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Extraction of ultratrace amounts of nelfinavir from biological samples and pharmaceutical formulations using surfactant-modified magnetite nanoparticles followed by spectrophotometric determination

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ABSTRACT

A new sensitive, selective, rapid, and simple magnetic solid phase extraction (MSPE)/spectrophotometry method was proposed for extraction and determination of nelfinavir in biological samples and pharmaceutical formulations using tetradecyltrimethylammonium bromide (TTAB)-coated Fe_3O_4 nanoparticles (Fe_3O_4 NPs) as an efficient adsorbent. The synthesized Fe_3O_4 NPs were characterized by X-ray diffraction (XRD) and scanning electron microscopy (SEM). Fourier transform infrared (FT-IR) spectrometry was used for confirmation of TTAB-coated Fe_3O_4 NPs. The influence of various parameters affecting the extraction of nelfinavir has been investigated. The maximum extraction efficiency for extraction of 100.0 μg nelfinavir from aqueous solution was obtained with 18.6 mg Fe_3O_4 NPs and 9 mg TTAB at pH = 9. The analyte was desorbed by 2.5 mL acetonitrile (AN) prior to the absorbance measurement. Under optimized conditions, quantitative extraction recoveries of nelfinavir were observed with the sample volumes up to 600 mL and hence an enrichment factor of 240 was obtained. This method was so rapid and complete recovery was obtained in 5 s. By applying this method for determination of nelfinavir in sample volume of 600 mL, the calibration curve was linear in the range of 15.4–500 μg L^{-1} ($r^2 = 0.994$) and the detection limit was obtained 0.83 μg L^{-1}. Also, the relative standard deviations (RSD) for determination of 100.0 and 300.0 μg L^{-1} of nelfinavir were 1.20% and 2.4% (n = 7), respectively. This method was successfully applied for determination of nelfinavir in serum samples, urine samples, and pharmaceutical formulations. The recoveries of spiked serum and urine samples were between 98.7% and 102.4%. All the obtained results are indicative of a convenient, fast, and economic method for extraction and determination of nelfinavir from biological samples and pharmaceutical formulations.

1. Introduction

Nelfinavir mesylate [1], with a chemical name of 2-[2-hydroxy-3-(3-hydroxy-2methyl-benzoyl)amino-4-phenylsulfanyl-butyl]-*N*-*tert*-butyl-1,2,3,4,4a,5,6,7,8,8a-decahydroisoquinoline-3-carboxamide methanesulfonic acid (Fig. 1), is recommended for the treatment of human immunodeficiency virus (HIV) infection. It is one of the most potent HIV-protease inhibitors that show activity against both HIV-1 and HIV-2 [2]. Nelfinavir acts by reversible binding to HIV-protease active site thereby preventing cleavage of the viral polyproteins. All protease inhibitors bind to the protease, but the precise way of binding determines the protease inhibition behavior of the molecule. The binding mode of nelfinavir to the enzyme may be sufficiently unique to reduce cross-resistance between it and other protease inhibitors. On the other hand, not all protease inhibitors inhibit both HIV-1 and HIV-2 proteases [3]. One of the most important side effects of nelfinavir is diarrhea or loose stool. Nelfinavir has been associated with elevated cholesterol and triglycerides levels and glucose intolerance [2].

Various analytical methods have been used for nelfinavir determination in biological samples and pharmaceutical formulations. Most of them are included various chromatographic methods such as HPLC-UV [4–7], HPTLC-UV [8] and LC-MS [9]. Also, a few spectrophotometric methods [3,10–12] have been reported for determination of nelfinavir. Due to the high complexity of the biological and real sample matrices, interference effects are inevitable. Meanwhile, concentrations of analyte in such samples are usually too low to be determined directly by instrumental techniques. So, for achieving the accurate quantitative determinations or lower detection limits, extraction and preconcentration of the target analytes are necessary before instrumental determination.

Various sample pretreatment methods such as liquid–liquid extraction (LLE) [4] and solid phase extraction (SPE) [2] have been investigated to extract and enrich trace levels of nelfinavir from complex matrices. However, in the most cases, the cleanup and recovery have been poor [2].

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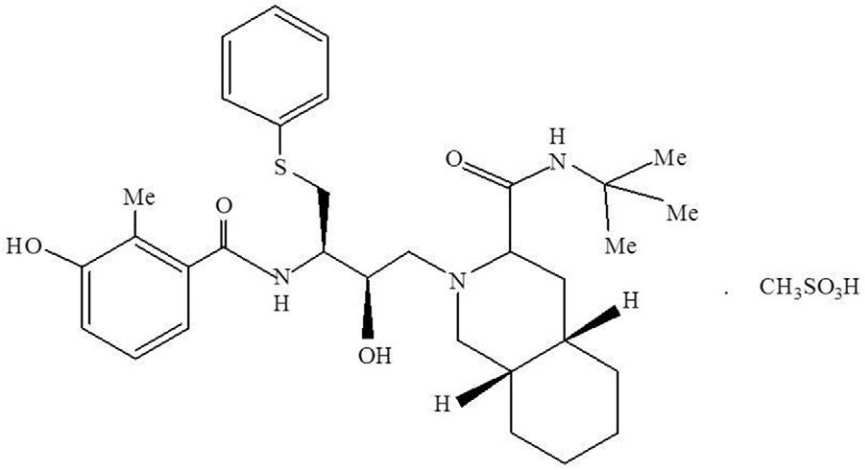

Fig. 1. Chemical structure of nelfinavir mesylate.

At present, SPE is one of the most suitable extraction techniques for extraction of trace levels of target compounds in real samples with complex matrices. It has received special attention and has outstanding advantages compared to conventional LLE method because of its simplicity, ability to achieve high recoveries and enrichment factors, high flexibility in choice of selective adsorbent and low consumption of hazardous organic solvents [13,14]. But, in some cases because of the limited rate of diffusion and mass transfer of the target analyte from the bulk adsorbents, the extraction time of ordinary SPE methods are relatively long [15]. So, it is important to increase the analysis speed of SPE procedure. Nowadays magnetic solid phase extraction (MSPE) method, specially by using nanomaterials as adsorbent, has attracted a great attention due to its obvious advantages such as short extraction time and ease of operation [13]. The high surface area-to-volume ratio of nanomaterials and their excellent dispersibility in sample aqueous phase result in reduced extraction time and increased adsorption capacity and extraction efficiency [16,17]. On the other hand, because of the magnetic properties of adsorbents in MSPE, the adsorbents can be separated easily from bulk sample solution by applying an external magnetic field and application of time-consuming filtration or centrifugation steps is not required. As a result, this leads to ease of phase separation [13,18].

In recent years, a new MSPE method based on the adsorption of ionic surfactant on magnetic nanoparticles surface has been developed extensively and applied for the extraction and preconcentration of organic pollutants from a variety of environmental samples with complex matrices [19–21]. The advantages of this method is high breakthrough volume, easy elution of analytes and no need to clean-up steps [19].

To the best of our knowledge, magnetic solid phase extraction of nelfinavir from biological samples and pharmaceutical formulations has not been reported till date. The aim of the present work is to develop a new simple, selective, and reliable MSPE method using hemimicelle formation for the extraction/determination of nelfinavir in biological samples and pharmaceutical formulations, with increased recoveries. Magnetite (Fe_3O_4) as suitable iron-oxide nanoparticle was chosen in this study due to its biocompatibility, low toxicity, and strong magnetization response. Using spectrophotometric method for detection of the nelfinavir has increased simplicity, low cost, and reliability of the method.

2. Experimental

2.1. Chemicals and reagents

All chemicals used were of analytical reagent grade and were used as received. Ferric chloride ($FeCl_3 \cdot 6H_2O$), ferrous chloride ($FeCl_2 \cdot 4H_2O$), hydrochloric acid (HCl), sodium hydroxide (NaOH), aqueous ammonia (NH_4OH), sodium chloride (NaCl), acetonitrile (AN), methanol, ethanol, acetone, and acetic acid were purchased from Merck (Darmstadt, Germany). Tetradecyltrimethylammonium bromide (TTAB) and nelfinavir mesylate hydrate were purchased from Sigma–Aldrich. Doubly distilled deionized water was used throughout the study.

The stock solution of TTAB (2000 mg L^{-1}) was prepared by dissolving 1.00 g of TTAB in 500 mL doubly distilled deionized water. The stock solution of nelfinavir (200 mg L^{-1}) was prepared by dissolving 0.050 g of nelfinavir in 250 mL HPLC-grade AN. The working solutions were prepared daily by diluting the standard stock solution with AN. The pH of the solutions was adjusted by dropwise addition of HCl (0.1 mol L^{-1}) and/or NaOH (0.1 mol L^{-1}) solutions. The nitrogen gas with high purity was used for providing the inert atmosphere necessary for synthesis of Fe_3O_4 nanoparticles (Fe_3O_4 NPs).

2.2. Apparatus

The UV–Vis spectra were recorded using a double-beam Perkin–Elmer Lambda 25 UV/Vis Spectrophotometer. FT-IR spectra were recorded with a Perkin–Elmer RXI FT-IR 2400, using KBr disk in the range 4000–400 cm^{-1}. The morphological features of magnetite nanoparticles were observed by scanning electron microscopy (SEM) using Zeiss–DSM 960A microscope. The X-ray diffraction (XRD) study was done by Italstracture X-ray diffractometer model MPD 3000 with a radiation source from Cu Kα ($\lambda = 0.1542$ nm). All pH measurements were carried out using a Metrohm-827 pH/mV meter at 25.0 °C. Magnetic separations were fulfilled by a Nd–Fe–B supermagnet with 1.2 Tesla magnetic field, N 35 model ($5 \times 3 \times 2$ cm) from Tehran Magnet (Tehran, Iran). Magnetic stirrer with heating Yellow Line Model MSH Basic was applied for stirring and heating of the solutions. The ultrasonic bath used was SONICA mod 2200 ETH. Shimadzu AEL-200, Japan, ± 0.0001 g electronic analytical balance was used for weighting the solid reagents.

2.3. Synthesis of Fe_3O_4 NPs

Fe_3O_4 NPs were prepared by the chemical coprecipitation method in an ammonia solution [22]. Prior to the synthesis, all water samples and solutions were deoxygenated by nitrogen bubbling. 5.84 g of $FeCl_3 \cdot 6H_2O$ and 2.92 g of $FeCl_2 \cdot 4H_2O$ were dissolved in 160 mL deoxygenated deionized water. The mixed solution was stirred under N_2 atmosphere at 80 °C for 30 min. Then, 40 mL of 25% ammonium solution was injected into the mixture gradually, stirred under N_2 atmosphere for another 30 min and then cooled to room temperature.