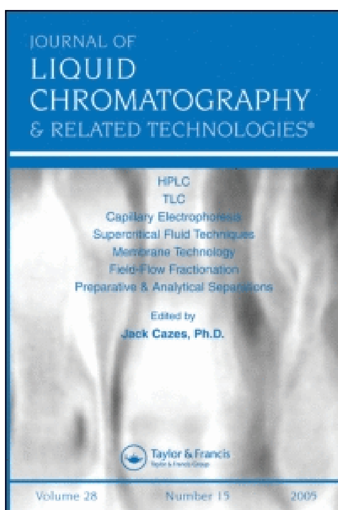
This article was downloaded by:
On: *23 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Molecularly Imprinted Solid-Phase Extraction of 1-Methyl-2-piperidinoethylesters of Alkoxyphenylcarbamic Acid from Human Plasma, Comparison with Classical Solid-Phase Extraction

M. Lachová[a]; J. Lehotay[a]; I. Skačáni[a]; J. 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

To cite this Article Lachová, M. , Lehotay, J. , Skačáni, I. and Čižmárik, J.(2009) 'Molecularly Imprinted Solid-Phase Extraction of 1-Methyl-2-piperidinoethylesters of Alkoxyphenylcarbamic Acid from Human Plasma, Comparison with Classical Solid-Phase Extraction', Journal of Liquid Chromatography & Related Technologies, 32: 16, 2293 — 2306
To link to this Article: DOI: 10.1080/10826070903187684
URL: http://dx.doi.org/10.1080/10826070903187684

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Journal of Liquid Chromatography & Related Technologies®, 32: 2293–2306, 2009
Copyright © Taylor & Francis Group, LLC
ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070903187684

Molecularly Imprinted Solid-Phase Extraction of 1-Methyl-2-piperidinoethylesters of Alkoxyphenylcarbamic Acid from Human Plasma, Comparison with Classical Solid-Phase Extraction

M. Lachová,[1] J. Lehotay,[1] I. Skačáni,[1] and J. Čižmárik[2]

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

Downloaded At: 15:53 23 January 2011

Abstract: Molecularly imprinted polymers for 1-methyl-2-piperidinoethylester of 2-methoxyphenylcarbamic acid (template) have been synthesised using a non-covalent imprinting approach. Acrylamid and methacrylic acid, respectively, were used as the functional monomers. Imprinted polymers were used as the sorbents for solid-phase extraction (MISPE). The capacity and selectivity of prepared imprinted polymers was investigated. The non-imprinted (blank) polymers were prepared by the same way without template to study the non-specific interactions. Molecularly imprinted polymer prepared from methacrylic acid was used to preconcentrate 1-methyl-2-piperidinoethylester of 2-methoxyphenylcarbamic acid and its analogues from spiked human plasma.

Keywords: HPLC, Molecularly imprinted polymer, SPE

Correspondence: Professor Jozef Lehotay, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, 812 37 Bratislava, Slovak Republic. E-mail: jozef.lehotay@stuba.sk

INTRODUCTION

Alkoxysubstituted esters of phenylcarbamic acid form a group of potential drugs employed local anaesthesias.[1] Some of them were extracted from human plasma using a molecularly imprinted polymer (MIP) as a selective sorbent for solid-phase extraction. Molecular imprinting techniques are very attractive because specific recognition sites for a target molecule could be easily moulded in synthetic polymer networks.[2] MIPs can be prepared in many different ways, for instance bulk polymerisation,[3,4] suspension polymerisation,[5,6] precipitation polymerisation,[7,8] multistep swelling polymerisation,[9] etc. MIPs are stable towards a wide range of solvents, are highly termostable, can be used over a range of temperatures.[10] They can be stored at ambient temperature and in dry state without loss of performance. In this study, MIPs were prepared by a bulk polymerization method, where the resultant monoliths had to be crushed, ground and sieved to produce microparticles for their applications.

The non-imprinted (blank) polymers (NIPs) were also prepared. These non-imprinted polymers possess the same chemical properties as those of the MIPs but without possessing cavities. Therefore, the nature of the interactions between a MIP and a template is the same as between the NIP and the template, the difference between the two sorbents being the strength of the interactions developed with the template. If well-defined cavities were created during the polymerization step, the strength of the interactions is higher on the MIP than on the NIP because the template can be retained by different points (sum of the interactions) due to the spatial complementarities of the template with cavities.[11]

Presented group of derivatives of alkoxyphenylcarbamic acid includes 30 derivatives with various alkoxygroup from methoxy- to decyloxygroup in different positions. Theoretical studies of physical-chemical, pharmacological and chromatographic properties and biological activity have been studied.[1,12,13]

The aim of this work was to find out the sorptive properties of the MIP when the analyte with shorter alkoxygroup is used as the template. We evaluated the binding capacity and selectivity of prepared MIPs (1-methyl-2-piperidinoethylester of 2-methoxyphenylcarbamic acid as template) towards structurally related compounds: with different length and position of alkoxy-group in 1-methyl-2-piperidinoethylester of alkoxyphenylcarbamic acid. MIPs were utilized as a molecular imprinted solid-phase extraction sorbent for preconcentration of derivatives of phenylcarbamic acid from the human plasma. Solid-phase extraction was performed also by using classical sorbent C18 and the results were compared.

Downloaded At: 15:53 23 January 2011

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 and co-workers.[1] Acetonitrile, methanol, methacrylic acid and diethylamine were purchased from Merck, acrylamide, azobisizobutylonithrile (AIBN) and ethyleneglycoldimethacrylate (EGDMA) were obtained from Fluka, and acetic acid was purchased from Lachema. SPE cartridges Sep-Pak 3cc C18 were bought from Waters.

HPLC Analysis

Downloaded At: 15:53 23 January 2011

An HP 1100 system (Hewlett-Packard, Germany), consisting of a pump with a degasser, a diode-array detector (DAD) a 50 μL injector and a HP ChemStation were used. Analyses were carried out on the analytical column Separon SGX C18 (125 × 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, v/v/v/v) at a flow rate of 0.5 mL/min. was employed. Isocratic 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 all's method.[14] Two MIPs were synthesized, using acrylamide (MIP1) and methacrylic acid (MIP2), respectively, as a functional monomers. The monomer (1.8 mmol), the template molecule 1-methyl-2-piperidinoethylester of 2-methoxyphenylcarbamic 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 and acetic acid (9:1, v/v) until template was not detected in the extract. Non-imprinted, control polymers (NIPs) were prepared in the same manner as MIPs but in the absence of template molecule in the polymerization mixture.

Evaluation of MIP

100 mg of each polymer was packed into 3 mL polypropylene cartridges. The cartridge capacity was tested in methanol, acetonitrile and water. Prior to applying the solution of ester of 4-methoxyphenylcarbamic acid (2-MPCA), which was used as a template during polymerization, the polymer was pre-equilibrated with 5 mL of methanol and then with 5 mL of solvent in which the capacity was studied. 2-MPCA solution was gradually applied onto the cartridge until release of 2-MPCA was detected. All effluents were collected in 1 mL fractions and measured by HPLC. The concentration of 2-MPCA in acetonitrile and methanol was 20 μg/mL.

Selectivity of MIP2 for esters of 2-decyloxyphenylcarbamic acid (2-DPCA) and of 4-methoxyphenylcarbamic acid (4-MPCA) was tested. Selectivity was studied by the same procedure as described for the template. The concentration of 4-MPCA and 2-DPCA was 20 μg/mL.

MIP2 and NIP2 cartridges were conditioned with 5 mL of methanol, 5 mL of solvent, which was used in the washing step, and 5 mL of acetonitrile. After the conditioning, the acetonitrile solution of 2-MPCA was applied onto a polymer cartridge. 1 mL of template solution was applied onto MIP2 (20 μg/mL in acetonitrile) and onto NIP2 (2 μg/mL in acetonitrile). Methanol, acetonitrile, water, mixture of methanol-acetic acid (99:1, v/v), mixture of methanol-acetic acid (95:5, v/v) and mobile phase (methanol-acetonitrile-acetic acid-diethylamine 80:20:0.1:0.1, v/v/v/v) were tested as the washing solvents. Each effluent was collected in 1 mL fractions. Only the first eluted millilitre was collected in two 0.5 mL fractions. In the case of mixtures of methanol/acetic acid, all fractions were evaporated to dryness and redissolved in methanol. All fractions were analysed by HPLC.

The same washing solvents were tested for 4-MPCA and 2-DPCA. Concentration of both solution and the procedure of washing solvents investigation was the same as in the case of the template (2-MPCA).

MISPE Procedure

100 mg of polymers MIP2 and NIP2 were packed into polypropylene cartridges. C18 sorbent was also used for SPE to compare the efficiency of the procedure. Cartridges were gradually conditioned with 5 mL of methanol, 5 mL of acetonitrile and 5 mL of water. Then 5 mL of spiked human plasma was applied onto each cartridge. The concentration of studied analytes (2-MPCA, 4-MPCA, 2-DPCA and 4-DPCA) in plasma was 1 μg/mL. Then the cartridges were washed with 2 mL of water and dried. Then dry sorbents were washed with 1 mL of acetonitrile and dried again. Analytes were eluted by 2 mL of mixture methanol-acetic acid (95:5, v/v).

Downloaded At: 15:53 23 January 2011

Effluents were evaporated to dryness, redissolved in 0.5 mL of methanol. In this way the sample of plasma can be preconcentrated 10 times. These extracts were filtered through the nylon microfilters (0.45 μm) and injected into the HPLC system.

RESULTS AND DISCUSSION

Capacity of Polymers

As it is described in Experimental, the capacity of MIPs was tested in different solvents: methanol, acetonitrile and water. Prior to the applying the solution of 2-MPCA, the cartridges were conditioned with methanol and then with solvent, in which the capacity was studied. The same experiment was performed also on relevant NIPs and the resultant values of polymer capacities are shown in Table 1. The specific binding capacity was calculated by the deduction of the amount non-specifically adsorbed on NIP from the amount adsorbed on MIP.

It is obvious from the Table 1 that the best recognition of target analyte was obtained on MIP2 in acetonitrile, which was used as the porogen during the polymerization. This solvent is able to recreate the interaction that took place during the polymerization.[11] Furthermore, acetonitrile has weak hydrogen-bonding capacity and the ability to compete for the hydrogen-bonding sites on the template or in polymer matrix is limited. In the case of MIP1, which was prepared using acrylamide as a functional monomer, there are no specific interactions between polymer and analyte in the acetonitrilic environment. It could be explained that there is no interaction between the functional group of the monomer and functional group of the template during the polymerization.

The capacity of MIP2 in methanol is much lower than in acetonitrile. It can be caused by stronger interaction between methanol and analyte (2-MPCA) than between polymer and analyte. Methanol is polar solvent

Table 1. Binding capacities of prepared polymers. RSD = ±5–14%, n = 3

	Capacity (μg of analyte/100 mg of polymer)					
Solvent	MIP1	NIP1	SBC*	MIP2	NIP2	SBC*
Acetonitrile	0.6	0.6	0	164	11.0	153
Methanol	0.5	0.5	0	29.6	8.5	21.1
Water	>200	>200		>200	>200	

*The specific binding capacity was calculated by the deduction of the amount non-specifically adsorbed on NIP from the amount adsorbed on MIP.

Downloaded At: 15:53 23 January 2011

which can influence the hydrogen bonding between analyte and monomer.

When the water was used for sample loading the highest values of binding capacities were obtained for all MIPs and also for NIPs. The whole amount of loaded analyte (200 μg) was sorbed onto the sorbents. In aqueous environments, hydrogen-bonding and electrostatic interactions could be disrupted, and hydrophobic interactions, which are non-specific, could govern analyte retention. This allows using water samples for extraction.

Selectivity of MIPs

The selectivity of MIP2 was tested towards two structurally related esters of alkolyphenylcarbamic acid with different length and position of alkoxy-group. The influence of alkoxy-chain length on the polymer capacity was tested by using the 1-methyl-2-piperidinoethylester of 2-decyloxyphenylcarbamic acid (2-DPCA). The influence of position of alkoxy-chain was investigated by measuring of MIPs' capacity for 1-methyl-2-piperidinoethylester 4-methoxyphenylcarbamic acid (4-MPCA). Polymers were conditioned with methanol and acetonitrile and then the solution of tested analyte was gradually applied onto the cartridge. The polymer capacities of MIP2 and relevant NIP2 are shown in Table 2.

MIP2 demonstrates the highest binding capacity for 2-MPCA, which was used as template molecule in the process of MIP synthesis. The value of specific binding capacity of 4-MPCA was the same as for 2-MPCA. These two molecules are structurally closely related, the difference is only in the position of the methoxy group on the benzene ring. MIP2 can not recognize these two molecules and 4-MPCA can be retained in the MIP2 cavities. The specific binding capacity of MIP2 for 2-DCPA is lower then for 2-MPCA, which can be caused by longer alkoxy chain on the

Table 2. Binding capacities of prepared polymers. RSD = ±4–10%, n = 3

	Capacity (μg of analyte/100 mg of polymer)		
Analyte	MIP2	NIP2	SBC*
2-MPCA (template)	164	11.0	153
4-MPCA	158	11.5	146
2-DPCA	121	10.8	110

*The specific binding capacity was calculated by the deduction of the amount non-specifically adsorbed on NIP from the amount adsorbed on MIP.

Downloaded At: 15:53 23 January 2011

molecule of 2-DPCA. Although the value of specific binding capacity is lower, the difference is not very significant.

Washing Step and Elution

After the determination of the cartridge capacity, the different washing and elution solvents were studied. Washing step is necessary for removing of interferences from the MIP. The clean-up solvent should suppress the non-specific interactions without disrupting the selective interactions between the MIP and the target molecule. Good elution solvent has to remove the sorbed analytes with maximal recovery in a minimal volume of this solvent. Cartridges with MIP2 and NIP2 were conditioned with 5 mL of methanol and with 5 mL of solvent, which was used in the washing step and 5 mL of acetonitrile. After the conditioning, the solution of 2-MPCA was applied onto a polymer cartridge. Methanol, acetonitrile, water, mixture of methanol-acetic acid (99:1, v/v), mixture of methanol-acetic acid (95:5, v/v) and mobile phase (methanol-acetonitrile-acetic acid-diethylamine 80:20:0.1:0.1, v/v/v/v) were used as washing solvent. Results are shown in Figs. 2 and 3.

Downloaded At: 15:53 23 January 2011

Some authors have stated that recognition is often better when the porogen is used as the solvent because the environment established during the synthesis is reproduced (solvent memory effect).[15,16] Acetonitrile was used as porogen and this solvent was also chosen for sample loading. No analyte was washed out after the cartridge washing with 5 mL of acetonitrile. Water was another solvent tested. It could be used for removing matrix components of hydrophilic properties from the cartridge. Significant amount of 2-MPCA was washed out by the application of 5 mL of methanol on MIP2 and NIP2 (13% from MIP2 and 22% from NIP2, respectively). Opposite effect can be observed in the case of methanol containing acetic acid (1%, 5%) and mixture of methanol–acetonitrile–diethylamine–acetic acid (80:20:0.1:0.1 – mobile phase). All

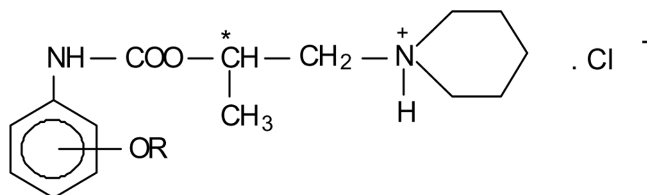


Figure 1. Structure of compounds under study. Template molecule: R = $-CH_3$ in 2-position (2-MPCA). Other analytes used in research: R = $-CH_3$ in 4-position (4-MPCA), R = $-C_{10}H_{21}$ in 2-position (2-DPCA), R = $-C_{10}H_{21}$ in 4-position (4-DPCA).

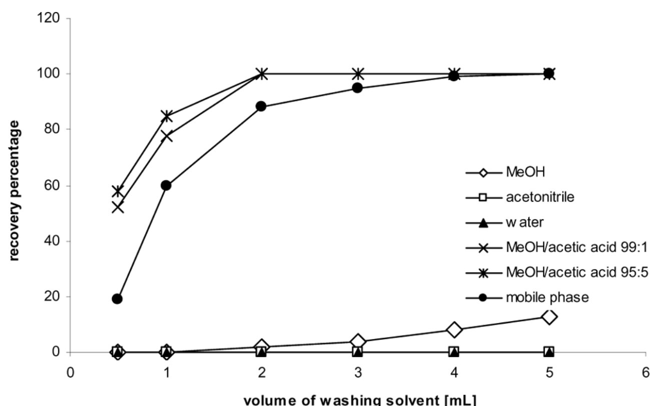


Figure 2. Release percentage of 2-MPCA after percolation of increasing volumes of different washing solvents through the MIP2.

Downloaded At: 15:53 23 January 2011

three solvents were chosen because whole amount of 2-MPCA was washed out by 5 mL of each of these solvents. Only 2 mL of the mixture of methanol and acetic acid was needed for complete elution of 2-MPCA.

The same solvents are used also for structurally related compounds, 4-MPCA and 2-DPCA. The influence of solvent volume on the release percentage is shown in Figs. 4 and 5.

Both tested analytes (4-MPCA and 2-DPCA) were washed out with small volume of methanol/acetic acid mixture and with mobile phase like in the case of 2-MPCA. Also after the washing of MIP2 by acetonitrile and water, the same results for 4-MPCA and 2-DPCA were achieved like

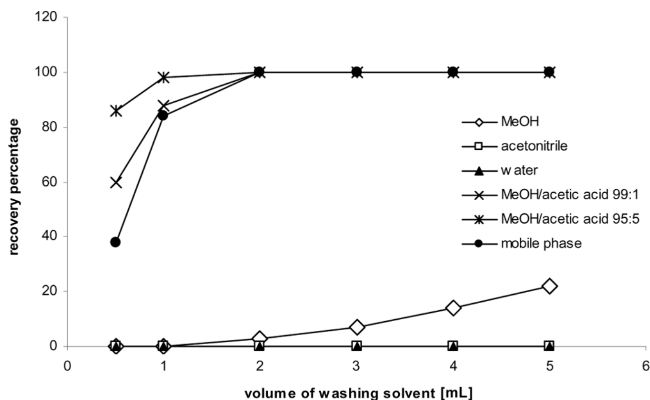


Figure 3. Release percentage of 2-MPCA after percolation of increasing volumes of different washing solvents through the NIP2.

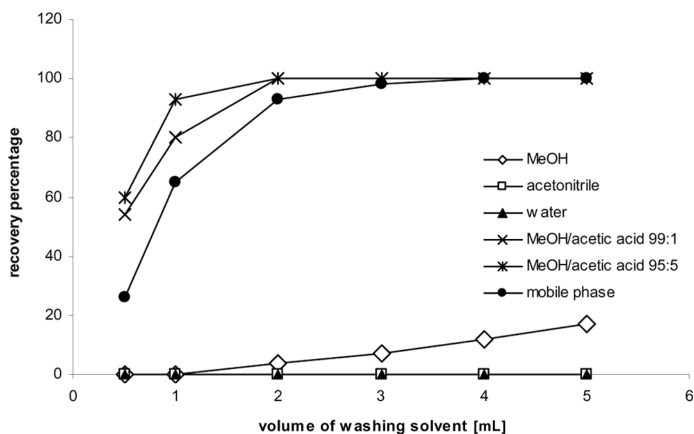


Figure 4. Release percentage of 4-MPCA after percolation of increasing volumes of different washing solvents through the MIP2.

Downloaded At: 15:53 23 January 2011

for the template molecule. The loss of 4-MPCA and 2-DPCA from cartridge during the washing of cartridges by water and acetonitrile was not observed. After the washing step of MIP2 by 5 mL of methanol, 17% of 4-MPCA and 25% of 2-DPCA was washed out. The sorption properties of 4-MPCA and 2-DPCA seem to be similar to the template, which can be caused by the similarity of analytes' structures.

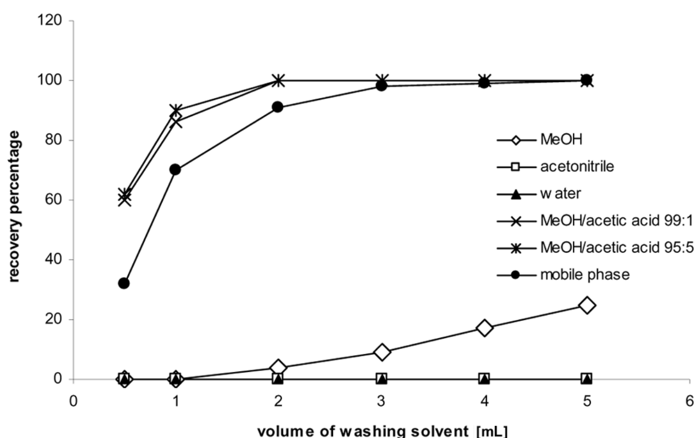


Figure 5. Release percentage of 2-DPCA after percolation of increasing volumes of different washing solvents through the MIP2.

Isolation of Analytes from Human Plasma

Matrix effect of spiked human plasma by using different sorbents (C18 MIP2 and NIP2) for SPE was investigated. Human plasma was spiked with 2-MPCA, 4-MPCA, 2-DPCA and 4-DPCA. Final concentration of each analyte was 1 μg/mL in plasma. After conditioning of cartridges with methanol, acetonitrile and water, 5 mL of plasma was percolated through each cartridge. After the washing of cartridges by water and acetonitrile, analytes were eluted by the mixture of methanol and acetic acid (95:5, v/v). Each effluent was evaporated to dryness, dissolved in 0.5 mL of methanol and analysed. The results were compared with results obtained in SPE on commonly used C18 sorbent and on NIP2. Chromatograms of blank and spiked human plasma after SPE on MIP2, NIP2 and C18 are shown in Figs. 6–8. Percentage recoveries of studied analytes from spiked human plasma after SPE using MIP2, NIP2 and C18 cartridges are given in Table 3.

Chromatograms of blank human plasma suggest that the better removing of interferences can be obtained by SPE on MIP2 than on

Downloaded At: 15:53 23 January 2011

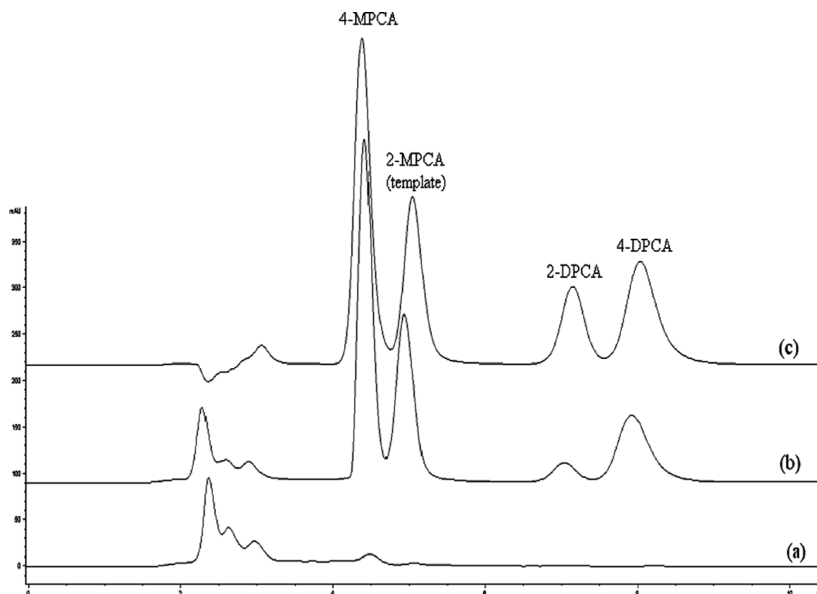


Figure 6. Chromatograms of blank human plasma (a) after SPE on MIP2, spiked human plasma (1 (g/mL)), (b) after SPE on MIP2 and the mixture of standards (solution in methanol, 10 μg/mL of each analyte). HPLC column: Separon SGX C18. Mobile phase: methanol/acetonitrile/acetic acid/diethylamine (80/20/0.1/0.1, v/v/v/v), izokratic elution. $F = 0.5$ mL/min. Detection: DAD, 240 nm. Injected volume: 50 μL.

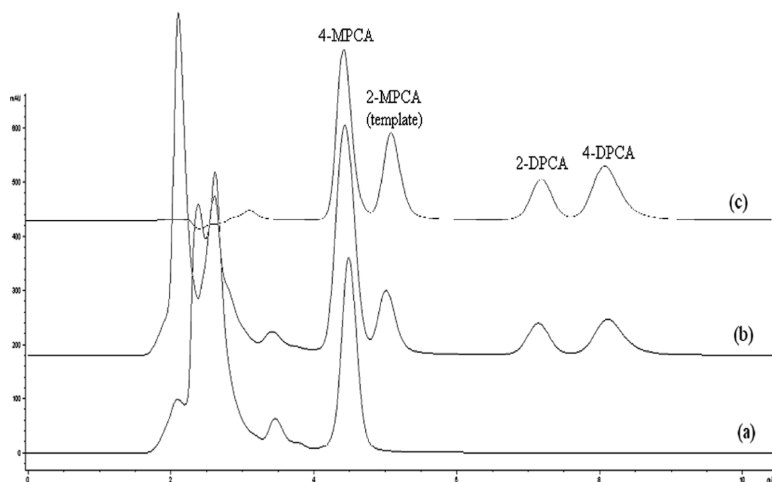


Figure 7. Chromatograms of blank human plasma (a) after SPE on C18, spiked human plasma (1 (g/mL)), (b) after SPE on C18 and the mixture of standards (solution in methanol, 10 μg/mL of each analyte). HPLC column: Separon SGX C18. Mobile phase: methanol/acetonitrile/acetic acid/diethylamine (80/20/0.1/0.1, v/v/v/v), izokratic elution. $F = 0.5$ mL/min. Detection: DAD, 240 nm. Injected volume: 50 μL.

Downloaded At: 15:53 23 January 2011

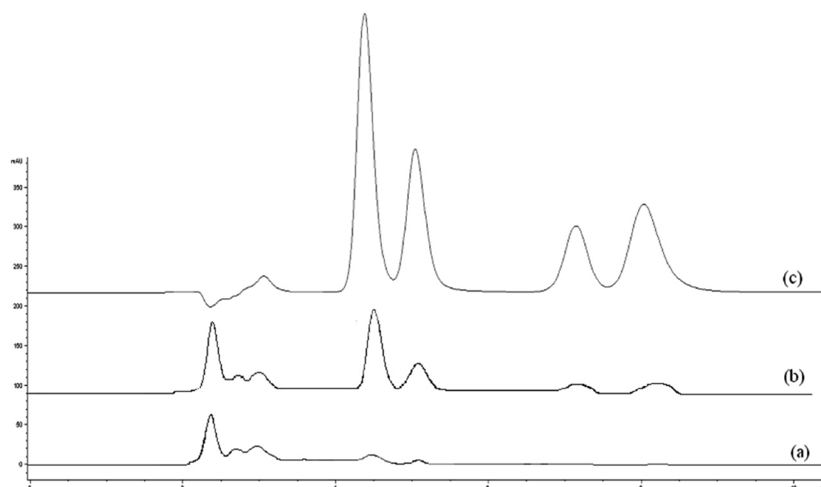


Figure 8. Chromatograms of blank human plasma (a) after SPE on NIP2, spiked human plasma (1 (g/mL)), (b) after SPE on NIP2 and the mixture of standards (solution in methanol, 10 μg/mL of each analyte). HPLC column: Separon SGX C18. Mobile phase: methanol/acetonitrile/acetic acid/diethylamine (80/20/0.1/0.1, v/v/v/v), izokratic elution. $F = 0.5$ mL/min. Detection: DAD, 240 nm. Injected volume: 50 μL.

Table 3. Recoveries of target analytes on investigated sorbents [%]. RSD = ±5–8%

	Analyte			
	2-MPCA (template)	4-MPCA	2-DPCA	4-DPCA
MIP2	97.1	94.7	27.6	75.4
C18	77.0	*	68.7	71.4
NIP2	17.7	20.9	10.0	10.1

*Coelution with another compound from plasma.

classical C18. On the chromatogram of blank plasma after SPE using C18, there is an interfering peak with the same retention time as the analyte 4-MPCA, therefore 4-MPCA can not be reliably quantified.

Recoveries of the template (2-MPCA) and its analogue with methoxy-group in 4-position from MIP2 are very good, more than 90%. The recoveries of derivatives with decyloxy-group in 2-(2-DPCA) and 4-(4-DPCA) position is lower. It can be explained by weaker retention on MIP2 sorbent. Weaker retention can be caused by the difference of the structure of the molecules. The recoveries of tested analytes on C18 range from 68.7 to 77%. In the case of NIP 2 the recoveries of target analytes is much lower than on MIP2. Probably it is caused by weaker the non-specific interaction between analytes and the NIP than the specific interaction between analytes and the MIP. The recoveries of analytes on NIP2 range from 10 to 20.9%.

Molecularly imprinted polymers can be successfully utilized as a sorbents for solid-phase extraction. In our work MIP prepared using methacrylic acid and acetonitrile as the functional monomer and porogen, respectively, was used. The derivatives of phenylcarbamic acid from spiked human plasma were extracted. The analytes were 10 times preconcentrated by this procedure and the recoveries for target analyte 2-MPCA was 97.1%.

ACKNOWLEDGMENT

Support from the Grant agency Slovak Republic (grants 1/0058/08), APVV project No. 20-035-205 and VEGA project 1/4291/07 is gratefully acknowledged.

REFERENCES

1. Pokorná, M.; Čižmárik, J.; Sedlárová, E.; Račanská, E. The relationship of the structure, physical-chemical properties and local anaestetic activity in a group

Downloaded At: 15:53 23 January 2011

of 1-Methyl-2-piperidinoethylester of 2-,3- and 4-alkoxyphenylcarbamic acid. Study of local anaesthetic. Part 147. Čes. a Slov. Farm. **1999**, *48*, 80–86.
2. Caro, E.; Marcé, R.M.; Borrull, F.; Cormack, P.A.G.; Sherrington, D.C. Application of molecularly imprinted polymers to solid-phase extraction of compounds from environmental and biological samples. Trac - Trends Anal. Chem. **2006**, *25*, 143–154.
3. Sun, Z.; Schüssler, W.; Sengl, M.; Niessner, R.; Knopp, D. Selective trace analysis of diclofenac in surface and wastewater samples using solid-phase extraction with a new molecularly imprinted polymer. Anal. Chim. Acta. **2008**, *620*, 73–81.
4. Yavuz, H.; Karakoc, V.; Türkmen, D.; Say, R.; Denizli, A. Synthesis of cholesterol imprinted polymeric particles. Int. J. Biol. Macromol. **2007**, *41*, 8–15.
5. Shi, X.; Wu, A.; Zheng, S.; Li, R.; Zhang, D. Molecularly imprinted polymer microspheres for solid-phase extraction of chloramphenicol residues in foods. J. Chromatogr. B **2007**, *850*, 24–30.
6. Peréz-Moral, N.; Mayes, A.G. Direct rapid synthesis of MIP beads in SPE cartridges. Biosens. Bioelectron. **2006**, *21*, 1798–1803.
7. Tasselli, F.; Donato, L.; Drioli, E.J. Evaluation of molecularly imprinted membranes based on different acrylic copolymers. J. Membr. Sci. **2008**, *320*, 167–172.
8. Turiel, E.; Martín-Esteban, A.; Tadeo, J.L. Molecular imprinting-based separation methods for selective analysis of fluoroquinolones in soils. J. Chromatogr. A **2007**, *1172*, 97–104.
9. Haginaka, J.; Tabo, H.; Kagawa, C. Uniformly sized molecularly imprinted polymers for d-chlorpheniramine: Influence of a porogen on their morphology and enantioselectivity. J. Pharm. Biomed. Analysis **2008**, *46*, 877–881.
10. Fischer, L.; Muller, R.; Ekberg, B.; Mosbach, K. Direct enantioseparation of beta-adrenergic blockers using a chiral stationary phase prepared by molecular imprinting. J. Am. Chem. Soc. **1991**, *113*, 9358–9360.
11. Pichon, V. Selective sample treatment using molecularly imprinted polymers. J. Chromatogr. A **2007**, *1152*, 41–53.
12. Ďungelová, J.; Lehotay, J.; Hroboňová, K.; Čižmárik, J.; Armstrong, D.W. Study of Local anaesthetics part CLVIII. Chromatographic separation of some derivates of substituted phenylcarbamic acid on vancomycin-based stationary phase. J. Liq. Chromatogr. **2002**, *25*, 299–312.
13. Rojkovičová, T.; Hroboňová, K.; Lehotay, J.; Čižmárik, J. Coupled column chromatography for separation and determination of derivatives of phenylcarbamic acid enantiomers in serum. Die Pharmazie **2003**, *58*, 108–110.
14. Zhang, T.; Liu, F.; Chen, W.; Wang, J.; Li, K. Influence of intramolecular hydrogen bond of templates on molecular recognition of molecularly imprinted polymers. Anal. Chim. Acta. **2001**, *450*, 53–61.
15. Chapuis, F.; Pichon, V.; Lanza, F.; Sellergren, B.; Hennion, M.C. Optimization of the class-selective extraction of triazines from aqueous samples using a

Downloaded At: 15:53 23 January 2011

molecularly imprinted polymer by a comprehensive approach of the retention mechanism. J. Chromatogr. A **2003**, *999*, 23–33.
16. Hosoya, K.; Yoshizako, K.; Sasaki, H.; Kimata, K.; Tanaka, N. Molecular recognition towards coplanar polychlorinated biphenyls based on the porogen imprinting effects of xylenes. J. Chromatogr. A **1998**, *828*, 91–94.

Received February 28, 2009
Accepted March 14, 2009
Manuscript 6498

Downloaded At: 15:53 23 January 2011