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Nano polypyrrole-coated magnetic solid phase extraction followed by dispersive liquid phase microextraction for trace determination of megestrol acetate and levonorgestrel

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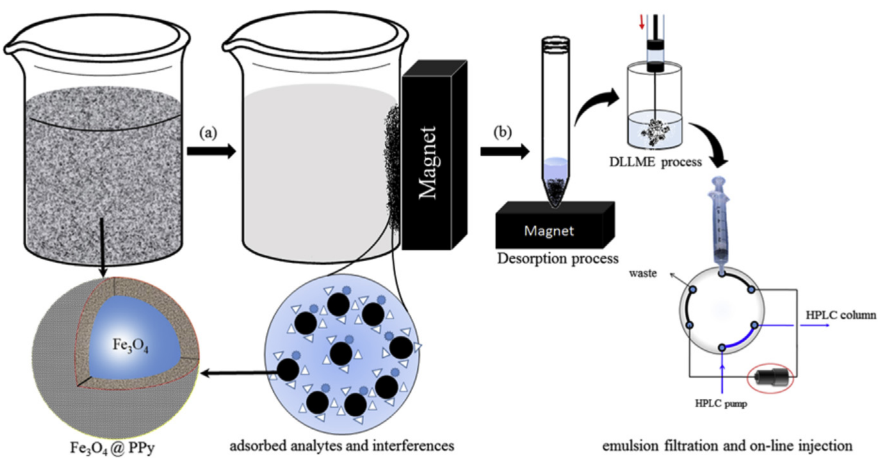
HIGHLIGHTS

- Magnetic solid phase extraction was followed by dispersive liquid phase microextraction.
- Sedimented phase was separated by filtration based phase separation.
- The separated phase was eluted and transferred to HPLC-UV for separation and analysis.
- Method was applied for detection of megestrol and levonorgestrel in biological samples.
- Under the optimal conditions, a linear range from 0.05 to 50 ng mL^{-1} was obtained.

GRAPHICAL ABSTRACT



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ABSTRACT

The aim of the present work is combination of the advantages of magnetic solid phase extraction (MSPE) and dispersive liquid phase microextraction (DLLME) followed by filtration-based phase separation. A new pretreatment method was developed for trace determination of megestrol acetate and levonorgestrel by liquid chromatography/ultraviolet detection in biological and wastewater samples. After magnetic solid phase extraction, the eluent of MSPE was used as the disperser solvent for DLLME. Emulsion resulted from DLLME procedure was passed through the in-line filter for phase separation. Finally the retained analytes in the filter was washed with mobile phase of liquid chromatography and transferred to the column for separation. This approach offers the preconcentration factors of 3680 and 3750 for megestrol acetate and levonorgestrel, respectively. This guarantees determination of the organic compounds at trace levels. The important parameters influencing the extraction efficiency were studied and optimized. Under the optimal extraction conditions, a linear range of 0.05–50 ng mL^{-1} ($R^2 > 0.998$) and limit of detection of 0.03 ng mL^{-1} were obtained for megestrol acetate and levonorgestrel. Under optimal conditions, the method was

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successfully applied for determination of target analytes in urine and wastewater samples and satisfactory results were obtained (RSDs < 6.8%).

1. Introduction

The main purposes of sample preparation are to make the analytes of interest discrete and concentrated, and to transfer them to a medium compatible with the instrument used in the final analysis [1]. To increase the throughput of sample preparation, development of time saving and highly efficient extraction techniques has drawn much attention in recent years. Solid phase extraction (SPE) has become a well-established sample preparation method to extract and preconcentrate the desired components [2–4]. Application of magnetic nanoparticles in SPE (MSPE) simplifies sample pretreatment and overcomes some limitations of conventional SPE [5]. It is not needed to pack the sorbent into the cartridge and the phase separation can be easily realized by applying an external magnetic field. Nanoparticles (NPs) possess large surface area, high adsorption capacity, and rapid adsorption rate; so, low amounts of sorbent and short equilibrium time are required to extract analytes from large volumes of samples [6–8].

According to reports available, surface modification stabilizes the NPs and prevents their oxidation [9–13]. Also, suitable surface modification increases their dispersibility and extraction capacity as well as their selectivity [14–16]. Therefore, a suitable coating is essential to overcome some limitations and realize the advantages of magnetic nanoparticles in sample pretreatment. There has been an obvious increasing interest in establishing new coating materials for MSPE. Recent researches suggest that conductive polymers are promising in separation science because of their versatile properties such as hydrophobicity, large π-conjugated structure, polar functional groups, and ion exchange characteristics [13–18]. Polypyrrole (PPy) is one of the most widely investigated materials, due to its useful features. The utilization of PPy as a coating material in MSPE has been reported previously for extraction of phthalate esters [19] and nitrophenols [20].

Dispersive liquid phase microextraction (DLLME) is a miniaturized sample pre-treatment technique that uses microliter volumes of the extraction solvent. Simplicity of the operation, rapidity, low sample volume, low cost, and high preconcentration factor are some advantages of this technique [21–25]. An important technical aspect of emulsification-based microextraction methods is separation and collection of the acceptor (extractant) phase containing the target analytes. Typically, this is accomplished by centrifugation, a procedure that prolongs the analysis time [26,27]. Most importantly, collection of the small volume of the extractant phase requires cautious sampling with micropipettes or cone tip needle syringes from appropriate or specialized apparatus, which are either home-designed or not readily available [28,29]. In our previous work [30], to improve the emulsification-based liquid phase microextraction (ELPME), filtration was successfully used instead of centrifugation, and any organic solvent immiscible with water (notwithstanding the density) can be used as the extractant in ELPME.

Megestrol acetate (MGA) and levonorgestrel (LEV) belong to one of the five classes of the steroid hormones called progestogen. Megestrol acetate is mainly used as an appetite stimulant in a variety of conditions and as a contraceptive in combination with an estrogen at relatively low doses [31,32]. Levonorgestrel is used as an active ingredient in some hormonal contraceptives, and hormone replacement therapy [33]. Steroid hormones are endocrine-disrupting compounds, which affect the endocrine system at very low concentrations. Since these compounds are widely used, there is an increasing interest in sensitive determination of steroids in the biological and environmental samples in recent years [34,35].

In this paper, combination of polypyrrole-coated Fe_3O_4-based MSPE and DLLME was applied as an efficient sample pretreatment method for determination of MGA and LEV at trace levels in biological and wastewater samples. The eluting solvent of MSPE was used as a disperser solvent in DLLME method. After completion of MSPE, the suitable volume of extraction solvent was added to the eluting solvent of MSPE. Then, the mixture was rapidly injected into the aqueous medium that resulted in an emulsified solution. After that, it was passed through an in-line filter and the extraction phase was separated from the emulsion by filtration. Finally, the extraction solvent was injected into the separation column of HPLC as on-line procedure. This combination represents a simple sample preparation procedure that lacks tedious steps such as solvent evaporation after extraction, centrifugation, and delicate collection of the extractant phase. Moreover, this approach can offer the high preconcentration factors that guarantee determination of the organic compounds at trace levels.

2. Experimental

2.1. Chemicals and reagents

Megestrol acetate (17-(acetyloxy)-6-methyl-pregna-4,6-diene-3,20-dione) and levonorgestrel (13-ethyl-17-ethynyl-17-hydroxy-1,2,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydrocyclopenta phenanthren-3-one) were kindly gifted by the Faculty of Pharmacy, Tehran University (Tehran, Iran). All the chemicals were of analytical reagent grade. Ferric chloride ($FeCl_3·6H_2O$), ferrous chloride ($FeCl_2·4H_2O$), ammonia solution (25 wt.%), 1-octanol, dihexyl ether (DHE), sodium perchlorate, sodium dodecylbenzenesulfonate (SDBS), methanol, acetonitrile, and 1-propanol were purchased from Merck (Darmstadt, Germany). Synthetic pyrrole was provided by Aldrich (Milwaukee, WI, USA). The stock standard solutions of the analytes were prepared in methanol at a concentration of 1000 mg L^{-1} and stored at 4 °C, protected from light. The standard working solutions were prepared daily by appropriate dilution of the stock standard solutions with ultra-pure water to the required concentrations. The ultra-pure water used throughout the process was produced by an Aqua Max-Ultra Youngling ultrapure water purification system (Dongan-gu, South Korea).

2.2. Instruments

Chromatographic analysis was performed with a HPLC instrument including a Varian 9012 HPLC pump (CA, USA), a six-port Cheminert HPLC valve from Valco (Houston, USA) that was equipped with a Varian 9050 UV–vis detector. The separations were carried out on an ODS-3 column (250 × 4.0 mm, with 5 μm particle size) from MZ-Analysentechnik (Mainz, Germany). A mixture of water:acetonitrile (30:70 V/V%) under an isocratic elution and flow rate of 1 mL min^{-1} was used as the mobile phase. The elution time of the analytes was 15 min and after elution of the analytes, the mobile phase composition was changed to 100% acetonitrile for 5 min to complete elution of the filter and the column. The detection was performed at the wavelength of 257 nm.