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Synthesis of molecular imprinted polymers for selective extraction of domperidone from human serum using high performance liquid chromatography with fluorescence detection

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ABSTRACT

In this study a novel method is described for selective quantization of domperidone in biological matrices applying molecular imprinted polymers (MIPs) as a sample clean up procedure using high performance liquid chromatography coupled with a fluorescence detector. MIPs were synthesized with chloroform as the porogen, ethylene glycol dimethacrylate as the crosslinker, methacrylic acid as the monomer, and domperidone as the template molecule. The new imprinted polymer was used as a molecular sorbent for separation of domperidone from serum. Molecular recognition properties, binding capacity and selectivity of MIPs were determined. The results demonstrated exceptional affinity for domperidone in biological fluids. The domperidone analytical method using MIPs was verified according to validation parameters, such as selectivity, linearity (5–80 ng/mL, $r^2 = 0.9977$), precision and accuracy (10–40 ng/mL, intra-day = 1.7–5.1%, inter-day = 4.5–5.9%, and accuracy 89.07–98.9%).The limit of detection (LOD) and quantization (LOQ) of domperidone was 0.0279 and 0.092 ng/mL, respectively. The simplicity and suitable validation parameters makes this a highly valuable selective bioequivalence method for domperidone analysis in human serum.

1. Introduction

Domperidone (6-chloro-3-[1-[3-(2-oxo-3H-benzimidazol-1-yl) propyl] piperidin-4-yl]-1H-benzimidazol-2-one) is a dopamine antagonist with antiemetic properties which can be used to relieve motility disorders. Domperidone does not readily cross the blood–brain barrier so rarely causes more extrapyramidal side-effects than other dopamine receptor antagonist such as sulpiride and metoclopramide. Whereas these specifications make it a useful drug in Parkinson's disease, caution is needed due to the cardiotoxic side-effects of domperidone (intravenously), in elderly people and high doses (>30 mg per day) [1]. Furthermore, it is recommended for treatment of nausea caused by chemotherapeutic agents and also associated with acute migraines following Canadian Headache Society guidelines [2]. However domperidone is a powerful prokinetic drug, there is very little information about its biodynamic and bioequivalence properties due to lack of selective method for its determination in biologic media. A number of analytical methods such as radio immunoassay [3–6] and HPLC coupled with fluorescence or mass detector [7–13] have been reported for the determination of domperidone in plasma. The mentioned methods apply a variety of pretreatment techniques including liquid–liquid back-extraction [8], post-column photo derivatization [9], protein precipitation [11] and solid phase extraction [13]. Expensive equipment is required for LC-mass methods which are not available in most of laboratories. So HPLC-fluorescence detection is still broadly used due to lower cost and greater robustness in bioequivalence studies. Concerning various possible interferences in methods using HPLC-fluorescence, it is necessary to develop a suitable and selective sample pretreatment step for the analysis of target compounds in biological samples. Solid-phase extraction (SPE) is commonly utilized as a clean up technique for target enrichment to assist analytical method. SPE can reduce time and solvent required (particularly for automated methods), manage with a small amount of target samples, and solvent compared to liquid–liquid extraction (LLE). However, the mechanism of extrac-

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tion is mainly based on hydrophobic interactions that lead to co-extraction of several endogenous compounds having similar physicochemical properties. A relatively new development in the field of SPE is applying molecularly imprinted polymers (MIPs) with high selectivity and affinity for a target analyte in a biological sample clean up procedure [14]. MIPs, as artificial receptors with excellent template recognition, are utilized for extraction of target molecules from complicated matrices [15]. MIP preparation includes the following steps: formation of a complex between the template molecule and the monomers, polymerization by thermal or photo initiation, and finally elimination of the template molecule from the polymer matrix which provides specific binding sites in the bulk or on the surface of the polymers [16]. An ideal MIP adsorbent is supposed to cover selectivity, high binding capacity, strong adsorption-desorption kinetics, and extensive solvent compatibility in addition to long-term pH, heat and organic solvent stability permitting more adaptability in analytical methods. The MIPs for SPE can be used in different ways, i.e. in conventional SPE where the MIP is filled into columns or cartridges and batch mode SPE where the MIP is equilibrated with the sample [14]. Most of earlier methods required a prolonged laborious extraction process of several steps by various solvents due to insufficient recovery using LLE in preconcentration of trace analytes. These methods use toxic and flammable organic solvents, such as chloroform, hexane and methyl-*tert*-butyl ether, and also long chromatographic run times. So development of a faster and simpler method using HPLC fluorescence is desirable. In our previously published papers, molecular imprinting polymers were successfully applied for analysis of different drugs such as lamivudine [14], adefovir [17] and efavirenz [18] in biological fluids. In this work, a novel domperidone MIP was developed as a simple and uncomplicated clean up sorbent, and its performance was evaluated within sorption-desorption procedures for determinations of domperidone in human serum using HPLC-fluorescence. The validated method can be adopted by bioequivalence studies.

2. Experimental

2.1. Materials

Pure reference standard of domperidone was purchased from (Sigma-Aldrich) and used without further purification. Levodopa, carbidopa, acetaminophen, caffeine, codeine, pimozide and Propranolol (as internal standard) were supplied from (Sigma-Aldrich). Ultrapure water was prepared using a milli-Q purification system from (Purelab UHQ Elga). 2, 2′-Azobis (2-methylpropionitrile) (AIBN) as the initiator was purchased from Merck (Darmstadt, Germany). Methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), acetic acid and chloroform were purchased from Merck (Darmstadt, Germany). HPLC grade methanol was obtained from Merck (Darmstadt, Germany).

Human serum without drug was purchased from the Iranian Blood Transfusion Organization (Tehran, Iran) and refrigerated at −20 °C until use after gentle thawing. Domperidone stock standard solution (100 μg/mL) was prepared in methanol monthly and stored at 4 °C. Intermediate standard solutions (1000 ng/mL and 100 ng/mL) were prepared weekly by dilution of the stock solution. Different spiked serums (5–80 ng/mL) were prepared daily by diluting the intermediate standard domperidone solution using phosphate buffer pH = 8.

2.2. Instrumentation

The analysis of the standard and test samples were performed using a Waters Alliance system equipped with a vacuum degasser, fluorescence detector and consisting of a Waters 717 auto sampler. Chromatographic separations were carried out using HPLC column ACE $5C_{18}$ (250 mm × 4.6 mm I.D.). Empower software was utilized for computer-controlled, data collection and data processing. A combination of methanol and monobasic potassium phosphate solution (0.02 M, pH = 3.5) (49:61) was used as the mobile phase at a flow rate of l mL/min in isocratic elution mode. The injection volume was 10 μL for all samples. The detector was set at 282 nm for excitation and 328 nm for emission wavelength [7]. The pH of the mobile phase and all solutions were measured by a Methrohm digital pH meter (744) equipped with a glass calomel electrode. Scanning electron microscopy (SEM) imaging was carried out by Vegatescan (USA). Polymer particles were coated by sputtering with gold prior to the SEM imaging. FT-IR spectra was recorded on a Bomem MB 155 S FT-IR spectrometer (Canada) using KBr pellets in the range of 400–4000 cm^{-1}.

2.3. Synthesis of MIP and NIP particles

Selective MIP particles were prepared according to the following procedure, domperidone (425 mg, 1 mmol), MAA (793 mg, 4 mmol) and chloroform (10 mL) were placed in a capped test tube and stirred for 60s. Adding EGDMA (3.77 mL, 20 mmol), the mixture was sonicated by ultrasonic radiation for 6 min and the reaction initiator AIBN (50 mg, 0.347 mmol) was added. Oxygen should be removed so the reaction mixture was purged with a gentle flow of N_2 for 10 min and the test tube was sealed under this atmosphere. The polymerization was carried out for 24 h at 60 °C. The obtained poly (MAA-co-EGDMA) were collected and dried. Then domperidone molecules were removed by several continuous washings with a mixture of methanol: acetic acid (9:1, v/v), then it was centrifuged at 5000 rpm for 20 min and the supernatant was analyzed by HPLC until domperidone could not be detected. The polymers were dried at room temperature for 72 h before subsequent studies. Blank polymers (NIP) were prepared using the same procedure, but in the absence of the template molecule to evaluate the binding affinity and selectivity of MIP polymers.

2.4. Batch rebinding procedure and isotherm study

The binding capacity for MIPs was estimated using batch adsorption experiments [19]. Domperidone (5 mL, 1–1250 ng/mL, phosphate buffer pH = 8) was added to the dried polymers (30 mg) in a glass centrifuge tube. The obtained solution was stirred at 400 rpm for 50 min at room temperature and centrifuged for 30 min at 9000 rpm. The free concentration of domperidone was recorded by HPLC-fluorescence at three replicate extractions for each concentration. After washing the polymer with acetonitrile, the adsorbed domperidone was desorbed from the MIP by stirring with methanol: acetic acid (9:1, v/v, 5 mL) at room temperature for 30 min. The bounded template was calculated by subtracting the concentration of free domperidone from the initial concentration. The same process was followed for NIP. The amount of domperidone at equilibrium (q_e, mg/g) was calculated from the following equation:

$$q_e = (C_i - C_e)\,V/W$$

where C_i (mg/L) is the initial concentration of the domperidone, C_e (mg/L) is the equilibrium concentration of domperidone, V (L) is the volume of the solution and W (g) is the mass of the adsorbent used.