Acta Chimica Slovaca, Vol.1, No 1, 2008, 175 – 179

Selective solid-phase extraction of phenylcarbamic acid derivatives from a rat serum by molecularly imprinted polymer

Miroslava Lachová[a]*, Jozef Lehotay[a], Ivan Skačáni[a], Jozef Čižmárik[b]

[a] *Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Slovak Republic*
[b] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University in Bratislava, Slovak Republic*

**miroslava.lachova@stuba.sk*

Abstract

Molecularly imprinted polymer (MIP) was synthesized using a non-covalent imprinting approach. Methacrylic acid was used as the functional monomer, acetonitrile was used as the porogen and 1-methyl-2-piperidinoethylester of 4-decyloxyphenylcarbamic acid as the imprint molecule (template). Imprinted polymer was utilized as the sorbents for solid-phase extraction (MISPE) to extract derivatives of alkoxyphenylcarbamic acid from spiked rat serum.

Keywords: HPLC, molecularly imprinted polymer, solid-phase extraction

Introduction

Molecularly imprinted polymers (MIPs) are synthetic materials with artificially generated recognition sites able to specifically rebind a target molecule in preference to other closely related compounds. These materials are obtained by polymerising functional and cross-linking monomers around a template molecule, leading to a highly cross-linked three-dimensional network polymer. The monomers are chosen considering their ability to interact with the functional groups of the template molecule. Once polymerisation has taken place, template molecule is extracted and binding sites with shape, size and functionalities complementary to the target analyte are established (Tamayo et al. 2007). MIPs possess many advantages, they are stable towards a wide range of solvents, are highly termostable, can be used over a range of temperatures (Fischer et al. 1991) They can be stored at ambient temperature and in dry state without loss of performance . MIPs can be easily and quickly

repared and can be applicated to a wide range of target molecules (Yang et al. 2005). Molecularly imprinted polymers have been applied as stationary phases in chromatography (Sellergren 1994, Jiang et al. 2006), sensors (Yan et al. 2007, Javanbakht et al 2008), sorbents for SPE (Claude et al. 2008, Jiang et al. 2008).

We prepared the molecularly imprinted polymer with 1-methyl-2-piperidinoethylester of 4-decyloxyphenylcarbamic acid as the imprint molecule (template). MIP was utilized as a molecular imprinted solid-phase extraction sorbent for isolation of derivatives of phenylcarbamic acid from the rat serum.

Experimental

Materials

1-methyl-2-piperidinoethylesters of alkoxyphenylcarbamic acid were synthesized on Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Comenius University in Bratislava according to Pokorná et al (Pokorná et al. 1999). Acetonitrile, methanol, methacrylic acid and diethylamine were purchased from Merck, azobisizobutylonithrile (AIBN) and ethyleneglycoldimethacrylate (EDMA) were obtained from Fluka, and acetic acid was purchased from Lachema.

HPLC analysis

An HP 1100 system (Hewlett-Packard, Germany), consisting of a pump with degasser, diode-array detector (DAD), 50 µl injector and HP ChemStation were used. Analyses were carried out on the analytical column Sepharon SGX C18 (125 x 4 mm, 7 µm) (Watrex, USA) at laboratory temperature. Mobile phase consisted of methanol, acetonitrile, acetic acid and diethylamine (80:20:0.1:0.1) at a flow rate of 0.5 ml/min. was employed. Izokratic elution was used. Diode-array detector worked in the range of 190 – 400 nm and the chromatograms were acquired at wavelength of 240 nm.

Polymer preparation

The molecularly imprinted polymers were prepared according to Zhang et al´s method (Zhang et al. 2001). Two MIPs were synthesized, using acrylamide (MIP1) and methacrylic acid (MIP2), respectively, as a functional monomer. The monomer (1.8 mmol), the template molecule 1-methyl-2-piperidinoethylester of 4-decyloxyphenylcarbamic acid (0.3 mmol) and the porogen acetonitrile (3 ml) were placed into a glass tube. Then the crosslinker EDMA (9

mmol) and the initiator AIBN (9 mg) were added. The polymerization was carried out in a water bath at 60 ˚C for 24 h. Prepared polymer was passed through 40 μm sieve, fine particles were removed by flotation in acetone and final product was dried under vacuum at 60 ˚C for 1 h. The template was removed from the MIP by Soxhlet extraction with 70 ml of a mixture of methanol/acetic acid (9:1, v/v) until template was not detected in the extract.

MISPE procedure

100 mg of polymer was packed into polypropylene cartridge. SPE procedure was performed on MIP and on the commonly used sorbent C18. Cartridges were gradually conditioned with 5 ml of methanol, 5 ml of acetonitrile and 5 ml of water. Then 0.5 ml of spiked rat serum was applied onto each cartridge. The concentration of studied analytes (4-MPCA, 2-DPCA and 4-DPCA) in serum was and 10 μg/ml. Then the cartridges were washed with 1 ml of water and dried. Then dry MIPs were washed with 1 ml of acetonitrile and dried again. Analytes were eluted by 1.5 ml of mixture methanol/acetic acid (95:5, v/v). Effluents were evaporated to dryness, redissolved in 0.5 ml of metanol and injected into the HPLC system.

Results and Discussion

1-methyl-2-piperidinoethylesters of phenylcarbamic acids are potential anaestethics (Pokorná et al. 1999). Basic chromatographic parameters are described in Renčová´s article (Rentová et al. 2002).

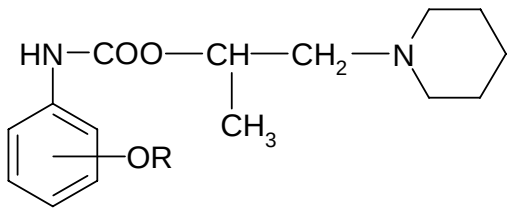

Fig. 1. Structure of compounds used in research. Template molecule: R= - $C_{10}H_{21}$ in 4-position (4-DPCA). Other analytes used in research: R= - $C_{10}H_{21}$ in 2- position (2-DPCA), R= -CH_3 in 4- position (4-MPCA).

Extracts obtained from SPE using the commonly used C18 cartridge and the MIP is shown on Figure 2. Percent recoveries of studied analytes after SPE using the MIP and C18 cartridges are shown in Table 1.

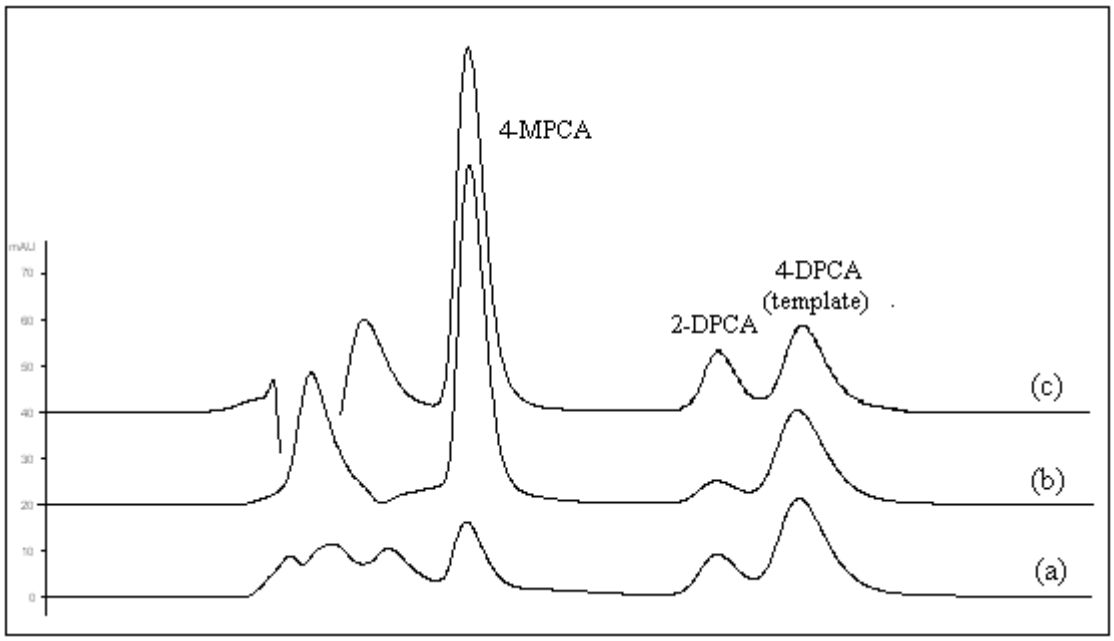

Fig.2. Chromatograms of rat serum after SPE on MIP2 (a) and on traditional C18 cartridge (b). Chromatogram of the mixture of standards (solution in methanol) (4-MPCA, 2-DPCA, 4-DPCA), c= 10µg/ml (c).

Table 1. Recoveries of target analytes on used sorbents [%]. RSD = 5-8%

	4-MPCA	2-DPCA	4-DPCA (template)
MIP	96,5	45,0	97,8
C18	22,3	86,1	98,2

The aim of this work was to check, if the MIP is able to recognize the template molecule in the presence of structurally related compounds and to compare the recoveries of SPE procedures by using MIP and C18 sorbents. As it is obvious from Figure 2 and Table 1, the recovery for the template (4-DPCA) is very good using both tested sorbent. The recovery of MISPE is much lower for 2-DPCA. It demonstrates that MIP2 can recognize template molecule from the compound with alkoxy- chain in other position.

References

Claude B, Morin P, Lafosse M, Belmont AS, Haupt K (2008) Talanta 75: 344-350.

Fischer L, Muller R, Ekberg B, Mosbach K (1991) J. Am. Chem. Soc. 113 (24): 9358-9360.

Javanbakht M, Fard SE, Mohammadi A, Abdouss M, Ganjali MR, Norouzi P, Safaraliee L (2008) Anal. Chim. Acta 612: 65-74.

Jiang M, Zhang J, Mei S, Shi Y, Zou L, Zhu Y, Dai K, Lu B (2006) J. Chromatogr. A 1110, 27-34.

Jiang X, Zhao Ch, Jiang N, Zhang H, Liu M (2008) Food Chem. 108: 1061-1068.

Pokorná M, Čižmárik J, Sedlárová E, Račanská E (1999) Čes. a Slov. Farm. 48: 80-86.

Renčová M, Čižmárik J, Lehotay J, Hroboňová (2002) Čes. a Slov. Farm. 51: 150-153.

Sellergren B (1994) Anal. Chem. 66 (9): 1578 – 1582.

Tamayo FG, Turiel E, Martín-Esteban A (2007) J. Chromatogr. A 1152.:32-40.

Yan S, Fang Y, Gao Z (2007) Biosens. Bioelectron. 22 (6): 1087-1091.

Yang HH, Zhang SQ, Tan F, Zhuang ZX, Wang XR (2005) J. Am. Chem. Soc. 127 (5): 1378-1379.

Zhang T, Liu F, Chen W, Wang J, Li K (2001) Anal. Chim. Acta 450: 53-61.