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Development of Molecularly Imprinted Polymers (MIP_S) nano particles for the selective determination of Carbamazepine in human serum and plasma

Behrouz Akbari-adergani[1] and Ahmad Heydarieh[2*]

[1]Food and Drug Laboratories Research Center, Ministry of Health and Medical Education, Tehran, Iran
[2]Food and Drug Laboratories Research Center, Ministry of Health and Medical Education, Semnan, Iran
ahmadheydarieh@yahoo.com*

Abstract

A new, simple, rapid, and sensitive solid-phase extraction with molecularly imprinted polymer (SPE-MIP) for determination of Carbamazepine in biological samples was developed. These nano particle polymers were synthesized via a non-covalent molecular imprinting approach through precipitation polymerization method using methacrylic acid (MAA) as a functional monomer, ethylene glycol dimethacrylate (EGDMA) as a cross-linker agent, carbamazepine as a target template molecule and azobisisobutyronitrile (AIBN) as an initiator. The optimal conditions for SPE consisted of conditioning the cartridge using a pH=3.0 water, loading 5.0 ml of the sample under basic aqueous conditions, clean-up using 2×2ml acetonitrile and elution with 3.0 ml methanol. After optimization of SPE procedure, an aliquot of extracted template was injected to the ACE, 5µm 250×4.6 mm analytical column with the mobile phase as the same as elution solvent. This method was used for extraction of carbamazepine from an anticonvulsant aqueous solution and the results revealed an extraction recovery of more than 90%. HPLC chromatograms show an efficient clean up, which supports the potential of MISPE for clean-up of trace amounts of carbamazepine from the drug formulation.

Keywords: Molecularly imprinted polymerl, Carbamazepine, Solid-phase extraction, Biological samples

Introduction

Carbamazepine (CBZ) is widely used as the first line treatment for partial and generalized Tonic-clonic seizures (Beghi, 2002). CBZ also has other indications, including bipolar disorder, trigeminal neuralgia and other neuropathic pain syndromes (Wyllie, 2001; Trimble, 2002). Clinical effects of CBZ bear a relatively close relation to serum drug concentrations in epileptic patients (Bertilsson & Tomson, 1986). Optimal use of CBZ and its appropriate serum concentration depend to different factors, which affect pharmacokinetics of the drug (Bertilsson & Tomson, 1986). Because of abnormalities in neurotransmitters, neuroendocrine and membrane transport in manic patients (Nathan *et al.,* 1995; Goodwin & Jamison, 1990; Janicak *et al.,* 1997), it is suggested that pharmacokinetics of some drugs may be influenced by these patients.

The molecular imprinting technique has emerged as a powerful approach for the creation of recognition site in highly cross-linked polymeric matrices. The imprinting methodology involves copolymerization of matacrylic acid (MAA) monomers with ethylene glycoldimatacrylate monomers in the presence of guest molecules to produce three dimensional network polymers. Removal of the template yields a functional polymeric matrix with recognition site complementary in functionality and shape to the print molecule structure. These imprints exhibit selectivity for rebinding the template with which it was prepared. These MIP_S nano particles have been employed in fields where a certain degree of selectivity is required such as sensors, chromatography, and catalysis. Solid-phase extraction (SPE) has become a routine tool in many laboratories for preconcentration and clean-up steps in the analysis analysis of complex samples. Due to the popularity of SPE, new solid sorbents have appeared as alternative to conventional sorbents with the aim of achieving more and more selective preconcentration of target analytes. Thus, immunosorbents and synthetic receptors such as molecularly imprinted polymers (MIP_S) appear as excellent candidates to accomplish this requirement. The materials routinely used in SPE are usually based on the on the non-specific binding of the targets. Molecularly imprinted polymers (MIP_S) offer the possibility of achieving selective extraction, analogous to those achieved by immuno-based extraction systems, and thus may represent an advance on conventional SPE materials (Goodwin & Jamison, 1990; Nathan *et al.,* 1995; Janicak *et al.,* 1997).

Materials and methods

Chemicals and reagents

Methacrylic acid (MAA) from Merck (Darmstadt, Germany) was distilled in vacuum prior to use in order to remove the stabilizers. Ethylene glycol dimethacrylate (EGDMA), 2,2-azobis isobutyronitrile (AIBN), 2,4-dibromo-aniline and dimethyl-cyclohexyl-amin from Sigma-Aldrich (Steinheim, Germany) were of reagent grade and were used without any further purification. All solvents used in chromatography analyses were HPLC grade and supplied by Merck. Carbamazepine reference standard was used for preparing stock and standard solutions. The Carbamazepine stock solutions (1000 g/L) were prepared monthly and stored at 4 °C. Intermediate standard solution of 10 g/L was prepared weekly by dilution of stock solutions with water. Working standard solutions of different concentrations were prepared daily by diluting the intermediate standard solution with mobile phase solution

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Instrumentation

Carbamazepine separation and quantification were conducted with an Alliance 2695 liquid chromatograph with an ACE C18 column (250×4.6 mm, 5μm). Measurements were carried out by using a Waters assembly equipped with a model 2996 Photodiode Array Detector. HPLC data were acquired and processed using a PC and Millennium 2010 Chromatogram Manager software (Version 2.1 Waters). pH of solutions were adjusted using amodel 630 digital Metrohm pH meter (Herisau, Switzerland) equipped with a combined glass-calomel electrode.

Procedures of MIP and NIP preparation with precipitation polymerization

For the preparation of the carbamazepine imprinted polymer nano particles, the template (94 mg, 0.25 mmol) was dissolved in asetonitril in a 25 mL thick walled glass tube. The functional monomer (MAA) (0.129mL, 1.5 mmol), the cross-linking monomer (EGDMA) (2.6 mL, 14 mmol), and the initiator (AIBN) (24 mg & 0.164 mmol) were then added to the above solution. The mixture was uniformly dispersed by sonication for 40 min to remove oxygen. Then the solution was placed in a water bath at 60 °C. The reaction was allowed to proceed for 24h. The hard polymers, P(MAA-Co-EGDMA), that were obtained were crushed. After the polymerization procedure and drying, the polymer nano particles were washed with methanol and acetic acid (10:1, v/v, of 98% methanol and pure acetic acid) for three times and with distillated water for two times.

Chromatographic conditions

Carbamazepine separation and quantification were conducted with an Alliance 2695 liquid chromatograph with an ACE C18 column (250×4.6 mm, 5μm). Measurements were carried out by using a Waters assembly equipped with a model 2996 Photodiode Array Detector. A methanol: water (55:45) at a flow rate of 1.0 ml/min was used as the mobile phase. All of the analyses were carried out at an operation wavelength of 225 nm.

Table 1. Assay of CBZ in human serum and plasma by means of the described MISPE procedure and the HPLC method

Sample	Spiked value (ng/ml)	Recovery% ± SD	
		MIP	NIP
Serum	2.0	91.3 ± 3.9	8.3 ± 1.7
	5.0	93.7 ± 4.2	12.1 ± 2.4
	10.0	95.3 ± 2.3	15.3 ± 3.1
Plasma	2.0	89.3 ± 3.7	6.3 ± 1.9
	5.0	90.1 ± 2.7	9.1 ± 2.1
	10.0	92.1 ± 3.1	8.3 ± 2.7

Sample pretreatment

Serum and plasma samples need to be pretreated for SPE. In many cases, analyses such as drug may be protein-bound which reduce SPE recoveries. To disrupt protein binding in these biological fluids, use one of the following methods for SPE procedures as following:

* Shift PH of the sample to extremes (PH≥ 9) with bases in the concentration range of 0.1 M. use the resulting supernatant as the sample for SPE.

* Sonicate the biological fluid for 15 minutes, add water or buffer, centrifuge, and use the supernatant for the SPE procedure.

Extraction procedure for serum and plasma samples

Drug free human serum was obtained from the Iranian blood transfusion service (Tehran, Iran) and stored at −20 °C until use after gentle thawing. Stock standard solutions of CBZ were prepared in water. Standard solutions were prepared by adding appropriate volumes of CBZ solution to a 10mL volumetric flask and the solution was diluted to the mark with biological fluids and vortexed for 3 min. 2mL of the serum and urine samples spiked by CBZ were diluted with 2mL buffer pH 8.5 and were centrifuged for 20 min at 8000rpm and then filtered through a cellulose acetate filter (0.20 m pore size, Advantec MFS Inc., CA, USA). The filtrate were collected in glass containers and stored at −20 °C until analysis was performed. 2mL of the filtered supernatant were collected to be directly percolated through the MIP or the NIP cartridges.

MISPE condition

Exactly 120 mg of the optimized MIP polymer nano particles was packed in a polypropylene cartridge, which was incorporated in a flow system prior to the HPLC analytical instrumentation. The principle of sequential injection was utilized for a rapid automated and efficient SPE procedure on the MIP. Samples, buffers, washing and elution solvents were introduced to the extraction cartridge via a vacuum pump and a multi-position Rehodyne valve. The method was optimized in terms of flow rates, extraction time, and volume. After extraction, the final eluent from the extraction cartridge was directed to the injection loop and was subsequently analyzed on HPLC. The optimal conditions for SPE consisted of conditioning the cartridge using a pH=3.0 water, loading 5.0 ml of the sample under basic aqueous conditions, clean-up using 2×2ml acetonitrile and elution with 3.0 ml methanol. After optimization of SPE procedure, an aliquot of extracted template was injected to the ACE C18 column (250×4.6 mm, 5μm) with methanol:water (55:45) at a flow rate of 1.0 ml/min was used as the mobile phase.

Results and discussion

In many bio-analytical and clinical applications where a certain degree of selectivity is required, MIPs could have been employed with many different therapeutic or health purposes. In order to enhance the selectivity of the detection in such a complex matrix, we need a systems, which can extract or response to target molecules individually. Results from the HPLC analysis showed that the recoveries of CBZ using MIP cartridges from human serum and plasma samples were higher than 90%. The programmed imprinted polymers were used as an efficient sorbent for the extraction of CBZ from human

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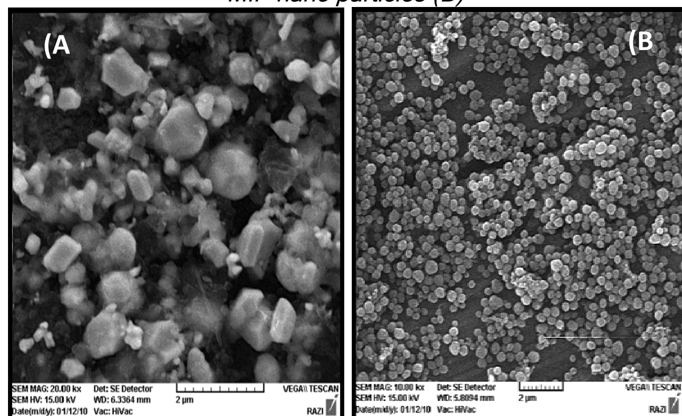
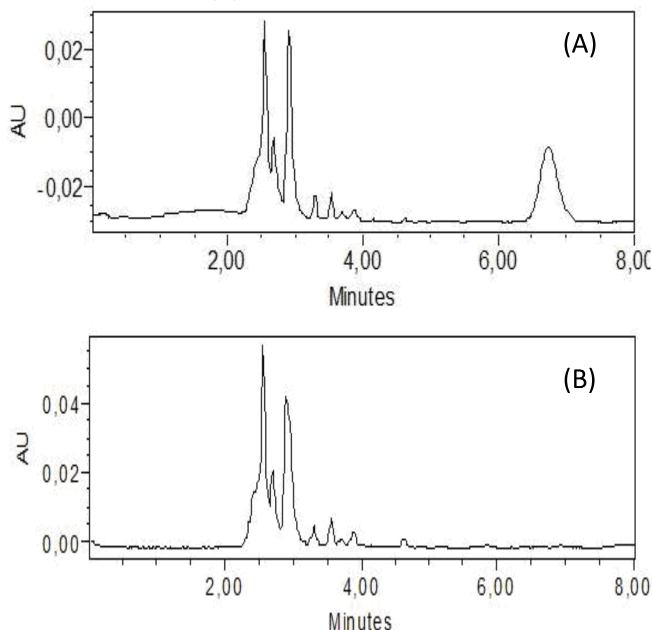
Fig. 1. Scanning electron microscopy image of NIP (A) and MIP nano particles (B)



biological samples. Various parameters affecting the extraction efficiency of the MIP cartridges were evaluated. The high selectivity of the media permitted a simple and rapid analysis of this antiepileptic drug in biological samples with good recovery and detection limit (Table 1, Fig. 1 and 2).

Fig. 2. HPLC chromatogram obtained after elution a 25 µl of CBZ in plasma samples with MIP nano particles (A) and NIP (B) monitored at 225 nm



Conclusions

In this paper, a polymer imprinted nano particles for CBZ has been synthesized via a non-covalent molecular imprinting approach. The MIP nano particles as new sorbents in SPE were successfully investigated for the clean up of human serum and plasma samples with an optimized procedure. A SPE-HPLC method based on MIP has been developed for the extraction of CBZ from biological solutions. This efficient method allowed cleaner extracts to be obtained and interfering peaks arising from the complicated biologic samples to be suppressed. The method was applied to the trace CBZ determination at three levels, and the recoveries for the spiked human serum and urine samples were higher than 90%. The results obtained show that the MIP-based approach to the solid-phase extraction is comparable or superior with the more traditional HPLC methods in terms of recovery and sample clean-up. It could be concluded that the technique has great potential in developing selective extraction method for other compounds.

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