Materials Science and Engineering C 71 (2017) 572–583

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Highly selective solid phase extraction and preconcentration of Azathioprine with nano-sized imprinted polymer based on multivariate optimization and its trace determination in biological and pharmaceutical samples

Saied Saeed Hosseiny Davarani [a,*], Zeinab Rezayati zad [a], Ali Reza Taheri [b], Nasrin Rahmatian [b]

[a] Faculty of Chemistry, Shahid Beheshti University, G. C., P.O. Box 19839-4716, Tehran, Iran
[b] Islamic Azad University, Ilam Branch, Ilam, Iran

ARTICLE INFO

Article history:
Received 30 April 2016
Received in revised form 19 September 2016
Accepted 29 September 2016
Available online 30 September 2016

Keywords:
Azathioprine
Nano molecularly imprinted polymer
Solid-phase extraction
Spectrophotometry
Box–Behnken design

ABSTRACT

In this research, for first time selective separation and determination of Azathioprine is demonstrated using molecularly imprinted polymer as the solid-phase extraction adsorbent, measured by spectrophotometry at λ_{max} 286 nm. The selective molecularly imprinted polymer was produced using Azathioprine and methacrylic acid as a template molecule and monomer, respectively. A molecularly imprinted solid-phase extraction procedure was performed in column for the analyte from pharmaceutical and serum samples. The synthesized polymers were characterized by infrared spectroscopy (IR), field emission scanning electron microscopy (FESEM). In order to investigate the effect of independent variables on the extraction efficiency, the response surface methodology (RSM) based on Box–Behnken design (BBD) was employed. The analytical parameters such as precision, accuracy and linear working range were also determined in optimal experimental conditions and the proposed method was applied to analysis of Azathioprine. The linear dynamic range and limits of detection were 2.5–0.01 and 0.008 mg L^{-1} respectively. The recoveries for analyte were higher than 95% and relative standard deviation values were found to be in the range of 0.83–4.15%. This method was successfully applied for the determination of Azathioprine in biological and pharmaceutical samples.

1. Introduction

Azathioprine (ATP) which is chemically known as 6-[(1-methy1-4 nitroimidazol-5-yl) thiol]purine, is official in the United States Pharmacopeia [1] and British Pharmacopeia [2]. It is primarily an immuno suppressive agent, used mainly in all transplantation procedures. Because there is always a chance that the body will try to reject new donor tissue, ATP helps to prevent this rejection by suppressing the body's immune or defense system [3–9]. It is also used in some auto-immune illnesses (e.g., rheumatoid arthritis and pemphigus), inflammatory bowel diseases (e.g., Crohn's disease andulcerative colitis) and multiple sclerosis [10]. The molecular is slightly soluble in water (0.272 mg mL^{-1}) and insoluble in alcohol and in chloroform (Fig. 1). In particular, the most common problems concerning this therapy are represented by relapses, medullar suppression, hepatic toxicity, and pancreatitis [11]. Due to the fact that ATP suppresses the bone marrow, patients will be more susceptible to infection. Unfortunately, its use is limited due to its associated high toxicity [12,13].

Several analytical methods involving UV–Visible spectrophotometry [14,15], HPLC [16,17], electrochemistry [18,19], titrimetry [20] and ^{1}H-NMR spectroscopy [21] have been reported for the determination of ATP in pharmaceutical preparations and body fluids. However, these methods suffer from some disadvantages such as high costs, long analysis times and requirement for complex and tedious sample pretreatment and low sensitivity and selectivity that makes them unsuitable for a routine analysis in some cases. Therefore, it is necessary to develop a new simple method with high sensitivity, simplicity and efficiency for the detection of this drug.

Solid-phase extraction (SPE) is widely used for the extraction and preconcentration of analyte in various environmental, food, and biological samples [22,23]. It is the most popular clean-up technique due to factors such as convenience, cost, time saving and simplicity and it is the most accepted sample pretreatment method today [24,25].

A relatively new development of molecularly-imprinted polymeric nanomaterials (MIPs) have been considered for the separation and preconcentration of different drugs and ions from other coexisting materials by solid phase extraction process (SPE). MIPs as part of molecularly imprinted solid phase extraction (MIPSPE) with higher stability, selectivity and sample loading capacity can provide a new way to

* Corresponding author.
E-mail addresses: ss-hosseiny@cc.sbu.ac.ir, s_davarani@yahoo.com (S.S.H. Davarani).

http://dx.doi.org/10.1016/j.msec.2016.09.075
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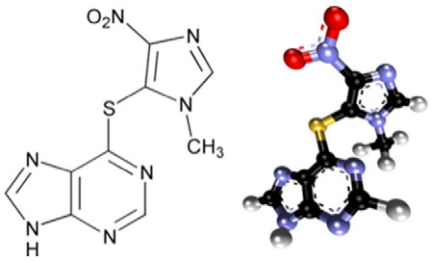

Fig. 1. The structural formula of Azathioprine.

overcome these limitations [26–28]. Some MIPs have been used for chromatographic separations biosensors and especially for selective recognition of templates from complicated biological matrices, such as plasma, urine and tissue samples [26,27]. MIPs are typically prepared by polymerizing a combination of a target molecule (template), functional monomers and an excess of cross-linker. Binding sites with molecular recognition properties are formed after removing template molecules from the polymer, leaving behind cavities complementary in size and shape to the template for the subsequent rebinding process [28]. In principle, the movements of a certain molecule (template) are frozen in a polymeric structure so that they are arranged in a desired style [28–30].

In the present work, for first time, the synthesized MIP was used to spectrophotometric determination of ATP after its selective extraction and pre-concentration from complex matrices, such as pharmaceutical and serum samples. In order to reduce the number of required experiment runs, survey the simultaneous effect of independent variables on the dependent variable, and determine the optimum condition for ATP recovery percentage, the Box–Behnken Design (BBD) under Response Surface Methodology (RSM) was employed. The performance of this method is comparable with most of the analytical instrumental methods reported for ATP determination such as ^{1}H-NMR spectroscopy[14] and HPLC [22–23].

2. Experimental

2.1. Instrumentation

Spectrophotometry measurements were performed on a Perkin-Elmer's LAMBDA 25 UV–Vis spectrophotometer with 1.0 cm quartz cell. The FT-IR spectra (4000–400 cm) using KBr were recorded with using a MB-154 model Bomem spectrometer. A Hitachi S-4160 scanning electron microscope (Tokyo, Japan) was used to study the morphology of the polymeric particles.

N_2 adsorption–desorption analysis was performed on the MIP by PHS-1020 system (PHSCHINA). Surface areas were obtained using the Brunauer–Emmentt–Teller (BET) method. The pore size calculated using the Barrett–Joyner–Halenda(BJH) formula. Also, a Metrohm model 713 pH-meter was used for pH measurements.

2.2. Materials and chemicals

ATP (>99.0% purity) was purchased from the Arastoo pharmaceutical company (Tehran, Iran), methacrylic acid (MAA) (Sigma-Aldrich) and ethylene glycol dimethacrylate (EGDMA) from Merck (Darmsctat, Germany). 2,2 azobis(isobutyronitrile) (AIBN) and methanol, ethanol, acetic acid, acetonitrile, ethylacetate (EA) from Merck (Darmsctat, Germany). All aqueous solutions were prepared with doubly distilled deionized water. Stock solutions of ATP were freshly prepared as required in 0.1 mol L^{-1} of appropriate buffer solutions. Fresh human blood serum samples were taken from cresent red, (*Ilam, Iran)*. The serum samples were centrifuged, filtered, diluted and applied for the recovery tests in spiked samples.

2.3. Preparation of nano-MIP and nano non-imprinted polymer

To prepare the MIP, 0.5 mmol of ATP and the appropriate amount of functional monomer (MAA) (Table 1) and 8.50 mL of acetonitrile, were placed in a 18 mL glass sample vial. This mixture was stirred at room temperature for 5 min, and then, cross-linker EGDMA and the reaction initiator AIBN according to the table, were added. Further addition of acetonitrile up to total volume of 10 mL was made, following sonication for 5 min. After being purged with nitrogen for 5 min, the bottle was sealed under nitrogen and then left to polymerize in an oil bath at 60 °C for 24 h. The tube was smashed and the bulk polymer was ground into fine particles using a mortar and pestle. The product, after drying overnight, possessed a rigid structure. A non-imprinted polymer (NIP), which contained no template, was also prepared using the same procedure. The most important step in perpetration of nano-MIP is the removal of the template molecules. Therefore, the synthesized MIP was washed with ethanol-water to remove unreacted monomer and the template (ATP) was removed by a Soxhlet extractor with 50.0 mL of methanol: acetic acid (9:1) for about 18 h. The elimination process of the template molecules was monitored by UV–Vis. spectrophotometery. In order to ensure complete removal of the target molecules, the synthesized polymer was rested in methanol overnight and then oven-dried at a temperature of 90.0 °C. The leached polymer particles were softly ground to pass through a 200-mesh stainless steel sieve (75 μm) sieve to remove bulk powders obtain particles sizes between 60 and 80 μm. Table 1 shows the recipes of the polymers synthesized in this study.

2.4. MIP-SPE analysis

Glassy columns with an i.d. 5 mm were packed with 0.10 g of dry nano-molecule imprinted polymers (MIP-or NIP). The end of column was fitted to make sure no loss of polymer particles and column was capped. Methanol (2.0 mL) was run through the column to guarantee uniform particle packing at flow rate of 1.0 mL min^{-1} then 16.0 mL sample solution containing ATP, in specified concentration and at pH = 8.00 was loaded onto the SPE column with a flow rate of 0.20 mL min^{-1}. The non-specific bound analyte was washed in a single washing step using 10 mL Methanol. The analyte was desorbed with 5.0 mL of methanol: acetic acid (9:1) solution at a flow rate of 0.25 mL min^{-1}, and the ATP content in the resulting solution was determined spectrophotometrically at wavelength of 286 nm. For determination of ATP in the eluate, a stock solution (100 μg mL^{-1}) was prepared by dissolving appropriate ATP in methanol: acetic acid (9:1) in a volumetric flask.

Fresh working solutions were prepared by an appropriate dilution of the stock solution in the aforementioned solvent before use. The

Table 1
Composition and evaluation of various synthesized polymers.

Adsorbent	ATP (mmol)	MAA (mmol)	EGDMA (mmol)	AIBN (mg)	Acetonitrile (mL)	Q (mg g^{-1})	IF*
MIP 1	0.5	2	10	0.3	10	6.15	2.28
NIP 1	–	2	10	0.3	10	2.70	
MIP 2	0.5	2	10	0.3	20	5.88	2.24
NIP 2	–	2	10	0.3	20	2.62	
MIP 3	0.5	2	15	0.3	10	7.40	2.67
NIP 3	–	2	15	0.3	10	2.77	
MIP 4	0.5	2	15	0.3	20	6.24	2.29
NIP 4	–	2	15	0.3	20	2.73	
MIP 5	0.5	2	20	0.3	10	6.81	2.53
NIP 5	–	2	20	0.3	10	2.69	
MIP 6	0.5	2	20	0.3	20	6.75	2.50
NIP 6	–	2	20	0.3	20	2.70	

ATP = Azathioprine; MAA = methacrylic acid; EGDMA = ethylenglygol methacrylate; IF = imprinting factor. Adsorption conditions: sample volume = 10 mL; concentration = 50 mg L^{-1} sorbent weight = 100 mg, flow rate = 0.2 mL min^{-1}