuw, 1998). From this study, it appears that all chemically bonded phases retain nonionized BZF more strongly than ionized form of drug (the recovery decreases with increasing in pH value). For SDB-1 sorbent, the values of recovery were stable at the pH range 2 - 5, but higher loss of analyte (nearly 30 %) in comparison to silica based materials was observed. It suggests that this kind of sorbent is capable of trapping more polar compounds than C_{18} material, due to of intense π - π^* interactions produced by aromatic rings in the polymeric matrix (Henion, 1999). For further SPE development, the samples were adjusted to pH 4.

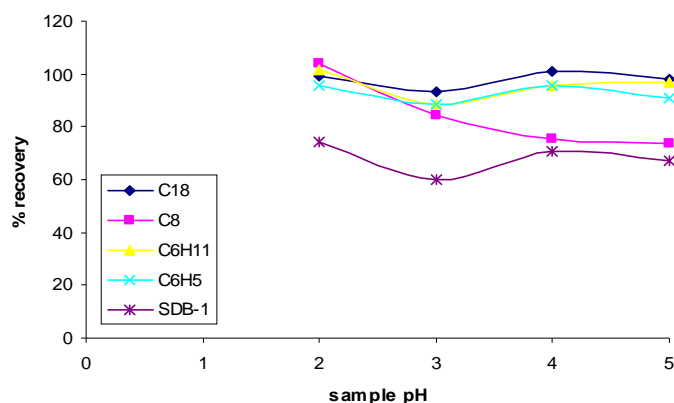


Fig. 2: Mean recoveries of BZF from aqueous standard samples as a function of the sample pH, on selected sorbents.

Conditioning steps

The purpose of the conditioning step is to open up the structure and increase the surface area available for interaction with the analytes. It also removes residues from the cartridges left by the previous extraction process. Conditioning (wetting) the sorbents is usually performed with water miscible organic solvents in the case of reversed-bonded phase and polymers, thus methanol and other water-miscible solvents were checked. The studies

showed that the proper cartridges conditioning is obtained by washing with methanol (1.5 mL) followed by HCl (1 mL, pH 4). A solution of HCl, at pH similar to the sample pH value (pH 4) applied onto columns after methanol, displaced the excess of organic solvent in the pores, preparing the sorbents to receive the sample (Gilar *et al.*, 2001).

Elution solvents

Eluting the retained analyte requires the use of a sufficiently strong solvent to disrupt the bonds between the sorbents and the compound of interest. Since one of the goals of extraction is to concentrate the compounds of interests, elution should be accomplished using the smallest volume of solvents as possible.

To select appropriate solvents to elute of BZF from the studied phases, four millilitres of the following solvents were tested: methanol, acetonitrile, water, tetrahydrofuran and solvents mixtures. The best recoveries of bezafibrate from the samples were achieved when methanol was used (76 – 103.6 %, depending on the sorbents used). As might be expected, pure methanol possess sufficient strength to disrupt the weak hydrophobic interactions which retained bezafibrate onto all modified silica and polymer phases. The best recovery of BZF from C_{18} sorbent (101.7 %) explains the previously suggested binding mechanism. It was also necessary to apply the precise volume of eluting solvent by testing 1 - 6 mL of methanol. The results obtained are shown in Fig. 3.

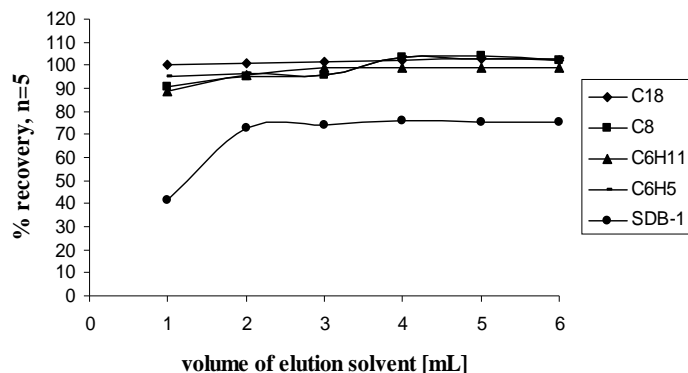


Fig. 3: Effects on recoveries of the volume of elution solvent passed through the selected sorbents.

The smallest volume of methanol which allowed the qualitative elution of BZF adsorbed onto all phases was 2 – 3 mL except of the SDB-1 material where the efficient extraction was achieved when more than 4 mL of methanol was used. The required higher volume of solvent resulted from the higher polarity of the SDB-1 sorbent than silica bonded-phases.

SPE procedures for spiked river water samples

Wash solvents

Matrix naturally present in real water samples can strongly influence the extraction efficiency of BZF SPE process using examined sorbents. Therefore it was necessary to find the proper solvents which remove the potential hydrophilic

interferents adsorbed onto extraction cartridges together with bezafibrate. To improve the cleanness of the extracts, pure distilled water, methanol:water mixtures (50:50, 30:70) and solutions of HCl (pH 2 - 6) were checked. All experiments were conducted using 10 ml of river sample spiked with 50 μg of BZF. The portion of such prepared solution was loaded onto column and then the naturally occurred in waters compounds were wash out using various samples. The best results were achieved when methanol-water mixture (30:70, v/v) was used for washing phenyl, cyclohexyl, copolymer phases. Solution of HCl at pH 2 appeared to be the best washing medium in the case of octadecyl and octyl materials. The selected solvents removed effectively almost all components present in water samples without disrupting the bonds between sorbent and analyte. Table 2 shows the results of extraction recoveries using different kind of solvents in washing step.

Table 2: Recoveries of BZF from the model solutions as a function of the used washing solvents.

Washing Solvents	C ₁₈	C ₈	C ₆ H ₅	C ₆ H ₁₁	SDB-1
	Mean recovery (%), n=3				
Pure distilled water	94.0	82.8	92.2	99.0	76.8
Methanol:water (50:50, v/v)	98.8	10.6	96.2	100.8	88.4
Methanol:water (30:70, v/v)	101.8	58.8	96.4	99.4	97.0
HCl, pH=2	101.2	93.6	105.2	101.8	92.8
HCl, pH=4	93.8	83.2	95.2	99.2	81.0

Next, the optimal volumes of methanol:water mixture (30:70, v/v) and HCl (pH = 2) were found. Various volumes in the range 1 – 10 mL of washing solution were examined. However it was observed no significant impact of used volume on recoveries of analyte. Therefore a further washing step was performed with 3 – 5 mL of selected washing solvents.

Columns capacity and breakthrough volume

It was necessary to determine the columns capacity and breakthrough volumes, because the matrix constituents (usually present at high concentration levels in water) may interact with the sorbent reducing the number of free bonding places available for the retention of analyte. Sometimes matrix compounds can interact with analyte forming complexes, preventing its interaction with the sorbents (Thuriel *et al.*, 2003). The column capacity and breakthrough volume were determined for C₁₈ cartridge as it exhibited the best efficiency of extraction process. Additionally the same parameters were assayed for SDB-1 column.

For this purpose the increasing water sample volumes (10 - 2000 mL) containing constant concentration of BZF 25 $\mu\text{g mL}^{-1}$ were loaded on C₁₈ and SDB-1 cartridges. Figure 4 presents the obtained results. The plotted curves, shows the correlation between amount of analyte in spiked various water sample volumes and percentage of the analyte appeared in effluents from extraction columns (**Fig. 4**). In this method columns capacity was estimated for 10 % acceptable loss of analyte which appears in effluent

(Baltussen, 2002). The empirically determined capacities of 200 mg of SDB-1 and C₁₈ columns for bezafibrate are 31.15 mg g⁻¹ and 30 mg g⁻¹, respectively. It showed that used adsorbents are adequate for extraction of μg levels of analyte from less than 241 mL and 250 mL. These volume values reading at the 10 % level were indicated as breakthrough volumes and they allowed to achieve the pre-concentration factor required for trace analysis (60 in the case of SDB-1 and 125 in the case of C₁₈).

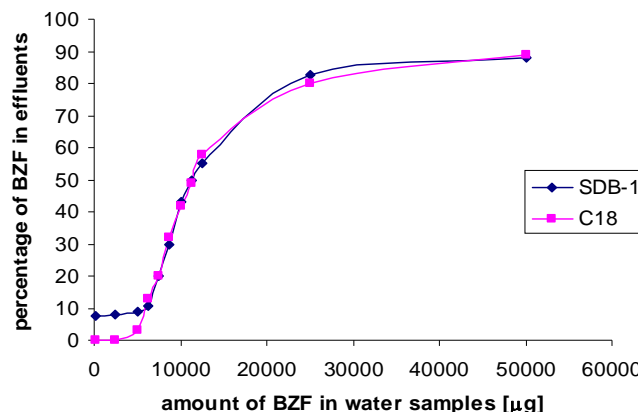


Fig. 4: Capacity and breakthrough volumes obtained for BZF on two sorbents, C₁₈ and SDB-1, at pH = 4.

As the use of C-18 sorbent allowed to obtain a very high enrichment factor, others analytical parameters of the elaborated SPE-procedure were assayed. Using the optimum SPE conditions, the absolute recoveries of BZF at two different concentration levels were examined. The precision of the SPE procedures, expressed by the coefficient of variation (CV %), was estimated by measuring the within-day repeatability at concentration 5 $\mu\text{g mL}^{-1}$ (0.01 %, n=10). The amount of bezafibrate in eluates was assayed by UV-spectrophotometric and HPLC-UV methods (Table 3).

Table 3: The results of determination of BZF in spiked river waters samples by UV- spectrophotometric and HPLC-UV methods after SPE isolation on C₁₈ sorbent.

Method of determination	Amount added [μg]	Mean recovery [%], n=3	CV [%]
UV-spectrophotometric	50	100.2	0.20
HPLC-UV	100	80	1.50

Selectivity of SPE procedures

The influence of some compounds naturally presented in water matrix on extraction process of BZF was checked next. For this purpose water samples spiked with 5 $\mu\text{g mL}^{-1}$ of BZF with addition of naturally occurring humic acids, phenols, glucose, saccharose, anionic and cationic surfactants and some inorganic cations Na⁺, K⁺, Mg²⁺, Ca²⁺, Zn²⁺, and anions Cl⁻, SO₄²⁻, NO₃⁻, PO₄³⁻ were used. Organic and inorganic compounds were added in similar or higher levels to those in natural waters. As expected, the obtained results showed no disturbances in extraction process of BZF and its further determination by UV- spectrophotometry and HPLC-UV method. The all tested compounds can be presented in the sample by BZF in excess 20 – 250 vs BZF.

CONCLUSION

The very selective SPE procedures were developed for the determination of bezafibrate from the river water samples. The conditions of extraction of BZF were optimised on different sorbents (C₁₈, C₈, C₆H₁₁, C₆H₅, SDB-1), however deep studies showed that C₁₈ material is preferable for the analysis of water samples. The enrichment step allows the assay of trace level of BZF in river water by UV-spectrophotometric as well as HPLC-UV methods. The best mean recoveries of the analysed compound were obtained on C₁₈ sorbent (100.2 %).

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