Talanta 134 (2015) 1–7

Contents lists available at ScienceDirect

Talanta

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

# Molecularly imprinted solid phase extraction of fluconazole from pharmaceutical formulations

S. Manzoor, R. Buffon, A.V. Rossi *

*Institute of Chemistry, University of Campinas, CP 6154, CEP 13083-970 Campinas, Brazil*

ARTICLE INFO

*Article history:*
Received 3 July 2014
Received in revised form
11 October 2014
Accepted 28 October 2014
Available online 8 November 2014

*Keywords:*
Molecularly imprinted polymer
MIP
Fluconazole

ABSTRACT

This work encompasses a direct and coherent strategy to synthesise a molecularly imprinted polymer (MIP) capable of extracting fluconazole from its sample. The MIP was successfully prepared from methacrylic acid (functional monomer), ethyleneglycoldimethacrylate (crosslinker) and acetonitrile (porogenic solvent) in the presence of fluconazole as the template molecule through a non-covalent approach. The non-imprinted polymer (NIP) was prepared following the same synthetic scheme, but in the absence of the template. The data obtained from scanning electronic microscopy, infrared spectroscopy, thermogravimetric and nitrogen Brunauer–Emmett–Teller plot helped to elucidate the structural as well as the morphological characteristics of the MIP and NIP. The application of MIP as a sorbent was demonstrated by packing it in solid phase extraction cartridges to extract fluconazole from commercial capsule samples through an offline analytical procedure. The quantification of fluconazole was accomplished through UPLC–MS, which resulted in LOD $\leq 1.63 \times 10^{-10}$ mM. Furthermore, a high percentage recovery of 91 ± 10% ($n=9$) was obtained. The ability of the MIP for selective recognition of fluconazole was evaluated by comparison with the structural analogues, miconazole, tioconazole and secnidazole, resulting in percentage recoveries of 51, 35 and 32%, respectively.

© 2014 Elsevier B.V. All rights reserved.

## 1. Introduction

Fluconazole (Fig. 1) is a synthetic triazole antifungal agent commonly used for the treatment of oropharyngeal, oesophageal, and deep candidiasis that works by inhibiting the C-14 demethylation of lanosterol, similar to other antifungal drugs of triazole and the imidazole class. It is predominantly excreted through the kidneys where approximately 11% of the administered dose is eliminated in the form of metabolites [1]. Commonly, the monitoring of the drug level is not necessary; however, it becomes important in patients suffering from renal inefficiency or undergoing dialysis [2]. Fluconazole is commonly commercialised in the form of capsules containing 150 mg of the active pharmaceutical ingredient (API) and excipients, which generally facilitate the release of the API inside the organism [3,4]. Excipients may also sometimes contain impurities having chemical structures similar to that of the API [5].

The methods frequently applied for the analysis of fluconazole in pharmaceutical formulations and biological samples include gas chromatography, high performance liquid chromatography (HPLC) and spectrophotometry [6,7]. Among these techniques, most of the HPLC methods utilise C18 columns for the analysis of fluconazole in samples prepared mainly using liquid–liquid extraction and solid phase extraction [8].

Solidphase extraction (SPE), for the purpose of sample pre-treatment, has gained attention in recent years because of its simplicity and active participation in the field of separation science [9,10]. Different types of SPE sorbents are available, and molecularly imprinted polymerisation is one of the prime and most promising techniques to synthesise sorbents with high selectivity [11,12].

Molecularly imprinted polymers (MIPs) are tailor-made polymeric materials with cavities complementary in shape, size and functional groups to the template molecule and offer highly selective molecular recognition properties [13–15]. Different techniques can be used for the synthesis of MIPs, including bulk, suspension, dispersion, multi-step swelling and precipitation polymerisations [16–19]. The low cost, stability, ease of preparation and increased selectivity of MIPs make them highly useful in SPE as well as several other applications, such as sensors, enantiomeric separations and analytical applications [20,21]. Moreover, the high affinity of these sorbents may result in the efficient extraction of the analyte from different matrices [22]. Keeping in mind the efficacy of this technique, we have successfully synthesised molecularly imprinted sorbents for the analysis of fluconazole in commercial pharmaceutical samples. The proposed scheme of the synthesis is represented in Fig. 1.

* Corresponding author.
*E-mail address:* adriana@iqm.unicamp.br (A.V. Rossi).

http://dx.doi.org/10.1016/j.talanta.2014.10.057
0039-9140/© 2014 Elsevier B.V. All rights reserved.

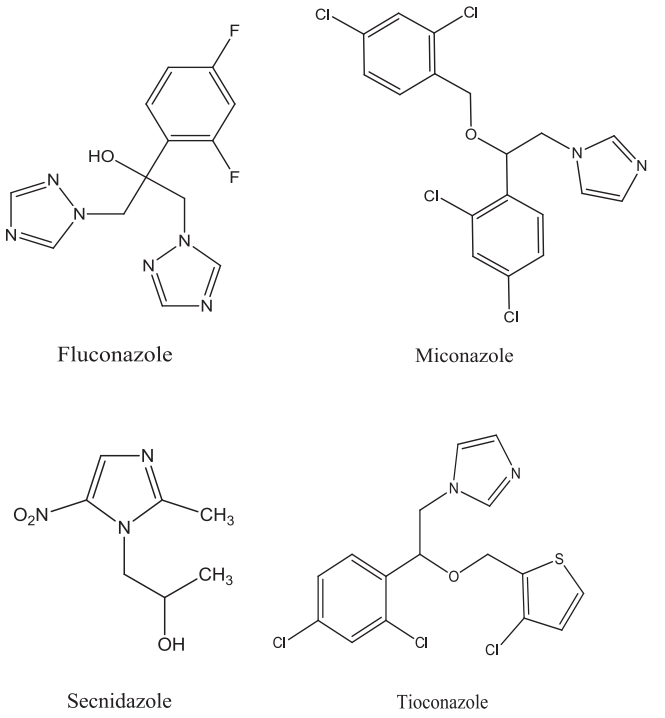


**Fig. 1.** Molecular structures of (a) fluconazole, (b) miconazole, (c) secnidazole and (d) tioconazole.

## 2. Experimental

### 2.1. Reagents

Fluconazole (98%), methacrylic acid (MAA, 99%), ethyleneglycoldimethacrylate (EGDMA, 98%) and 2,2′-azo-bis-isobutyronitrile (AIBN, 98%) were obtained from Sigma-Aldrich (St. Louis, USA). Miconazole (99.6%) and tioconazole (99.8%) were supplied by Evonik Degussa Brasil Ltda (São Paulo, Brazil), whereas secnidazole (98.8%) was supplied by Galena (Campinas, Brazil). Methanol (99.8%) was purchased from Synth (São Paulo, Brazil) and acetonitrile (99.5%) was purchased from Vetec (Duque de Caxias, Brazil). Methanol and acetonitrile were of ultra-high performance liquid chromatography (UPLC) grade and dimethylsulfoxide (DMSO) was purchased from Merck (Schwalbach, Germany) with 99% purity. Ultrapure water (18.3 MΩ × cm) was generated by a Milli-Q Plus system (Millipore, Billerica, USA).

### 2.2. Preparation of polymers

Fluconazole imprinted polymer (FLUMIP) was synthesised through the precipitation polymerisation method. The template (1 mmol), the monomer MAA (4 mmol), the cross-linker EGDMA (20 mmol) and the initiator AIBN (0.07 mmol) were dissolved in 25 mL of acetonitrile in a Schlenk flask. The solution was purged with argon for 5 min, and the flask was sealed with a septum. The polymerisation was performed at 60 °C for 24 h in a thermostatic bath. The polymer, obtained in the form of precipitates, was washed with methanol in a Soxhlet apparatus for 24 h to remove fluconazole and unreacted monomers.

The non-imprinted polymer (NIP) was synthesised using the same procedure mentioned above, but without adding the template molecule.

### 2.3. Characterisation

Infrared (IR) spectra from 4000 to 400 $cm^{-1}$ were obtained on an FTIR BOMEM NB instrument in transmission mode. Thermogravimetric analysis (TGA) was performed using a TA instruments TGA 2050 using 4.3 mg of MIP and NIP at a heating rate of 5 °C/min. Scanning electron microscopy (SEM) analysis was performed using a JEOL SEM 6360-LV microscope. Nitrogen adsorption/desorption isotherms were measured using a Nova 4200 apparatus using 500 mg of each polymer dried under vacuum at 120 °C for 5 h before analysis. The Brunauer–Emmett–Teller (BET) plot and the Barrett–Joyner–Halenda (BJH) method were used to determine the specific area, the pore volume and the pore size distribution.

### 2.4. MIP–SPE conditions

A FLUMIP–SPE cartridge was prepared by packing 50 mg of FLUMIP in an empty SPE cartridge. A NIP SPE cartridge was also prepared following the same procedure. Thereafter, the cartridges were conditioned with 2 mL of acetonitrile, followed by 2 mL of an acetonitrile/water (1:15 v/v) mixture.

The washing conditions for the FLUMIP–SPE were studied. For this purpose, washing solutions were prepared by adjusting the pH of deionised water from 4 to 8 by the dropwise addition of 0.001 M HCl or NaOH solutions as required. The addition of a 2% (v/v) aqueous DMSO solution was also tested. Elution was performed using 1 mL of methanol, and the eluate was analysed using UV spectroscopy. Five replicates for each washing condition were performed.

### 2.5. MIP–SPE analysis

A fluconazole capsule (local pharmaceutical commercial sample) was uncoated, and 6.00 mg of the powder (containing 2.5 mg of API) was dissolved in 3 mL of acetonitrile and then diluted to 50 mL with deionised water in a volumetric flask. The solution was filtered through a 0.5 μm Millipore filter, and 1000 mL was then loaded onto the FLUMIP and NIP cartridges. The cartridges were washed with 0.50 mL of 2% aqueous DMSO solution and then eluted with 1 mL of methanol. Three replicates were performed.

### 2.6. UPLC–MS analysis

The liquid chromatographic system consisted of an Ultra Performance Liquid Chromatograph Acquity-Waters with a C18 column (Acquity BEH; length 5.0 × 2.1 mm and particle diameter 1.7 μm) coupled with a mass detector Quattro Micro API Waters.

The column oven temperature was maintained at 40 °C. The mobile phase consisted of 0.1% (v/v) formic acid in water (A) and methanol (B). The elution gradient is described in Table 1.

The flow rate was set to 0.35 mL/min and an injection volume of 3 μL was used. The mass spectrometer was operated in positive mode with 1000 V spray voltage. The electrospray ion source was heated to 150 °C, and an ion ratio ($m/z$) of 307 was observed.

### 2.7. Cross-reactivity study

The selectivity of the FLUMIP was evaluated by comparing the percentage recovery of fluconazole with that of structurally related analogues, i.e., miconazole, tioconazole and secnidazole (Fig. 1),

**Table 1**
Description of gradient elution chromatography UHPLC–MS.

| Time (min) | Solvent A (% v/v) | Solvent B (% v/v) |
|---|---|---|
| 0 | 97 | 3 |
| 4.0 | 5 | 95 |
| 4.2 | 5 | 95 |
| 5.0 | 97 | 3 |

Download English Version:

https://daneshyari.com/en/article/1244044

Download Persian Version:

https://daneshyari.com/article/1244044

Daneshyari.com