Chinese Chemical Letters Vol. 16, No. 11, pp 1503-1506, 2005 1503
http://www.imm.ac.cn/journal/ccl.html

A New Molecularly Imprinted Polymer for Solid-phase Extraction of Cotinine from Human Urine

Jun YANG[1,2], Xiao Lan ZHU[1,2], Ji Bao CAI[1,2], Qing De SU[1,2,*], Yun GAO[2], Liang ZHANG[2]

[1] Department of Chemistry, University of Science and Technology of China, Hefei 230026
[2] Research Center of Tobacco and Health, University of Science and Technology of China, Hefei 230026

Abstract: A molecularly imprinted polymer (MIP), prepared around a cotinine template, has been synthesized. The feasibility of using the polymer for solid-phase extraction (SPE) of cotinine from biological samples has been investigated. The results show that cotinine can be quantitatively retained and eluted from the polymer. Experiments with human urine samples indicate that clean target analyte is obtained for HPLC with UV detection using the protocol.

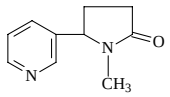
Keywords: Molecularly imprinted polymer, solid-phase extraction, cotinine, urine.

Cotinine (COT), with molecular structure presented in **Scheme 1**, the main metabolite of nicotine in man, is widely used as an abstention marker to *Nicotiana tabacum* smoke, as well as to evaluate passive inhalation of tobacco smoke by non-smokers[1]. Several methods using high-performance liquid chromatography (HPLC), gas chromatography (GC) or capillary electrophoresis (CE) are available for quantification of cotinine in body fluids, with liquid-liquid extraction or reversed-phase sorbents for SPE steps[2]. Though these methods offer good detection sensitivity, they generally suffer from low selectivity in sample preparation steps. Hence, a selective sample pretreatment procedure for the subsequent quantification of cotinine is highly desirable.

Molecular imprinting is a technique for obtaining very selective binding sites in highly cross-linked macroporous polymers. Among the applications proposed, the development of stationary phases to selectively extract trace analytes present in complex samples is very promising[3, 4]. SPE procedures using molecularly imprinted polymers (MI-SPE) have been reported for target analytes of biological and environmental samples[5, 6]. The aim of the present work was to demonstrate the feasibility of using MI-SPE for the selective clean-up and quantification of cotinine from human urine. To the best of our knowledge this is the first time that a MIP，synthesized following a non-covalent imprinting protocol using cotinine as the template molecule，has been used as a sorbent in SPE of biological samples to extract cotinine selectively.

* E-mail: qdsu@ustc.edu.cn

Scheme 1 Chemical structure of cotinine



Experimental

In the MIP synthesis, cotinine (1 mmol), methacrylic acid (4 mmol), ethylene glycol dimethacrylate (20 mmol), azo-N, N′-diisobutyronitrile (0.24 mmol) and 5.6 mL of dichloromethane were placed in a 20 mL thick-walled glass tube. The solution was degassed by sparging with N_2 gas for 5 min and the tube was sealed and heated in a water bath at 60℃ for 24 h. The resulting polymer was washed with methanol–acetic acid (9:1, v/v) in a Soxhlet apparatus for 16 h until cotinine was not detected in the washing solution. Afterwards it was ground and wet-sieved to 25-36 μm particle size, dried overnight at 105℃. A blank polymer (NIP) was prepared and treated in the same manner, but without cotinine.

Equilibrium adsorption experiments were used to evaluate the binding affinity of the MIP. 20 mg polymers were added to a 2 mL acetonitrile solution of cotinine of known concentrations (0.01 to 4.0 mmol/L) in vials and was rotated for 16 h at 25 ℃. The concentration of free cotinine was determined by HPLC.

Chromatographic separation of the cotinine and its structural analogs was performed to study the selectivity of the MIP. The MIP and the NIP were slurry packed into 150 ×2.1 mm stainless-steel columns, respectively. Retention and elution on the templated and blank columns were evaluated by injecting 20 μL 10 μg/mL of cotinine, nicotine, nornicotine, N-nitrosonornicotine(NNN), caffeine in acetonitrile onto the columns. The mobile phase was a mixture of MeCN-H_2O-HOAc (92.5/2.5/5, v/v) at a flow rate of 0.6 ml/min. Void time was measured using acetone. The capacity factor *k'*, and the imprinting factor *K*, and the selective factor *I*, were defined by the literature[7].

SPE columns were prepared by packing 200 mg suspensions of the MIP and NIP respective polymers in methanol into empty 3 mL SPE cartridges. The cartridges were conditioned with 1 mL CH_3COONH_4-$NH_{3(aq)}$ buffer，pH = 9.0. The urine samples, adjusted to pH = 9.0 using the alkaline buffer, were filtered and applied to the conditioned SPE column. The column was washed with 1 mL buffer and 2 mL MeCN, and eluted with 2 mL MeCN-H_2O-HOAc (95/2.5/2.5, v/v). All the fractions from the sample loading, washing and elution steps were analyzed by HPLC, using UV detector at 260 nm.

Results and Discussion

Equilibrium adsorption experiments and subsequent Scatchard analysis showed the MIP had higher binding association constants and more apparent binding sites. The calculated dissociation constant (K_D) were 5.21×10^{-4} mol/L (low concentration section) and 4.06×10^{-3} mol/L (high low concentration section) while the apparent maximum

number of binding sites (Q_{max}) were 44.3 μmol/g and 135.4 μmol/g, respectively.

Then, the selectivity of the MIP was evaluated by measuring its ability to resolve structural analogs in the HPLC process. The selectivity factors obtained from the experiments are listed in **Table 1**. The MIP exhibited a pronounced selectivity for cotinine, while the structurally unrelated compound, such as caffeine, co-eluting with cotinine from commercial C18 columns[2], was less retained on the MIP. For the compounds with more tenuous structural relationship, such as nicotine, nornicotine, NNN, some recognition was shown, but the recognition was greatly reduced compared with the template. Therefore it appears that the selectivity of MIP for cotinine was clearly induced during the imprinting process. The results of the selectivity test may also give aspects of the molecular recognition mechanisms. The base structure of cotinine, nornicotine or nicotine, did not show long retention times. This result agrees with the presumption that the lactam group in cotinine, stronger hydrogen bond acceptors, is likely to be the predominant interaction with the functional monomer. The nitroso group in the NNN and the size of the molecular possibly accounts for the fine retention on the MIP. It is obvious that not only the substituent but also the molecular size and shape plays the important roles in selective recognition process of MIP. Thus, the affinity and selectivity evaluation indicated that MIP is a potential SPE separation material can be taken forward and applied to selectively extract cotinine from human urine.

The aim of the subsequent work was to evaluate the feasibility of using MIP sorbents for SPE of cotinine. Loading in aqueous environment resulted in strong retention of cotinine in both MIP and NIP polymers, but this was attributed to non-specific binding in the polymers. In such a case, optimization of the washing steps is critical to disrupt non-specific interactions between the analytes and polymeric stationary phase. The first washing step, 1 mL pH = 9.0 alkaline buffer was applied to

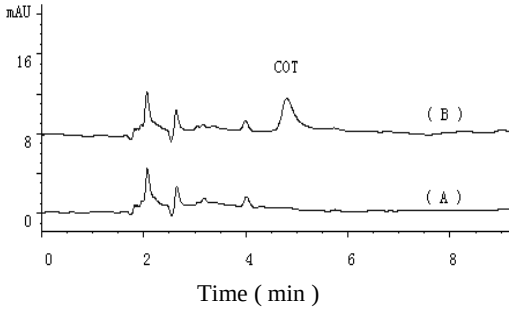
Table 1 Selectivity of cotinine-imprinted polymer

Compounds	$k'_{\text{blank column}}$	$k'_{\text{templated column}}$	K	I
Cotinine	2.5	15.6	6.24	1.00
Nicotine	3.8	11.5	3.03	0.48
Nornicotine	3.5	8.9	2.54	0.41
NNN	3.3	13.8	4.18	0.67
Caffeine	2.6	3.0	1.15	0.18

Table 2 Recovery of cotinine (10 μg/mL in alkaline buffer) on MI-SPE procedure

Fractions	Recovery (%)	
	NIP	MIP
Loading (1 mL)	0.0	0.0
Washing (1 mL, CH_3COONH_4-NH_3(aq), pH=9.0)	4.3	0.0
Washing (2 mL, MeCN)	64.6	10.2
Elution (1 mL, MeCN-H_2O-HOAc, 95/2.5/2.5, v/v)	22.9	61.8
Elution (1 mL, MeCN-H_2O-HOAc, 95/2.5/2.5, v/v)	6.5	29.6
Total	98.3	101.6

Figure 1 HPLC chromatograms of cotinine extracted from human urine by the MI-SPE



elute polar matrix components. Then the second washing step, 2 mL MeCN was used as the solvent to enhance the selective imprinting effect. The results (**Table 2**) showed that cotinine was retained by the MIP through the loading and washing steps, and could be quantitatively eluted using MeCN-H_2O-HOAc (95/2.5/2.5,v/v). In contrast, on the NIP most of the cotinine eluted in the wash steps. Expriments with human urine indicated that clean target analyte was obtained using the MI-SPE protocol (**Figure 1)**. Cotinine peak at 4.805 min. **Figure 1 (A)** is the chromatogram of 1.0 mL urine blank extracted on the MIP and **Figure 1 (B)**, 1.0 mL urine blank spiked with 2.5 μg cotinine extracted on the MIP, with the recovery of cotinine was 90.2%.

In conclusion, this study shows the synthesis and the application in SPE of the cotinine MIP. Due to the minimal sample preparation required and short time of analysis, this approach appears to be very well-suited for the control of cotinine in urine samples of passive smokers.

Acknowledgments

This research work has been supported by the National Natural Science Foundation of China (No. 20405013) and the Scientific Foundation of State Tobacco Monopoly Administration of China (No. 110200201017).

References

1. J. Deleon, F. J. Diza, T. Rogers, *et al*., *J. Clin. Psychopharmacol.*, **2002**, *22*, 496.
2. P. B. Doctor, V. N. Gokani, P. K. Kulkarni, *et al*., *J. Chromarogr. B*, **2004**, *802*, 323.
3. P. Dhar, *J. Pharm. Boimed. Anal.*, **2004**, *35*, 155.
4. T. Y. Guo, Y.Q. Xia, G. J. Hao, *et al*., *Chin. Chem. Lett.*, **2004**, *15*, 1339.
5. S. G. Hu, S. W. Wang, X. W. He, *Actal. Chim. Sinica*, **2004**, *62*, 864.
6. J. P. Lai, R. Niessner, *Anal. Chim. Acta*, **2004**, *522*, 137.
7. J. A. Tarbin, M. Sharman, *Anal. Commun.*, **1999**, *36*, 105.

Received 28 January, 2005