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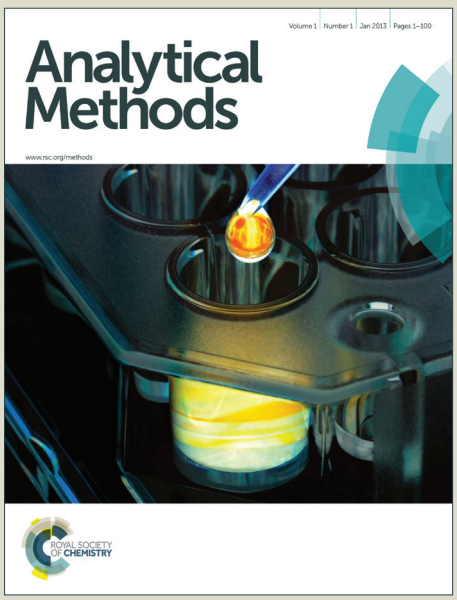

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Optimization of Magnetic Extraction by Experimental Design Methodology for the Determination of Antidepressants in Biological Samples

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Abstract

In this study, a novel and sensitive magnetic solid phase extraction (MSPE) method based on Fe_3O_4-$MgSiO_3$ magnetic nanocomposite was developed for extraction and preconcentration of venlafaxine, escitalopram, paroxetine, sertraline and fluoxetine in biological samples followed by liquid chromatography-ultraviolet detection (LC-UV). The effects of some factors influencing the extraction efficiency of antidepressants including adsorbent amount, extraction pH, and desorption solvent volume were optimized using experimental design methodology. The optimum conditions were found to be adsorbent amount: 12.5 mg, extraction pH: 7.4 and desorption solvent volume: 1.3 mL. Under the optimum experimental conditions, a good linearity was observed for all the analytes, with the square of correlation coefficients (r^2) ranging from 0.9986 to 0.9994. The limit of detection and limit of quantification for the antidepressant drugs were found to be in the range of 1.73-2.83 and 5.21-8.53 ng mL^{-1}, respectively. This method was successfully applied to analyzing real biological samples at different spiked concentrations, and the obtained recoveries ranged from 72 to 115 % with the relative standard deviations (RSDs) below than 4.75 %.

Keywords: Magnetic nanocomposites; antidepressants; chemometric optimization; liquid chromatography; biological samples.

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1. Introduction

In recent years, the studies on the nanomaterials have significantly increased as a result of the development of nanotechnology. [1] Fe_3O_4 nanoparticles have been used in MSPE because of its super paramagnetism, high magnetic saturation, and simple preparation process. However, naked Fe_3O_4 nanoparticles tend to aggregate, are prone to oxidation and are not selective toward complex matrices. [2] Therefore, many researchers have focused on preparation of organic and inorganic composite magnetic nanoparticles to make them selective and appropriate sorbents. [3, 4-6] Compared with organic composite analogues, inorganic composite magnetic nanoparticles are considered to be easier to prepare and more safe to be put into use. [7] The size and shape of composite magnetic nanoparticles can be controlled by the synthesis methods. [8-11] A variety of methods have been reported in the literature on the synthesis of composite magnetic nanoparticles, such as micro emulsions, [12] chemical co-precipitation method, [13-15] ultrasonic spray pyrolysis, [16] hydrolysis, [17] hydrothermal method, [18] microwave plasma, [19] and sol-gel method. [20] Each preparation method has its advantages and disadvantages, which is mainly related to distribution of particles size, production scale and cost.

Selective serotonin reuptake inhibitors (SSRIs), such as escitalopram, fluoxetine, paroxetine, venlafaxine and sertraline, are commonly used as antidepressant drugs in the treatment of depression, anxiety disorders and some personality disorders. [21-23] Analytical methods for the determination of antidepressant drugs in biological samples are not only of interest in the field of clinical toxicology, but also in forensics investigations as they are often involved in intoxications. Several analytical methods for the determination of antidepressants and their

metabolites in biological samples such as liquid chromatography (LC), [24-27] capillary electrophoresis (CE), [28] gas chromatography-mass spectroscopy (GC-MS), [29, 30] and liquid chromatography-mass spectroscopy (LC-MS) [31] have been developed. The liquid chromatography (LC) coupled with different detectors such as ultraviolet, mass spectrometry, electrochemical, chemiluminescent and fluorescence detection are the most widely used techniques to determine antidepressants in biological samples. [32] However, sample preparation and isolation of related compounds from various samples prior to instrumental analysis is one of the most important steps in a whole analytical method. For this purpose, generally, many sample preparation techniques have been used for the extraction and preconcentration of antidepressant drugs in biological samples such as liquid-liquid extraction (LLE), [29] ultrasound-assisted emulsification microextraction (UAME), [24] solid-phase extraction (SPE), [30] solid-phase microextraction (SPME), [25] and dispersive liquid-liquid microextraction (DLLME). [26] In recent years, much attention has been focused on the use of magnetic solid-phase extraction (MSPE), as a relatively new mode of SPE, which is suggested as a promising alternative to conventional methods for the extraction and preconcentration of a variety of inorganic and organic compounds from different samples. [33] Furthermore, MSPE offers some important advantages over the traditional extraction techniques such as rapid extraction and easy separation without additional centrifugation or filtration for the large volume samples by employing a strong external magnetic field. Up to now, this technique has been employed for the analysis of PAHs, pesticides, fungicides, acidic and basic drugs and metal ions in biological, food and environmental samples. [15, 34, 35]

This study presents chemometrically optimized analytical isolation procedure, proposed to enable the precise determination of new generation antidepressant drugs in biological fluids. The study was performed in three steps: synthesis of magnetic nanocomposites,

characterization of the synthesized composites and analytical applications. Magnetic nanocomposite (Fe_3O_4-$MgSiO_3$) was synthesized by the in situ-chemical co-precipitation of Fe^{2+} and Fe^{3+} in an alkaline solution in the presence of $MgSiO_3$. This material was first time employed as an SPE adsorbent for separating and concentrating trace amounts of antidepressant drugs from biological samples.

2. Experiment

2.1. Reagents and solutions

All of the standard reagents used in the experiments were of analytical grade. Five pharmaceutical formulations commercially available in Turkey were analyzed: Lustral tablet 50 mg (active ingredient: sertraline; expiry date 09/2016) and Efexor capsules 37.5 mg (active ingredient: venlafaxine; expiry date 04/2016) by Pfizer (İstanbul, Turkey), Paxil tablet 25 mg (active ingredient: paroxetine; expiry date 08/2016) by Glaxo Smith Kline (İstanbul, Turkey), Prozac capsules 20 mg (active ingredient: fluoxetine; expiry date 08/2016) by Lilly (İstanbul, Turkey) and Losiram 10 mg tablet (active ingredient: escitolopram; expiry date 10/2014) by Bilim (İstanbul, Turkey). Hydrochloride acid and ethanol were purchased from Merck (Darmstadt, Germany). Florisil60-100 mesh ($MgSiO_3$), NH_4OH, NaOH, $FeCl_3$, $FeCl_2$ and LC grade acetonitrile were purchased from Sigma-Aldrich (Steinheim, Germany). Double distilled water was prepared with a Direct-Q3 water purification system (Millipore, Bedford, MA, USA). The primary standard stock solutions of sertraline (0.50 mg mL^{-1}), venlafaxine (0.375 mg mL^{-1}), paroxetine (0.25 mg mL^{-1}), escitalopram (0.10 mg mL^{-1}) and fluoxetine (0.20 mg mL^{-1}) were prepared by dissolving appropriate amount of drug in water and all samples were filtered prior to analysis by Polypropylene filter cartridge (0.25 μm). Working solutions were obtained by diluting the stock solutions with double distilled ultra-pure water. All the standard solutions were stored at - 4 °C. Human serum and urine samples obtained

from different volunteers were stored at -20 ˚C until analysis. 1 mL of acetonitrile was added to the samples to precipitate proteins followed by centrifugation at 3000 rpm for 10 min at room temperature. The supernatants were diluted to 1:1 and 1:2 for serum and urine samples respectively, using double deionized water, and filtered before extraction. Biological samples were obtained from patients receiving daily various antidepressant drug doses with the permission of University Research Hospital. All experiments were performed in compliance with the relevant laws and institutional guidelines.

2.2. Instrumentation and chromatographic conditions

The LC analyses were performed using Thermo Finnigan LC system (San Jose, USA) consisting of a P1000 pump equipped with a Rheodyne injection valve (20 µl injection loop), a SCM 1000 degasser and a UV1000 ultraviolet detector. The system was controlled by a Specta System Controller SN 4000 and a software package Chromquest 4.0 program. Separation was performed by means of a Phenomenex Synergi Polar-RP column (250 mm × 4.6 mm, 4 µm) protected by a C_{18} guard column (4 mm × 3 mm, Phenomenex). A gradient elution program was optimized by using the mobile phases of acetonitrile and 40 mmol L^{-1} phosphate buffer (pH 5). The separation was performed at room temperature with a constant flow-rate of 1.0 mL min^{-1} by employing the elution program as follows; 0-10 min acetonitrile/buffer 45:55 v/v and then a linear gradient elution from 45 % acetonitrile at 10 min to 100 % acetonitrile at 20 min, followed by isocratic elution with acetonitrile for 5 min. Finally, 5 min was necessary in re-establishing the initial conditions. The detection wavelength was set to 230 nm. A Crison GLP 22 pH-meter (Barcelona, Spain), a vortex shaker (Velp Scientifica, Milan, Italy) and a NF 200 centrifuge (Nüve, Ankara, Turkey) were used in the sample preparation and isolation steps.

The IR spectra of Fe_3O_4 and Fe_3O_4-$MgSiO_3$ were carried out using a Fourier Transform Infrared (FTIR) Spectrometer (Perkin-Elmer-Frontier, Inc.CA, USA). X-ray diffraction (XRD) measurements were carried out using a PANalytical Emperial X-ray diffractometer (Netherlands) with Cu Kα radiation operated at 40 kV and 60 mA. The obtained XRD patterns were readily compared with the reference data. The morphology and size of the magnetic nanoparticles were observed by scanning electron microscopy (SEM) using FEIQuanta450 Environmental Scanning Electron Microscope (High Resolution FE-ESEM, USA).

2.3. Preparation of Fe_3O_4-$MgSiO_3$ magnetic nanocomposites

Fe_3O_4-$MgSiO_3$ magnetic nanocomposites were synthesized by the in situ chemical co-precipitation of Fe^{2+} and Fe^{3+} in an alkaline solution in the presence of $MgSiO_3$. The molar ratio of Fe^{2+} and Fe^{3+} was 1:2. The magnetic composite was prepared by suspending 0.5 g $MgSiO_3$ in 100 mL of solution containing 2.54 g (20 mmol) $FeCl_2$ and 3.25 g (40 mmol) $FeCl_3$ at 50 °C under N_2 atmosphere. After the solution was sonicated (200 W, 40 kHz) for 10 min, 10 mL of 8 mol L^{-1} NH_4OH aqueous solution was added drop-wise to precipitate the iron oxides while the mixture solution was stirred for 10 min. The color of bulk solution changed from orange to black immediately. The pH of the final mixture was adjusted within the range of 11-12 by drop-wise addition of 1 mol L^{-1} NaOH. To promote the complete growth of the nanoparticle crystals, the reaction was carried out at 60 °C for 30 min under constant mechanical stirring. After the system was cooled to room temperature, the precipitate was separated in the magnetic field by a permanent magnet, and then the supernatant was removed from the precipitate by decantation. The impurities (such as unreacted chemicals and ammonia) in the Fe_3O_4-$MgSiO_3$ were removed by washing with double-distilled water and the precipitate was isolated by a strong magnet. The obtained Fe_3O_4-$MgSiO_3$ nanocomposite was

then washed with 10 mL of absolute alcohol for three times. Subsequently, the resulting product was dried under vacuum and finally Fe_3O_4-$MgSiO_3$ (~ 5 g) was obtained.

2.4. Extraction procedure

The magnetic solid phase extraction procedure was carried out as follows; firstly 12.5 mg of Fe_3O_4-$MgSiO_3$ was transferred into a 10 mL glass test tube containing 5 mL aqueous solution of the drugs (100 ng mL^{-1} of each drugs). The pH of the aqueous phase was again adjusted to 7.4 by drop-wise addition of 1M Na_2HPO_4. The mixture was shaken by vortex mixing for 2 min at room temperature. Subsequently, the Fe_3O_4-$MgSiO_3$ adsorbent was isolated from the solution by placing a strong magnet at the bottom of the conical flask. The supernatant was discarded and 1.3 mL of slightly acidic methanol (85 % methanol containing 0.1 % HCl) was added as eluent and elution was completed during 2 min by vortex mixing. Finally, 20 µL of this 1.3 ml was injected into the LC system for chromatographic analysis.

2.5. Development of chemometric optimization approach

In conventional analytical studies, univariate optimization requires long time and not even takes into account of the possible factors interactions in experimental studies. Therefore, experimental design and optimization considering all factor effects together with factor interactions allow finding better factor settings (optimal experimental conditions) as well as the setup of a robust analytical method or chemical process. [36] In this context, before applying LC-UV to the analysis of antidepressants in biological samples, the central composite design (CCD) and optimization methodology were utilized for the determination of the optimal extraction conditions of the compounds from serum and urine samples. According to design and optimization results, the analysis procedures were done using LC-UV.

An experimental design and optimization approach were applied to find the optimal experimental extraction conditions for the determination of antidepressants in serum and urine samples. A CCD with three factor variables (adsorbent amount, extraction pH and solvent volume) with five levels was used for the optimization of the extraction conditions. Adsorbent amount (mg), extraction pH and solvent volume (mL) were coded as X_1, X_2 and X_3, respectively. The independent factor variables and experimental domain in their original and coded forms are shown in Table 1.

To obtain the mathematical model for three independent factor variables and their corresponding response variable, the second order-degree polynomial equation (1) is given as:

$$y = b_0+b_1X_1+b_2X_2+b_3X_3+b_{11}X_1^2+b_{22}X_2^2+b_{33}X_3^2+b_{12}X_1X_2+b_{13}X_1X_3+b_{23}X_2X_3+\varepsilon \quad (1)$$

where Y is a dependent variable (peak areas of related compound obtained by applying LC-UV method); b_0 is a constant; b_1, b_2 and b_3 are the linear coefficients; b_{11}, b_{22} and b_{33} are the quadratic coefficients; b_{12}, b_{13} and b_{23} are the interaction coefficients; X_1, X_2, and X_3 are the coded values of the independent variables; and experimental errors are modelled by ε.

3. Results and discussion

3.1. Characterization of magnetic nanocomposite

X- ray diffraction (XRD) measurements were employed to investigate the phases of Fe_3O_4 and structure of the synthesized magnetic nanocomposite (Fe_3O_4-$MgSiO_3$). The obtained XRD graphs are shown in Fig. 1. The experimentally obtained graphs were identified through comparison with standard Fe_3O_4 and Fe_3O_4-$MgSiO_3$.

Figure1a shows the characteristic peaks of the magnetic nanoparticles at 2θ = 18.4°, 30.27°, 35.66°, 37.30°, 43.34°, 53.78°, 57.33°, 62.96°, 71.45°, 74.51°, and 79.52°, which can be

indexed as 111, 220, 311, 222, 400, 442, 511, 440, 533, 620, and 444 planes of magnetite cubic crystalline phase (Fe_3O_4) (ICSD, 98-015-8745).

The obtained results from XRD analysis of Fe_3O_4-$MgSiO_3$ nanocomposites indicate that the resultant particles and Fe_3O_4 nanoparticles have similar eight characteristic peaks. The XRD peaks of Fe_3O_4-$MgSiO_3$ nanocomposites are similar to those reported by Zhou et al. [4] There is an additional broad peak in Figure 1b at around $2\theta = 23$ which indicates the existence of an amorphous structure of $MgSiO_3$ and all other diffraction peaks can be readily indexed to the orthorhombic phase of Fe_3O_4.

The SEM images of Fe_3O_4 nanoparticles and Fe_3O_4-$MgSiO_3$ nanocomposite are shown in Fig. 2. It can be seen in Figure 2 that Fe_3O_4 magnetic nanoparticles were successfully impregnated on the surface of $MgSiO_3$ to form Fe_3O_4-$MgSiO_3$ nanocomposite. SEM image of Fe_3O_4 showed that the nanoparticles exhibited spherical morphologies with an average diameter of 39 nm. SEM image of the synthesized Fe_3O_4-$MgSiO_3$ magnetic nanocomposites indicated that the uniform grey $MgSiO_3$ shell was impregnated with dark magnetite particles. Fe_3O_4 nanoparticles were well distributed on $MgSiO_3$ layers, and had a big area up to several square micrometers.

X-ray microanalysis was employed to determine the composition of Fe_3O_4 and Fe_3O_4-$MgSiO_3$ using Silicon Drift Detector (SDD; EDAX Apollo X) The EDX spectra (Fig. 2) indicated that the respective percent weight of oxygen and iron were found to be 62.8 and 37.2 %. The EDX data displayed only the peaks for Fe and O atoms, which thus confirmed the absence of any impurities during the preparation of desired material. As shown in Figure 2, O (50.07 %), C

(31.78 %), Si (11.40 %), Mg (3.91 %) and Fe (2.84 %) elements were detected. Carbon may be caused by the surrounding adhesive tape placed on the sample holder.

Figure 3a and b show the comparison of FT-IR spectra of Fe_3O_4 nanoparticles and Fe_3O_4-$MgSiO_3$ nanocomposites, respectively. The positions of vibrational band at around 566 cm^{-1} is related to the ν (Fe-O) lattice vibration. 1636 cm^{-1} and 1400 cm^{-1} belong to physisorbed water and residual ammonia, respectively. The strong adsorption band at 1107 cm^{-1} is associated with stretching vibration of Si-O-Si. A broad peak is due to the presence of van der Waals interactions between the hydroxyl groups of H_2O with an exterior layer of $MgSiO_3$ and the partial positive charge on the surface of Fe_3O_4. Some weak adsorption bands at 798 and 950 cm^{-1} are corresponding respectively to the stretching vibrations of ν (Si-OH) and ν (Si-O-Fe). The FT-IR spectra indicate the rigidity of silicate layers and nonbonding chemical interaction between the silicate layers and distributed Fe_3O_4 nanoparticles in Fe_3O_4-$MgSiO_3$ nanocomposites.

3.2. Experimental design and statistical analysis

In analytical method, experimental design and optimization approaches have been used to examine the relationship between one or more response variables and a set of experimental factor variables. Twenty-four experiments were generated by CCD and executed in randomized order and three experimental parameters were varied on five levels. In order to obtain the required data, the range of values of the three variables was defined as follows: adsorbent amount of 8-20 mg, extraction pH of 5-9 and solvent volume of 1-3 mL. The 24 run of three factor**s** variables and corresponding responses with predicted responses in the design of experiments are shown in Table 2.

For the selected three factors and their factor interactions with response variables, the analysis of variance (ANOVA) was performed to identify the significance of the main factors and their interactions and to estimate the adequacy of the model. The ANOVA of CCD model and the test results of the significance for each coefficient of second order-degree polynomial equations are shown in Table 3. It was determined by the corresponding Fisher's F-values and *p*-values and sum of square (SS). The higher Fisher's F-values than F-critical values and small *p*-values ($p<0.05$) indicated that factors and factors interactions had high effects on the response variables in the extraction of antidepressants from samples. ANOVA results demonstrated that the proposed experimental design can be effectively applied to the optimization of the selected factors in extraction processes of the related compound.

As seen from Table 3, *p* values of adsorbent amount (mg), extraction pH (X_1) and desorption solvent volume (X_3) are very low; therefore, the main factors have significant effects on the response variables (chromatographic peak area). Interactions of adsorbent amount and desorption solvent volume (X_1*X_3) have *p* values lower than 0.0001, which indicate that these interactions are also significant on the model. On the other hand, interactions between adsorbent amount-extraction pH (X_1*X_2) and extraction pH-desorption solvent volume (X_2*X_3) (*p* values, 0.152 and 0.619; respectively) are not significant. All of the quadratic terms of model are significant (*p* values are 0.000).

The T-test values and low *p*-values ($p< 0.05$) indicated that all the model terms had strong effects on the response variables (chromatographic peak area) corresponding to the extraction yield except for interactions between adsorbent amount-extraction pH (X_1*X_2) and extraction pH-desorption solvent volume (X_2*X_3). It was concluded that significant model terms provided fitting models for the investigated experimental space and optimization of factor

variables corresponding to adsorbent amount (X_1), extraction pH (X_2) and desorption solvent volume (X_3) in order to get maximum extraction efficiency of antidepressants from serum samples.

By applying multiple regression analysis on the experimental data, the quadratic polynomial model (Equation 2) for the predicted response values (peak areas of antidepressants obtained by applying method, *Y*) of venlafaxine are shown as follows (in the form of coded values):

$$Y = -576858 + 8118\ X_1 + 187442\ X_2 + 120453\ X_3 - 561\ X_1^2 - 12798\ X_2^2 - 44602\ X_3^2 + 268\ X_1*X_2 + 1610\ X_1*X_3 + 539\ X_2*X_3 \quad (2)$$

Generally, a model fits the data well if the differences between the observed values and the model's predicted values are small and unbiased. The applicability of the model is verified by the coefficient of determination (R^2). There is a high correlation ($R^2 = 0.9955$) between the predicted and actual responses, which indicates that only less than 1 % of the total variations are not explained by the model. Moreover, the high correlation between experimental and predicted values shows that the quadratic model developed in this study is suitable for determining the optimum conditions.

The 3D response surface, which is a three dimensional graphic representation was used to determine the individual and cumulative effect of the variable and the mutual interaction between the variable and the dependent variable. The response surface analyses the geometric nature of the surface, the maxima and minima of the response and the significance of the coefficients of the canonical equation. [37] The relationships between independent and

dependent variables were graphically represented by surface graphs and contour plots generated by the model (Fig. 4). These graphical representations were derived from the models of Eq1.

Figure 4a shows the interaction between adsorbent amount (X_1) and extraction pH (X_2) on the response of venlafaxine. The increase of adsorbent amount from 8 to 20 improved the response of venlafaxine. However, the adsorbent amounts lower than 8 mg caused gradual decline in the response. The extraction pHs higher than 6 showed an increased effect on response. Figure 4b describes the effect of adsorbent amount (X_1) and solvent volume (X_3) on the response of venlafaxine. The maximum response values were obtained at around adsorbent amount: 12 mg and solvent volume: 1.4 mL. The surface graph given in Figure 4c shows the relative effects of two variables when adsorbent amount is kept constant at around 12 mg. It is clear that the response reached maximum level for the solvent volume of 1.4 mL and extraction pH of 7.5.

In the same way, Response Surface Methodology (RSM) based on CCD was applied to determination of optimum extraction parameters for the analysis of antidepressant drugs from urine samples. In this work, the optimized experimental conditions required for maximum extraction efficiency of venlafaxine, escitalopram, paroxetine, sertraline and fluoxetine from serum and urine samples were found and the optimum extraction efficiency conditions with the average values for both related compounds were evaluated to be 12.7 mg for adsorbent amount, 7.4 for pH, and 1.5 mL for solvent volume in serum samples, 12.3 mg for adsorbent amount, 7.4 for pH, and 1.2 mL for solvent volume in urine samples using LC-UV. The surface charge of $MgSiO_3$ is neutral at selected pH, which is about 7. At neutral pHs, both the functional groups of analytes and the functional group on the sorbent surface is uncharged,

hence, hydrophilic interactions of analytes with sorbent surface can occur. Adsorbent plays a role for making the sample more homogeneous during vortex mixing. At the same time, polar components in the sample were easily adsorbed on the surface of the magnesium silicate based on the polar characteristic of the adsorbent. The selectivity of the present method can be attributed to extraction way in which the analytes are eluted with slightly acidic methanol, while the polar matrix components remained on the adsorbent surface. [38]

3.3. Analytical method validation

The proposed analytical method for determination of antidepressants in biological samples was validated under the optimized conditions for the linear range, correlation coefficient, limit of detection (LODs), limit of quantification (LOQs), repeatability, enrichment factor, and extraction recovery. The results are listed in Table 4. The calibration curves were linear over a wide range for all analytes, with correlation coefficients ranging from 0.9986 to 0.9994. LODs and LOQs as calculated based on the signal-to-noise ratio (S/N) of 3 and 10, ranged from 1.73 to 2.83 ng mL^{-1} and 5.21-8.53 ng mL^{-1}, respectively. The repeatability of the proposed method was evaluated by investigating the intra-day and inter-days precisions from five replicate analyses of the spiked samples at a concentration level of 50 ng mL^{-1}. The relative standard deviations (RSDs) were satisfactory, remaining lower than 4.9 % (intra-day) and 5.23 % (inter-days) for all compounds.

Equations (3) and (4) were applied for the calculation of enrichment factor (EF) and extraction recovery (ER), respectively.

$$EF = C_{col}/C_s \quad (3)$$

where C_{col} and C_s were the concentration of analyte in the collected phase and initial concentration of analyte in sample solution, respectively. C_{col} was calculated from the

calibration graphs of antidepressant standard solutions in the concentration range of 50-500 ng mL^{-1}

$$ER = (C_{col} \cdot V_{col}) / (C_s \cdot V_s) \times 100 = EF \times (V_{col}/V_s) \times 100 \quad (4)$$

where V_{col} and V_s were the volume of the collected phase and volume of sample solution, respectively.

Under the optimized conditions the enrichment factors and extraction recoveries were ranged between 3.5-6.0 and 71-90 % for biological samples, respectively (Table 4). The performance of the proposed method is comparable with those of other sample preparation techniques such as UAME, [24] SPME, [25] DLLME, [26] and SPE [30] from the viewpoint of LOD, RSD, linearity, and extraction time.

3.4. Sample analysis

The proposed method was successfully applied to determine the concentration of five antidepressants in serum and urine samples. The precipitated proteins were separated by centrifugation at 3000 r.p.m. for 10 min. The clear supernatant layers were filtered through Millipore filter (0.45 mm). In order to reduce the matrix effect, the serum and urine samples were diluted to 1:1 and 1:2, respectively, using double deionized water and then each real sample was extracted at optimal conditions with the proposed isolation procedure. The LC-UV chromatograms of the serum and urine samples are shown in Fig. 5. These chromatograms show that there is no interference co-eluting with antidepressants.

The relative recoveries were performed at three spiked concentration levels of 5, 10 and 50 ng mL^{-1} by adding standard solution into real samples. For each sample, the extraction was repeated for three times. Relative recoveries and RSDs of the analytes were calculated and listed in Table 5. As shown in Table 5, the recoveries were in the range of 72-113 % for serum samples and 75-115 % for urine samples with the precisions (RSDs) lower than 4.75 %

for all compounds, indicating that the method is feasible for the determination of antidepressants in biological samples.

In order to confirm the viability, the proposed method was applied to the analysis of serum and urine samples from depressed patients receiving daily various antidepressant drug doses. The serum and urine samples were collected from depressed patients in therapy with Lustral tablets (50 mg day^{-1}), Efexor capsules (37.5 mg day^{-1}), Paxil tablets (25 mg day^{-1}), Prozac capsules (20 mg day^{-1}) and Losiram tablets (10 mg day^{-1}). Twenty-six real serum and urine samples were analyzed for the contents of antidepressant by the proposed method in triplicate and the most of the target compounds were determined. The concentrations of antidepressants determined in serum and urine samples are shown in Table 6. The LC-UV chromatograms of antidepressants isolated from serum and urine samples of patients treated with 37.5 mg of venlafaxine, 25 mg of paroxetine and 10 mg of fluoxetine per day are shown in Fig. 5.

4. Conclusion

In this study, Fe_3O_4-$MgSiO_3$ magnetic nanocomposite was successfully prepared by a simple co-precipitation of Fe^{+3} and Fe^{+2} in alkaline solution in the presence of $MgSiO_3$. Fe_3O_4-$MgSiO_3$ has been firstly applied in magnetic solid phase extraction as adsorbent for preconcentration of some antidepressants such as venlafaxine, escitalopram, paroxetine, sertraline and fluoxetine in biological samples prior to LC-UV. The strong adsorption ability of Fe_3O_4-$MgSiO_3$ nanocomposite result in high adsorption capacity, low limit of detection, and extraction efficiency to target compounds, therefore, satisfactory results are achieved using lower amount of nano material than common sorbents. Under the optimal extraction conditions, the proposed method displayed a good precision with RSDs < 4.9 % and reliable analytical results with spiked recoveries in the range of 72-115 %. LODs were in the range of

1.73- 2.83 ng mL^{-1}, which are better than or comparable with other reported approaches applied to the determination of the same compounds. Consequently, the presented method described in this study has been shown to be suitable with satisfactory accuracy and good reproducibility for the quantitative determination of five antidepressants at trace levels in biological samples.

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Figure Captions

Fig. 1. XRD patterns of (a) Fe_3O_4 magnetic nanoparticles and (b) Fe_3O_4-$MgSiO_3$ magnetic nanocomposite.

Fig. 2. SEM image with EDX of (a) Fe_3O_4 nanoparticles and (b) Fe_3O_4-$MgSiO_3$ magnetic nanocomposite.

Fig. 3. FT-IR spectra of (a) Fe_3O_4 and (b) Fe_3O_4-$MgSiO_3$ magnetic nanocomposite.

Fig. 4. 3D-response surface graphs and contour plots of venlafaxine. The effect of (a) extraction pH and adsorbent amount; (b) solvent volume and adsorbent amount; (c) solvent volume and extraction pH.

Fig. 5. Chromatograms of (A) serum and (B) urine samples, (a) non-spiked, (b) spiked with 10 ng mL^{-1} standards, and (c) 20 ng mL^{-1} standards, (C) biological samples from patients

subjected to therapy with drugs. (1) Venlafaxine, (2) Escitalopram, (3) Paroxetine, (4) Sertraline, (5) Fluoxetine.

Table 1. Central composite design factors, experimental range and levels (coded and uncoded) of the variables used in the experimental design.

Factors/levels	Variables	-α	-1	0	1	α
Adsorbent amount (mg)	X_1	4.0	8.0	14.0	20.0	24.0
Extraction pH	X_2	3.6	5.0	7.0	9.0	10.4
Solvent volume (mL)	X_3	0.3	1.0	2.0	3.0	3.7

Design points: center (0), cubic (−1, 1), axial (−α, α).

Table 2. Actual and predicted responses of central composite design matrix.

	Coded variables			Venlafaxine		Escitalopram		Paroxetine		Sertraline		Fluoxetine	
Run No.	X_1	X_2	X_3	Response Actual	Response Predicted	Response Actual	Response Predicted	Response Actual	Response Predicted	Response Actual	Response Predicted	Response Actual	Response Predicted
1	-1	-1	-1	176688	171575	144809	144096	167845	165311	86315	89629	157817	159610
2	-1	-1	1	87698	86813	73149	74857	71457	71843	89720	87718	76970	77036
3	-1	1	-1	219267	215372	151428	153967	205933	206560	218954	212773	199784	194162
4	-1	1	1	138587	134919	146135	143094	94206	95901	125830	126163	92078	85498
5	1	-1	-1	121591	115916	243112	246839	128901	128169	129927	127627	171949	171920
6	1	-1	1	75241	69794	111211	109358	90505	90841	90471	94685	99365	98379
7	1	1	-1	181015	172558	185163	184140	209185	209763	193852	193887	219316	212641
8	1	1	1	134974	130744	103628	105026	151746	155244	81527	76246	121412	113010
9	-1.682	0	0	217685	221245	159954	159991	204568	204929	194348	196097	191926	194890
10	1.682	0	0	161279	170932	215382	214376	225323	223599	185041	186074	221995	228377
11	0	-1.682	0	58687	64362	129969	128594	66180	68158	30668	27802	67511	63824
12	0	1.682	0	144908	152444	132845	133251	160341	157001	110201	115849	92148	105182
13	0	0	-1.682	171006	180261	209786	207423	167800	169490	180718	182822	223577	226654
14	0	0	1.682	69867	73825	81279	82673	48101	45048	81612	82291	67169	73438
15	0	0	0	253420	253195	306623	306254	214887	214764	220287	226309	308729	308563
16	0	0	0	253422	253195	306635	306254	214768	214764	229083	226309	308732	308563
17	0	0	0	253421	253195	306646	306254	214121	214764	227498	226309	308726	308563
18	0	0	0	253435	253195	306641	306254	214887	214764	216969	226309	308719	308563
19	0	0	0	253426	253195	306626	306254	215769	214764	229083	226309	308735	308563
20	0	0	0	253428	253195	306635	306254	214121	214764	227486	226309	308724	308563
21	0	0	0	253420	253195	306249	306254	212882	214764	229083	226309	308711	308563
22	0	0	0	253431	253195	306327	306254	213765	214764	227498	226309	308715	308563
23	0	0	0	253419	253195	303631	306254	214121	214764	229083	226309	308716	308563
24	0	0	0	253427	253195	306357	306254	218089	214764	227492	226309	308723	308563

Table 3. ANOVA test and regression analysis for the quadratic model.

ANOVA test					Regression analysis				
Source	SS[a]	DF[b]	MS[c]	F		Coefficients	Std. Error	T-test	*p*-value
Model	2.61E+10	9	8.70E+09	80.26	b_0	-576858	32418.19	-17.7943	0.0000
X_1	3.060E+09	1	3.06E+09	84.96	b_1	8118	1855.69	4.3744	0.0006
X_1^2	6.61E+09	1	6.61E+09	18.88	b_3	187442	6199.31	30.2359	0.0000
X_2	9.37E+09	1	9.37E+09	260.39	b_5	120453	10846.82	11.1049	0.0000
X_2^2	4.25E+10	1	4.25E+10	1182.05	b_2	-561	41.36	-13.5602	0.0000
X_3	1.37E+10	1	1.37E+10	380.23	b_4	-12798	372.24	-34.3810	0.0000
X_3^2	3.23E+10	1	3.23E+10	897.30	b_6	-44602	1488.96	-29.9550	0.0000
X_1*X_2	8.25E+07	1	8.25E+07	2.29	b_7	268	176.69	1.5145	0.1522
X_1*X_3	7.47E+08	1	7.47E+08	20.76	b_8	1610	353.38	4.5559	0.0005
X_2*X_3	9.28E+06	1	9.28E+06	0.26	b_9	539	1060.15	0.5081	0.6193
Error	5.04E+08	14	3.59E+07						
Total	1.11E+11	23	**R^2:** 0.996						

[a] Sums of squares; [b] Degree of freedom; [c] Mean square.

Table 4. Analytical performance of the proposed method for the determination of antidepressants in serum and urine samples.

Antidepressants	Linear Range (ng mL^{-1})	R^2	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	Intra-day RSD	Inter-days RSD	EF [a]	EF[b]	ER [a]	ER[b]
Venlafaxine	10-500	0.9994	1.97	5.92	3.79	4.17	3.6	4.9	71	73
Escitalopram	10-500	0.9993	2.83	8.53	2.08	2.39	3.6	4.9	72	74
Paroxetine	10-500	0.9986	2.17	6.13	3.32	3.41	3.5	4.8	70	72
Sertraline	10-500	0.9991	1.83	6.11	4.90	5.23	3.8	5.2	76	78
Fluoxetine	10-500	0.9994	1.73	5.21	2.97	3.40	4.4	6.0	87	90

[a]: serum; [b]: urine

Table 5. Recovery (%) and repeatability (RSD) of the proposed method for the antidepressants in samples spiked at different levels.

Antidepressants	Concentration Added (ng mL^{-1})	Serum			Urine		
		Concentration Measured (ng mL^{-1})	Recovery (%)	RSD	Concentration Measured (ng mL^{-1})	Recovery (%)	RSD
Venlafaxine	5	3.75	75	3.04	3.9	78	4.75
	10	8.72	87	2.69	9.2	92	2.90
	50	47.3	96	2.14	51	102	2.68
Escitalopram	5	4.16	83	3.08	4.25	85	4.22
	10	8.53	85	2.24	8.62	86	2.43
	50	44.52	89	1.53	44.65	89	1.64
Paroxetine	5	3.63	72	3.12	3.75	75	2.85
	10	7.71	77	2.86	8.1	81	3.14
	50	42.16	84	2.51	44.0	88	2.90
Sertraline	5	4.09	81	1.76	4.15	83	2.25
	10	8.83	88	1.42	9.1	91	2.89
	50	50.57	101	1.84	52	104	3.15
Fluoxetine	5	4.47	89	1.81	4.55	91	2.24
	10	9.66	97	1.63	10.3	103	2.45
	50	56.61	113	1.88	57.5	115	2.78

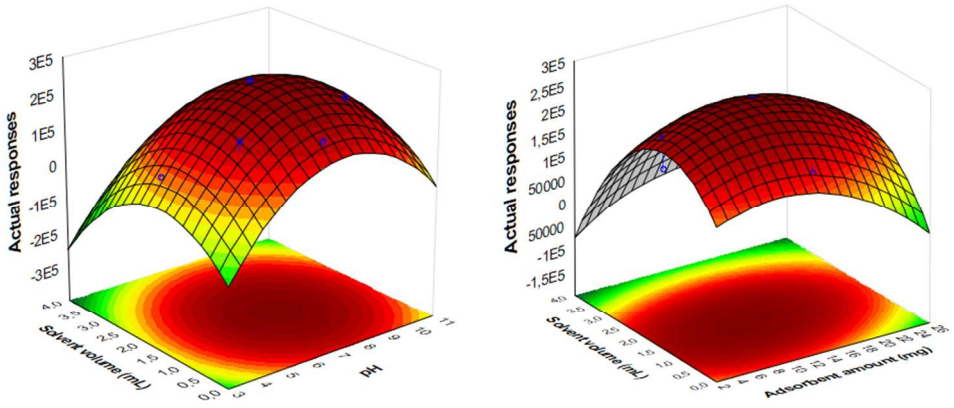
Experiment number: 3

Table 6. The concentrations of antidepressants determined in serum and urine samples.

Compounds	Serum (ng mL^{-1})					Urine (ng mL^{-1})				
	N	Min.	Max.	Mean	SD	N	Min.	Max.	Mean	SD
Venlafaxine	6	29.1	315.6	176.7	106.1	6	< LOD	238.8	205.8	38.6
Escitalopram	6	17.7	210.9	90.3	69.5	6	< LOD	101.2	46.8	36.2
Paroxetine	5	17.1	124.4	54.2	39.2	5	6.19	65.6	24.1	21.7
Sertraline	4	59.7	149.2	94.1	34.9	4	51.53	195.8	100.2	57.5
Fluoxetine	5	72.5	261.7	152.7	66.7	5	14.56	140.7	65.2	47.4

N: number of samples including compounds

SD: standard deviation; Min.: minimum; Max.: maximum



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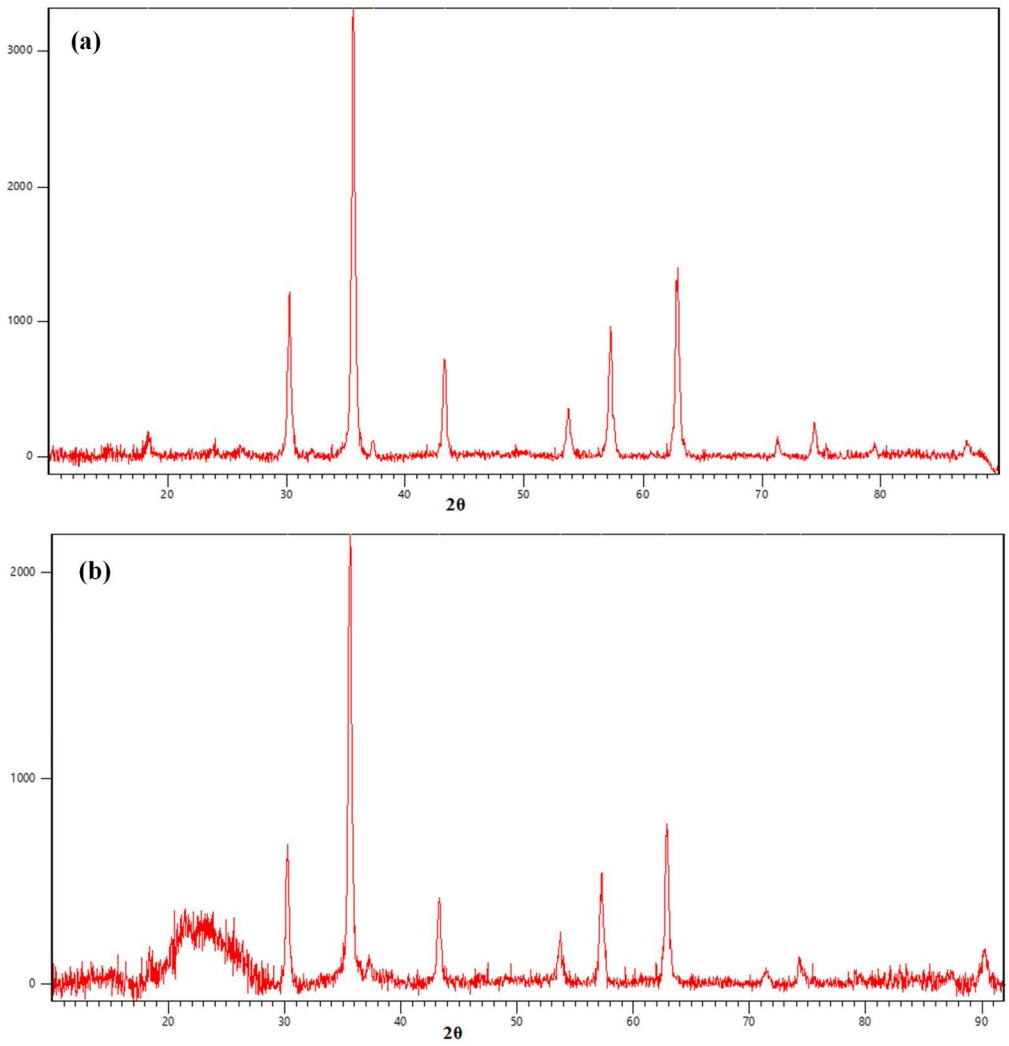

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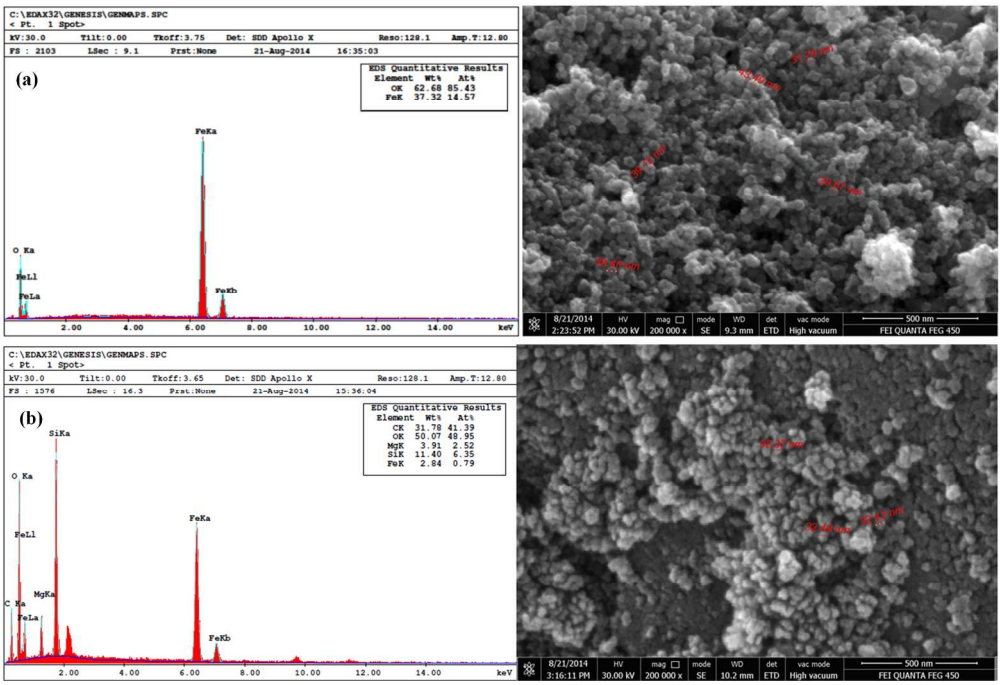

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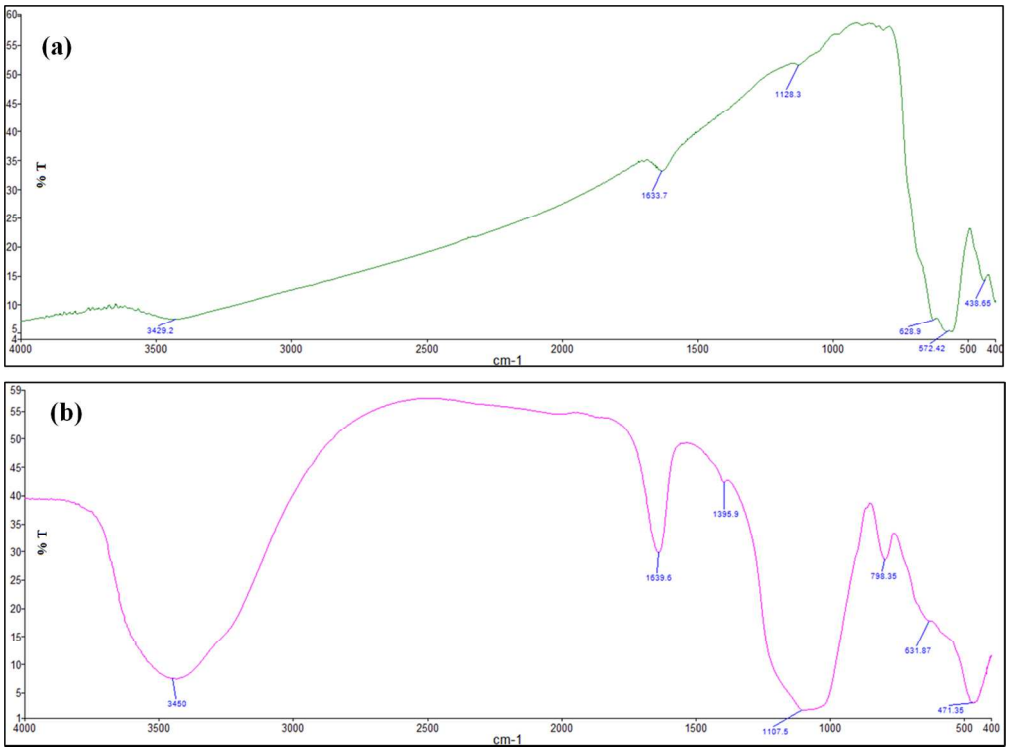

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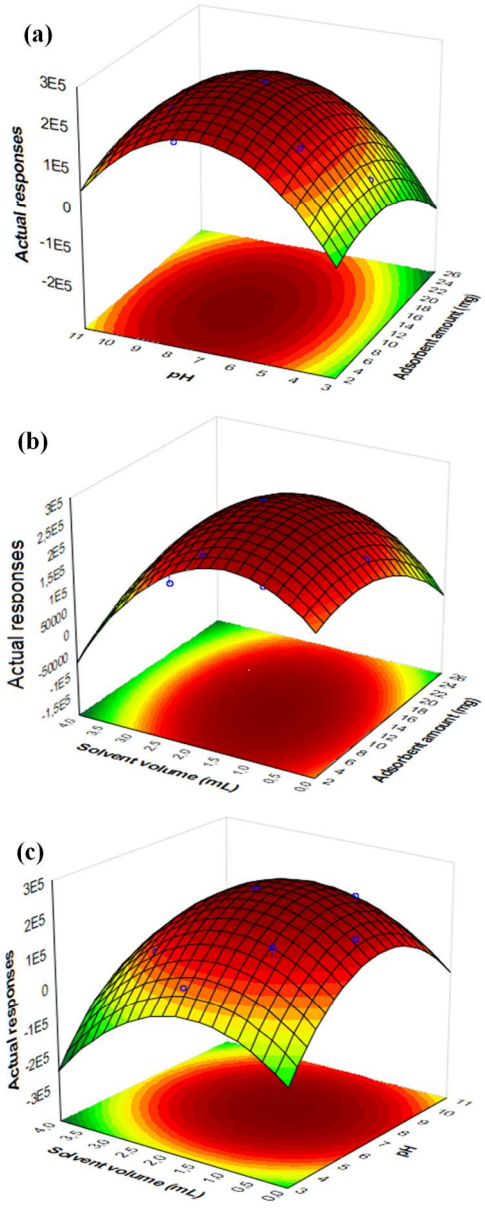

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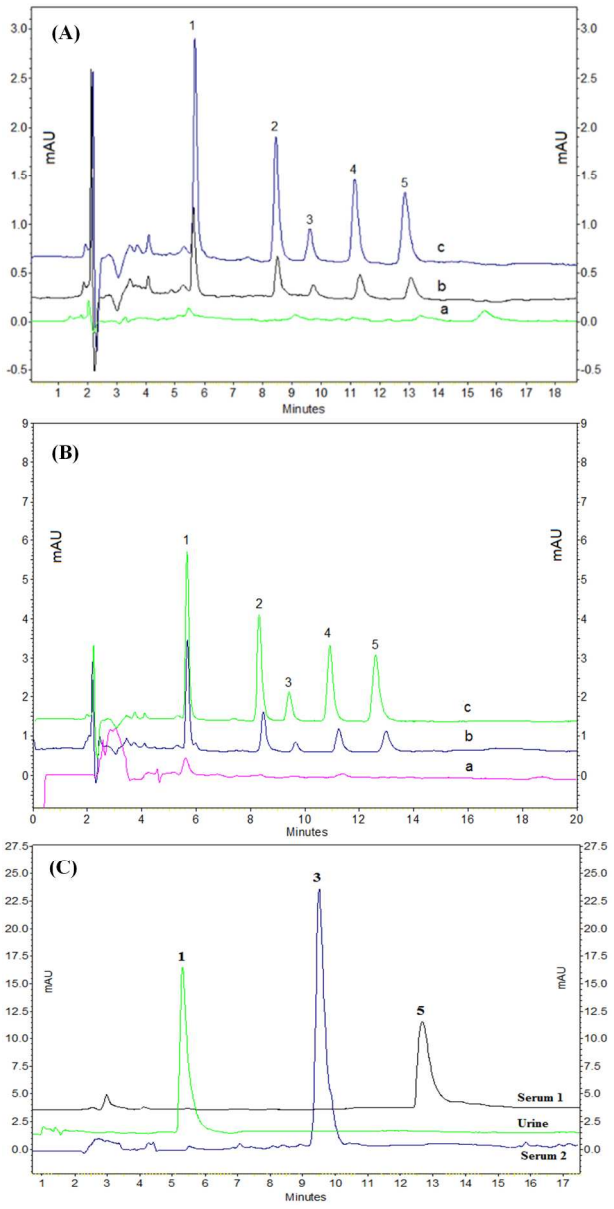

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