Journal of Chromatography A, 1318 (2013) 43–48

Contents lists available at ScienceDirect

Journal of Chromatography A

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

In-tube solid-phase microextraction with molecularly imprinted polymer to determine interferon alpha 2a in plasma sample by high performance liquid chromatography

Andréa Rodrigues Chaves, Maria Eugênia Costa Queiroz *

Departamento de Química da Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP 14040-903, Brazil

ARTICLE INFO

Article history:
Received 24 June 2013
Received in revised form
28 September 2013
Accepted 5 October 2013
Available online 15 October 2013

Keywords:
In-tube SPME
High performance liquid chromatography
Molecularly imprinted polymers
Interferon alpha 2a

ABSTRACT

A molecularly imprinted sol–gel polymer (MIP) based on protein (biopharmaceutical) template with a mild template removal condition using protease was synthetized and evaluated as stationary phase for in-tube solid phase microextraction (in-tube SPME) of the interferon alpha 2a from plasma samples, followed by high performance liquid chromatography analysis with fluorescence detection (HPLC-FD). The developed MIP exhibited high selectivity for the analyte in a complex matrix. The in-tube SPME variables such as draw/eject cycles, draw/eject volume, and desorption conditions were optimized to establish the equilibrium conditions in a short time. The MIP in-tube SPME/HPLC-FD method presented linear response over a dynamic range of 8–300 ng mL^{-1}, with a correlation coefficient of 0.997. The inter-assay precision presented coefficient of variation lower than 9.2%, and accuracy values between 92% and 98%. The developed MIP performed as well as other selective interferon alpha 2a stationary phases (e.g., immunosorbent and restricted access material), with the advantage that it is robust, easy to handle and cheap to synthesize, in a addition to requiring smaller sample volume (50 μL). Based on the analytical validation results, the proposed method (MIP in-tube SPME/HPLC-FD) can be a useful tool to determine interferon alpha 2a in plasma samples from patients receiving therapeutic dosages.

1. Introduction

Selective methods to analyze protein are highly desirable, especially in the areas of bioseparation, diagnostic applications, and controlled drug delivery [1].

Immunosorbents, which follow a separation mechanism based on specific antibody-antigen bounds, have been well employed to extract and preconcentrate analytes from complex matrices such as blood, plasma and serum [2–5]. However, developing immunosorbents has its drawbacks: this process is expensive and time-consuming, it is difficult to raise natural antibodies against toxic compounds or immunosuppressant, it is impossible to perform binding assays in nonaqueous media, and it is usually necessary to sacrifice animals to obtain specific antibodies [6].

An alternative to immunosorbent extraction phases has been molecularly imprinted polymers (MIP). The synthesis of MIP materials typically involves copolymerization of functional and crosslinking monomers in the presence of a template molecule that is subsequently removed from the polymer matrix, creating a receptor cavity that is complementary to the template [7]. Compared with immunosorbents, these polymers represent a cheaper and more versatile platform with molecule-specific recognition properties [8,9].

MIPs based on organic acrylate polymers are the most often used imprinted polymers [10–12]. However, these systems can be complex, and their preparation usually requires inert atmosphere. It is possible to synthesize MIPs based on modified silica via a conventional sol–gel process that incorporates the template molecules into the silica matrix. After removal of the template, molecular cavities with distinct pore size, functionality, and shape remain in the host. The sol–gel route provides several interesting physical properties – higher porosity and rigidity, improved mechanical properties, and chemical inertness. These molecularly imprinted cavities display high affinity and selectivity toward the target analyte, as well as fast mass transfer rate [6,13]. MIPs effectively separate molecules that are large and have multiple binding sites, such as nucleotide bases, carbohydrates, steroids, and proteins, among others [14].

The main challenge when preparing MIPs that are selective for biomolecules or macromolecules is to ensure their conformational integrity (three-dimensional structure) during the synthesis. Moreover, MIPs intended to target large molecular – weight

* Corresponding author. Tel.: +55 16 3602 4465; fax: +55 16 3633 8151.
E-mail address: mariaeqn@ffclrp.up.br (M.E. Costa Queiroz).

0021-9673/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.chroma.2013.10.018

biomolecules need to have access to the binding sites, contrary to conventional MIPs, for which diffusion of the template into the polymer matrix is expected during rebinding [15].

Interferon alpha 2a (IFN α2a) is a recombinant form of the natural protein and comprises 165 amino acids. This protein has been widely used to treat a variety of diseases such as hepatitis B and C, melanoma, renal cell carcinoma, and leukemia [16]. Under standard clinical therapy with interferon alpha 2a (180 μg once a week), the plasma concentrations of this compound reach values of 16–32 ng mL^{-1} in the steady state [17].

The current standard treatment with IFN α2a is usually extensive and expensive, with frequent adverse events [18,19]. Bioassay [20], immunoassay [21], isoelectric focusing [22], and gel electrophoresis [23] are the techniques that are commonly used to analyze IFN α2a. Nevertheless, they demand long analysis time and lack specificity as well as sensitivity.

In previous papers, our group developed a capillary packed with restricted access materials (RAM, C18-BSA) as well as an immunosorbent (monoclonal anti-interferon alpha 2a) fused silica capillary, to determine IFN α2a in plasma samples by in-tube SPME/HPLC-FD [24,25].

Aiming at therapeutic drug monitoring, in this paper a sensitive high-throughput methodology (MIP in-tube SPME/LC-FD) was developed to determine therapeutic levels of IFN α2a in plasma samples. To this end, glycoprotein-imprinted biomaterials that preferentially bind to IFN α2a molecules were synthetized by sol-gel route with mild template removal by protease.

2. Material and methods

2.1. Reagents

Tetraethoxysilane (TEOS; Fluka, Milwaukee, WI) was used as a crosslinker, 3-Aminopropyltriethoxysilane (APS; 99%, Sigma, Steinheim, Germany) as a functional monomer, HCl (Merck, Darmstadt, Germany) as a catalyst, and Sodium Docecylsulfate (SDS; Sigma, St. Louis, MO) as a foaming agent to generate macroporosity during MIP growth. Acetonitrile (ACN), Ethanol (EtOH), and Methanol (MeOH) were HPLC grade (J.T. Baker, USA). Trifluoracetic acid (TFA) provided by Fisher Scientific (Leics, UK). The Interferon alpha 2a that was employed as standard was acquired from Merck KGaA (Darmstadt, Germany). The water used to prepare the solutions had been purified in a Milli-Q system (Millipore, Brazil).

2.2. Chromatographic conditions

High performance liquid chromatography was conducted on an HPLC system (Shimadzu LC-20AT; Kyoto, Japan) equipped with a CBM-20A system controller and fluorescence detector (Shimadzu RF-10 AXL) was employed. IFN α2a was analyzed in an RP 18 LichroCART® (125 mm × 4 mm × 5 μm particle size – Merck, Darmstadt, Germany) column, at room temperature (25 °C), with a mobile phase consisting of TFA solution (0.1% pH 2.5) and ACN (86:14, v/v), using the isocratic mode at a flow rate of 0.6 mL min^{-1}. The column effluent was monitored at λ_{ex} = 235 nm and λ_{em} = 393 nm. The mobile phase had been filtered and degassed prior to use.

2.3. Plasma samples

To optimize and validate the developed MIP in-tube SPME method, drug-free plasma samples from patients that had not been exposed to any drug for at least 72 h (blank plasma) were used. These samples were kindly supplied by Hospital das Clínicas de Ribeirão Preto, University of São Paulo, Brazil.

The stock standard plasma sample was prepared by using reference plasma (blank plasma) spiked with IFN α2a at a concentration of 1 mg mL^{-1}. The working standard interferon plasma samples were obtained by diluting the stock sample (1 mg mL^{-1}) in drug free plasma, which resulted in interferon concentrations of 8, 10, 20, 50, 100 and 300 ng mL^{-1}. These solutions were stable for five days, at 4 °C.

2.4. Synthesis of the MIP and non-imprinted polymer

To develop the MIP particles, a two-step sol–gel procedure was conducted. In the first step, 1.32 mL of TEOS, 0.235 mL of deionized water, 0.33 mL of 0.1 M HCl, and 0.4 mL of absolute (EtOH) were mixed in a cylindrical plastic vial. Before being mixed with the TEOS solution, the labeled template molecules were added to the APS-containing template solution (1 mg of the interferon alpha 2a), for incorporation in the scaffolds. After pre-hydrolysis of TEOS at room temperature for 24 h, this solution was mixed with a solution containing 0.33 mL of APS and the template as well as 1 mL of 0.1 M SDS. Vials were vortexed to mix the components of the silanes and to foam the sol.

After gelation, the vials were kept at room temperature for 24 h and dried at 50 °C for 48 h. To remove non-uniform glassy layers, the top and bottom surfaces of the scaffolds were ground. After grinding, all the samples were gently shaken in a solution of 0.4 mg mL^{-1} protease (Pronase E; Sigma) in carbonate–bicarbonate (0.1 mol L^{-1}, pH 8.5) buffer solution, for up to 24 h, to remove the template. The amount of protein released from the MIP surfaces was quantified by measuring fluorescence at 1 to 24 h. A non-imprinted polymer (NIP) was also prepared in the same way but without the addition of the IFN α2a (template) to the mixture.

2.5. Characterization of the MIP and NIP

Scanning electronic microscopy (SEM) images of MIP and NIP were recorded on a Zeiss EVO50 SEM microscope (accelerating potential = 20 kV). The Fourier transform infrared spectroscopy (FTIR) absorption spectra of the MIP and NIP in KBr pellets were acquired between 400 and 4000 cm^{-1} on a Bombem MB-102 FTIR spectrometer (ABB, St-Laurent, Canada).

2.6. In-tube solid phase microextraction

Capillaries were prepared with either MIP or NIP phases. In both cases, the particles were slurred in phosphate buffer solution (pH 6.8, 0.025 M) and packed into 50 mm (length) of polyether ether ketone (PEEK) tubing (O.D. = 1/16 in. and I.D. = 0.02 in.). On the basis of the capillary geometry and dimensions, the estimated volume was 10 μL. The capillary column was capped at both ends using a 1/16-in. zero-volume union fitted with a 10-μm frit. After this procedure, the capillary was conditioned for 30 min with phosphate buffer (0.025 mol L^{-1} pH 6.0) at a flow rate of 0.50 mL min^{-1}.

The MIP or NIP capillary was fixed in the place of injection loop of the HPLC chromatographic system. Placing MicroTight sleeves at each end of the capillary facilitated the connections.

In a glass vial (1.5 mL), 50 μL of phosphate buffer (0.05 mol L^{-1}, pH 6.8) was added to 50 μL of plasma sample spiked with IFN α2a, resulting in a concentration of 300 ng mL^{-1}. The sample was vortexed for 10 s before extraction, which was carried out with the six-port valve in the load position. Simultaneously, the analytical column was conditioned with the mobile phase. The plasma sample was directly injected into the MIP capillary. After the extraction, the valve was switched to the inject position; the analyte was eluted (desorption process using the mobile phase-TFA) from the MIP capillary and transferred to the analytical column.