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A novel restricted access material combined to molecularly imprinted polymers for selective solid-phase extraction and high performance liquid chromatography determination of 2-methoxyestradiol in plasma samples

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ABSTRACT

A feasibility study was performed in order to ensure the possibilities in using a restricted access material combined to molecularly imprinted polymers (RAM-MIP) as sorbent material in solid phase extraction (SPE) for clean-up of 2-methoxyestradiol (2-ME) from plasma samples. The MIP with hydrophilic external layer was designed by precipitation polymerization. The polymer was characterized by thermogravimetric analysis (TGA) and scanning electron microscope (SEM). The use of analogs of 2-ME as templates, in combination with a chromatographic separation of the analytes in the sample, overcame the problem of the template bleeding. To demonstrate the property of the RAM-MIP obtained, a comparison of commercially available C_{18} SPE was performed. The results showed that the RAM-MISPE recoveries were significantly higher than that of C_{18} SPE for 2-ME in trace concentration. During the extraction process, 2-ME was sufficiently cleaned for further chromatographic analysis with no interferences from template leakage and matrix. Good linearity was obtained from 0.06 to 20 μg mL^{-1} with the correlation coefficient $r > 0.9991$. The coefficient of variation of the inter-assay precision was less than 11.9%. The recoveries of 2-ME in rat plasma at three spiked levels were in the range of 99.10–101.00%. Based on the analytical validation results, the proposed method (RAM-MIP off-line SPE/HPLC) can be a useful tool to determine 2-ME in rat plasma samples.

1. Introduction

For the liquid chromatographic determination of drugs and their metabolites in plasma, tedious and time-consuming pre-treatment procedures such as the removal of proteins by precipitation, liquid–liquid extraction or solid phase extraction (SPE) have been required in the past. Recently, restricted access matrix-solid phase extraction (RAM-SPE) has attracted increasing attention for the direct extraction of analytes from biological fluid [1,2]. With RAM large molecules such as proteins are eluted in the void volume without destructive accumulation because of restricted access to some surfaces, while allowing small molecules such as drugs to reach the hydrophobic, ion-exchange or affinity sites and to be separated [3]. However, the RAM developed could not be used for selective enrichment of an analyte, because of its separation mechanism. On the other hand, the molecularly imprinted polymers (MIPs), which can afford specific recognition against an imprint molecule and moderate recognition against the structurally related compounds, are used for SPE. Since Sellergren1 [4] firstly reported the use of MIP as SPE sorbents, a wide range of analytes of biological, pharmaceutical, food and environmental samples have been involved [5–7]. However, protein adsorption negatively interfered with their recognition properties [8]. To overcome this difficulty, numerous techniques have been used to deproteinize biological fluids before analysis, which is more time-consuming and can add sample artifacts [9,10]. The development of a special and selective extraction support, allowing the direct cleanup of biological samples, is required.

Recently, the RAM combined to molecularly imprinted polymers (RAM-MIP) was developed. Owing to the hydrophilic modification of MIP surface, the materials avoid the destructive deposition of biomacromolecules on the polymeric surface [11,12]. Puoci [13] has obtained RAM-MIP for selective recognition of *p*-acetaminophenol in gastrointestinal simulating fluids. But whether the RAM-MIP can be directly applied to pretreatment of the drug from plasma is not

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clear. The aim of our study was to prepare a RAM-MIP with hydrophobic inner surface and hydrophilic outer surface, which was a hydrophilic modification of MIP surface. The obtained RAM-MIP was used as an adsorbent for solid-phase extraction of 2-ME from the plasma.

2-Methoxyestradiol (2-ME), an endogenous metabolite of 17β-estradiol (Fig. 1), prevents the formation of new blood vessels, acts as a vasodilator and induces apoptosis in some cancer cell lines. Several analytical methods have been reported for the determination of 2-ME, including LC-UV linked solid-phase extraction [14], GC–MS [15], UPLC/QTOF-MS [16] and radioimmunoassay using a 125I-labeled ligand [17]. Although most of these methods are specific, their applications have been limited due to high cost, insufficient selectivity and time-consuming sample preparation. For its potential pharmaceutical applications and low blood drug concentration, the development of a simple, rapid, sensitive and selective method for the determination of 2-ME is therefore highly desirable.

In this study, the polymer was prepared by the precipitation polymerization and chemical modification [18]. We briefly described the synthetic routes and methods available to evaluate the polymers. RAM-MISPE protocol was optimized and applied for cleanup and enrichment of 2-ME from rat plasma samples. To date, the most successful way to avoid any unwanted leaching during pre-concentration resulting in clean and selective extraction of the analyte is the use of an analog of the target molecule during MIP design and production, known as "dummy molecularly imprinted polymer" (DMIP) [19]. The influence of template bleeding in trace analysis is avoided, since template used in the proposed non-covalent approach is only a close analog of analyte. The proposed method is a basis of online SPE (RAM-MIP as sorbents) in combination with HPLC.

2. Experimental

2.1. Chemicals and animals

Methyl methacrylate (MMA), ethylene glycol dimethacrylate (EGDMA), 2,2-azobisisobutyronitrile (AIBN) and glycidyl methacrylate (GMA) of reagent grade were purchased from Aldrich (Steinheim, Germany). 2-ME, estradiol, hydrocortisone acetate and glibenclamide were from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Their chemical structures are shown in Fig. 1. All the other reagents used in the experiment were obtained from Tianjin Shield Specialty Chemical Co. Ltd. (Tianjin, China). Age-matched Sprague-Dawley (SD) rats (250 ± 10 g) were obtained from the Zhengzhou University Medical Laboratory Animal Center (Zhengzhou, China).

2.2. Instrumentation and analytical conditions

HPLC analysis was performed on Agilent 1100 HPLC (Agilent, Palo Alto, USA) equipped with autosampler, thermostated-column device and a fluorescence detector. The chromatographic separations were carried out on a Dikma C_{18} column (200 mm × 4.6 mm, 5 μm), with a mobile phase consisting of acetonitrile–water–methanol (50:40:10, v/v/v), at a flow rate of 1.0 mL min^{-1}. Column temperature was maintained at 30 ± 1 °C. Aliquots of 20 μL were injected into the column and the chromatograms were recorded at excitation wavelength 285 nm and emission wavelength 325 nm. Agilent ChemStation® software was used for data acquisition and integration.

The approximate size distributions were determined by Nano-ZS90 laser nanometer particle sizer (Malvern, England). JSM-7500F scanning electron microscope (SEM) (Tokyo, Japan) and the thermogravimetric analysis (TGA) (Selb, Bavaria, Germany) were used for characterization of polymers. Solid phase extraction was performed using a Supelco 12-position SPE manifold and Supelclean LC-18 columns (Pennsylvania, Bellefonte, USA).

2.3. Preparation of the imprinted polymers

Estradiol (0.2 mmol), the analog of 2-ME, as the template molecule, was dissolved with 25 mL acetonitrile in a 50 mL glass tube fitted with a screw cap [20–23]. The functional monomer (MMA, 1.2 mmol) was then added. The tube was sonicated for 5 min and pre-polymerized at room temperature for 4 h to facilitate template–monomer complex formation. Thereafter, the co-monomer (GMA, 1.2 mmol), the cross-linking monomer (EGDMA, 6.0 mmol) and the

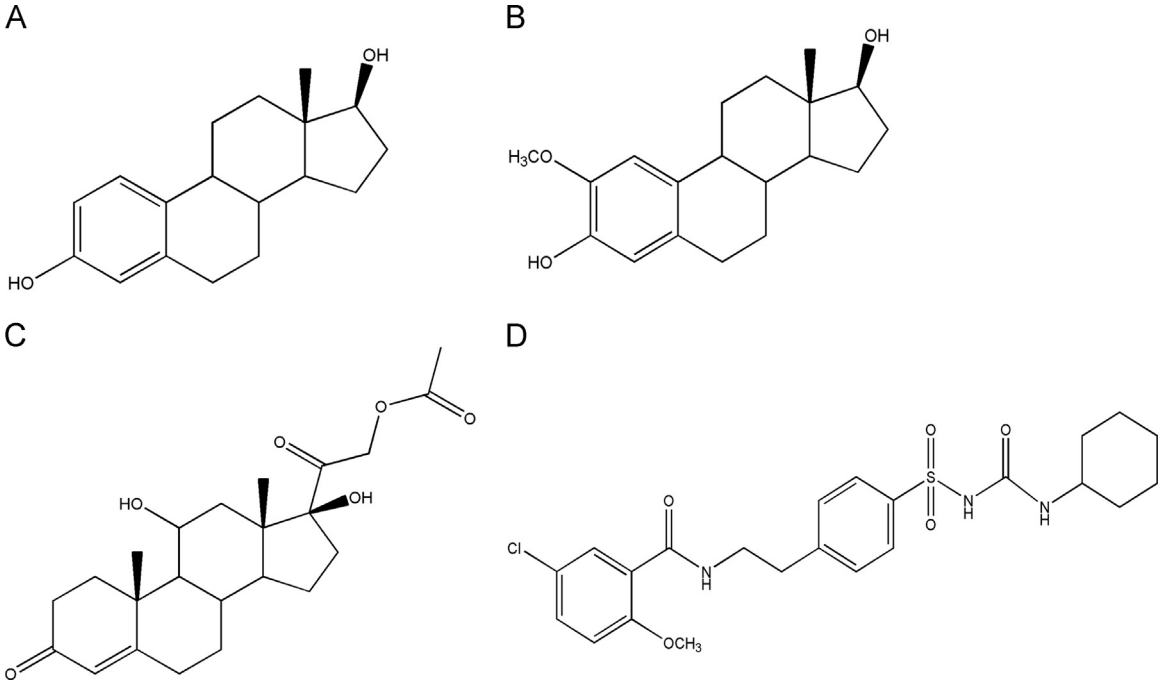

Fig. 1. The chemical structures of (A) estradiol, (B) 2-ME, (C) hydrocortisone acetate and (D) glibenclamide.