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Magnetic molecularly imprinted polymer nanoparticles for simultaneous extraction and determination of 6-mercaptopurine and its active metabolite thioguanine in human plasma

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ABSTRACT

Cytotoxic drugs used in cancer chemotherapy require the continuous monitoring of their plasma concentration levels for dose adjustment purposes. Such condition necessitates the presence of a sensitive technique for accurate extraction and determination of these drugs together with their active metabolites. In this study a novel solid phase extraction technique using magnetic molecularly imprinted nanoparticles (MMI-SPE) is combined with liquid chromatography tandem mass spectrometry (LC–MS/MS) to extract and determine the anti-leukemic agent; 6-mercaptopurine (6-MP) and its active metabolite thioguanine (TG) in human plasma. The magnetic molecularly imprinted nanoparticles (Fe_3O_4@MIP NPs) were synthesized via precipitation polymerization technique and were characterized using different characterization methods A computational approach was adopted to help in the choice of the monomer used in the fabrication process. The Fe_3O_4@MIPs NPs possessed a highly improved imprinting efficiency, fast adsorption kinetics following 2nd order kinetics and good adsorption capacity of 1.0 mg/g. The presented MMI-SPE provided the optimum approach in comparison to other reported ones to achieve good extraction recovery and matrix effect of trace levels of 6-MP and TG from plasma. Chromatographic separation was carried out using a validated LC–MS/MS assay and recovery, matrix effect and process efficiency were evaluated. Recovery of 6-MP and TG was in the range of 85.94–103.03%, while, matrix effect showed a mean percentage recovery of 85.94–97.62% and process efficiency of 85.54–96.18%. The proposed extraction technique is simple, effective and can be applicable to the extraction and analysis of other pharmaceutical compounds in complex matrices for therapeutic drug monitoring applications.

1. Introduction

Nowadays, there is a crucial need for reliable quantitative methods to estimate anti-leukemic drugs in the blood. Obtained data on the levels of these drugs in plasma or serum can give valuable guides to more effective therapy [1] 6-mercaptopurine (6-MP); which is widely used as antileukemic and immunosuppressive agent, has created a necessity for its selective determination together with its active metabolite 6-thioguanine (TG) in biological fluids [2]. Generally, therapeutic drug monitoring (TDM) of 6-MP and its metabolite TG is performed to evaluate toxicity and efficacy especially when the treatment is not achieving the desired therapeutic outcomes at the proposed doses for treatment [3]. Thus, various methodologies had been proposed to determine 6-MP and its metabolites in biological fluids to monitor their kinetics. These methods include paper and thin-layer chromatography, thin-layer electrophoresis, high performance liquid chromatography with UV detector [2,4–8]. However, such techniques didn't offer the optimum selectivity or resolution of target analytes (6-MP and its metabolites) during the quantitation process, which is a must in TDM applications. This can be attributed to the very close chemical structure of the investigated analytes.

Liquid chromatography/mass spectrometry (LC–MS/MS) is considered a powerful, highly specific technique for the determination of organic molecules in complex biological matrices as the detection process is via molecular mass of compounds [7,9]. LC–MS/MS also allows the reduction of analysis times due to its selectivity and sensitivity [7].

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Several studies have proposed the use of LC–MS/MS for the determination of 6-MP and its metabolites in human plasma [3,10,11].

Nevertheless, the reliability of such quantitative assays on LC–MS/MS detection methods may not be absolute [12]. Results may be adversely affected by lack of specificity and selectivity due to ion suppression or enhancement caused by co-eluted extracted substances from the biological matrix [13].

Therefore, an efficient extraction method (clean-up) usually precedes injection of the sample in LC–MS/MS assays to avoid the effect of potentially interfering biological matrix components [14]. The most commonly used sample preparation techniques are protein precipitation, Liquid–Liquid Extraction (LLE) and Solid Phase Extraction (SPE) [15]. Yet, these techniques suffer some disadvantages during the quantitation of analytes using LC–MS/MS assays., Protein precipitation; for example, can only remove gross levels of proteins from biological samples with no removal of phospholipids resulting in huge matrix interference [12]. The separation process in SPE is mainly based on physicochemical retention of target analytes on the functionalized surface of sorbents [16]. Thus, the SPE column not only retains the target analyte(s) but also other matrix components and a considerable amount of work is usually spent on optimizing the complete analytical method [16]. Consequently, serious attention was targeted towards the development of new technologies to improve current extraction techniques aiming to provide cleaner extracts with good recovery from complex biological matrices.

Molecular imprinting polymers (MIPs) are one of the newly proposed tools to provide required selectivity in extraction to overcome the problems of interfering components and low therapeutic levels of target analytes in complex matrices [14]. Generally, molecular imprinting technology presents smart polymers which have recognition sites that can be paired with a target molecule called a template [17,18]. Cavities formed in the polymer network during the polymerization step matches the template in size, shape and functional groups. These cavities then act as a specific binding site that selectively recognizes the template from the complex matrix [17]. Such selective recognition and template affinity, as well as easy and low cost preparation have grabbed considerable attention to MIPs in a wide range of applications including solid-phase extraction (SPE) [17,19,20].

One drawback of MIPs is that the procedure of their separation from solutions is time consuming and suffers the loss of target molecule. Such problem can hinder the use of MIPs in SPE for therapeutic drug monitoring applications [21]. Thus, the introduction of magnetic nanoparticles where the molecularly imprinted technique is combined with the magnetic separation technique can not only avoid the separating operation of centrifugation but can also reduce both the loss of liquid and solid during the separation [21]. Such nano-magnetic molecularly imprinted polymers also offer improved enrichment factors due to the large surface-to-volume ratio of the nanoparticles [22–26]. In addition, they can be isolated by an external magnetic field where the target molecules can easily be eluted using a suitable solvent [27,28]. Therefore, the combination of magnetic molecularly imprinted NPs as a specific, dispersive, easy recyclable sample clean-up technique along with LC–MS/MS as a selective, sensitive analytical method can provide a reliable, accurate protocol for the determination of drugs and their metabolites in complex matrices.

In previous work by our research group, an LC–MS/MS assay for the simultaneous determination of 6-MP and its active metabolite TG in human plasma was developed and validated. Ion exchange chromatography along with LC–MS/MS was applied for extraction and determination of the studied compounds [3]. Yet, ion exchange chromatography suffered a few limitations [3,29]. Thus, we here present a new approach for extraction of 6-MP along with its active metabolite (TG) from human plasma to overcome the problems of our previous developed assay. The new extraction technique is based on magnetic molecularly imprinted NPs (Fe_3O_4@MIP NPs) which were synthesized with the help of computational modeling. The extracted samples using the new technique are then quantified via our validated LC–MS/MS assay and obtained recoveries and matrix effect are compared to those obtained in our previous study.

2. Experimental

2.1. Materials

6-Mercaptopurine (6-MP) and thioguanine (TG) were purchased from Sigma-Aldrich (USA). Ferric chloride anhydrous ($FeCl_3$), ferrous sulphate heptahydrate ($FeSO_4{\cdot}7H_2O$), ammonia solution (25%, w/w) and tetraethoxysilane (TEOS) were purchased from Fisher Scientific (USA). Oleic acid was purchased from Central Drug House (India). Methacryloxypropyl trimethoxysilane (MPS) was obtained from Sigma Aldrich (China). Methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA) were purchased from Aldrich (Germany) and azobisisobutyronitrile (AIBN) from Levochem (USA). Human blank plasma was obtained from the Holding Company for Biological Products and Vaccines (VACSERA), Egypt and stored at −20 °C until use after gentle thawing. HPLC grade acetonitrile, acetone and methanol were purchased from Fisher scientific (UK). Diethyl ether, ethanol and chloroform were obtained from Loba Chemie (India). All other chemicals and reagents were of analytical grade or higher.

2.2. Instrumentation

The morphologies and dimensions of the synthesized nanoparticles were characterized using scanning electron microscopy (SEM) in a Zeiss instrument (Germany) and the transmission electron microscopy (TEM) on Tecani G20, FEI transmission electron microscope (USA). The structure of the synthesized nanoparticles was characterized by X-ray diffractometer (a Rigaku model Geigerflex apparatus using Cukα radiation from 10 to 70◦ (2θ) at a scan rate of $4^{\circ}min^{-1}$ and silicon as an external standard. Fourier transform infrared (FT-IR) spectra (KBr pellets) over the range of 400–4000 cm^{-1} were performed on a Perkin Elmer Spectrum GX spectrophotometer. Magnetic properties were analyzed using a vibrating sample magnetometer (Princeton EG and G Applied Research VSM, Model 155). The thermal degradation and crystallization process of the prepared molecularly imprinted NPs were investigated using a TGA 2050 thermogravimetric analyzer (TA Instruments, New Castle, DE). The measurement was conducted under N_2 atmosphere from 20 to 600 °C at a heating rate of 10 °C/min. The concentrations of 6-MP and TG in the solutions were determined by a Single beam UV–vis model AE-S90-MD form A & E Lab (UK). LC–MS measurements were achieved using Waters Aquity UPLC connected to Waters Aquity Ultra Performance LC autosampler. Mass spectrometric detection was performed using Waters Aquity TM TQD (triple quadrupole detector) in multiple reaction monitoring (MRM) mode. An electrospray ionization (ESI) interface in positive ionization mode was used. Hardware control and data acquisition and treatment were carried out using MassLynx 4.1 SCN805 software.

2.3. Computational approach

In order to save time and effort, computational chemistry tools were employed to estimate the interaction energy between the template drug, 6-MP, and different monomers in different solvent conditions. The monomers tested in this study were acrylamide

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