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Preconcentration and Determination of Theophylline in Water Samples using Magnetic Nano-Cellulose with Dispersive Solid Phase Extraction

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Abstract

Theophylline (1,3-dimethyl-2,3,6,7-tetrahydro-1-hydrogen-purine-6,2-dione) is a bronchodilator drug used in the treatment of asthma and chronic obstructive pulmonary disease (COPD). Due to the narrow therapeutic strip of this drug, it is important to measure its blood level.To achieve a simple, safe and sensitive method based on dispersive solid phase extraction (DSPE) using magnetite nanoparticles covered with nanocellulose and beta-cyclodextrin for preconcentration and measurement of theophylline in aqueous samples and its validation, this method was used. In this research, variables affecting the extraction such as pH, ionic strength, and amount of adsorbent, temperature, time, limit volume and type of solvent were optimized. After extraction, the adsorbent was easily separated from the aqueous sample using an external magnetic field without filtration or centrifugation.

The results showed that optimum conditions were obtained with pH=5, 1 mg of sodium chloride, 0.2 g of adsorbent with a capacity of 0.53 mg/g, temperature of 40 degrees Celsius,

20 minutes of contact between the adsorbent and the sample and 5 mL of methanol as the desorption solvent. In optimal conditions, the suitable concentration factor is 12, low detection limit (0.17 mg/L), wide linear range and in accordance with the therapeutic strip of the drug (5-50 mg/L), good correlation coefficient (R2=0.9991) and good accuracy (RSD=1.03%) was obtained.Urine sample was used to validate the above method. Also, it was found that the proposed method is suitable, efficient and usable for measuring of theophylline.

Key words: Theophylline, Dispersive Solid Phase Extraction, Preconcentration, Measurement, Magnetic nanoparticles, Nanocellulose.

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Introduction

Theophylline (1,3-dimethylxanthine, Figure 1) is widely used as a bronchodilator in the treatment of asthma and chronic obstructive pulmonary disease; Because it is effective and relatively cheap. There is a strong correlation between bronchodilator effects and blood levels of theophylline. It is very important to keep its blood level between 10-20 mg/L for maximum bronchodilator effect. Its serious side effects occur in blood concentrations higher than 20 mg/L, and its effectiveness decreases in blood concentrations lower than 10 mg/L. In addition to the narrow therapeutic strip, changes in theophylline pharmacokinetics due to individual differences, other drugs, diet, and habits such as smoking and alcohol consumption have also led to the need for a simple, sensitive, and reproducible method to determine the amount of theophylline and its metabolites [1].

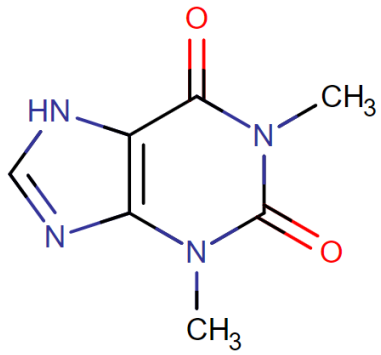

Figure 1. Structure formula of Theophylline.

Theophylline was first extracted from tea leaves and its chemical structure was discovered in 1888 [2]. Theophylline, theobromine and caffeine are three important methylxanthines. Their main sources are tea, cocoa and coffee respectively. Among the xanthines, theophylline is the most effective bronchodilator and has been repeatedly shown to relieve airway obstruction in acute asthma [3].

Theophylline should only be used where there are facilities to measure its blood level because this drug has a narrow therapeutic strip and its therapeutic and toxic effects are related to the blood level of the drug. Improvement of pulmonary function is achieved with plasma concentration in the range of 5-20 mg/L. Anorexia, nausea, vomiting, abdominal discomfort, headache and anxiety are common at concentrations of 15 mg/L and in some patients at concentrations higher than 20 mg/L. Higher levels (<40 mg/L) can cause seizures or arrhythmias that may not be preceded by any gastrointestinal or neurological warning signs.

Therefore, the need to develop sensitive bioanalytical methods for the quantitative determination of this drug is well felt [4].

According to reference USP41, HPLC method has been used to measure theophylline, which absorbs light in the range of 270 nm and is identified and measured in this way [5]. Also, different methods have been described to determine the amount of theophylline in different pharmaceutical forms [6-10].

Until today, several analysis methods have been used to determine the amount of theophylline. In addition to these, electrochemical methods have also attracted the attention of some researchers; because they give a quick answer; are cheap; they have high sensitivity and include simple operations. However, they do not have a low detection limit and cannot measure small amounts. Due to the high preconcentration factor, low cost, low solvent consumption, simplicity and flexibility, solid phase extraction is considered as a very effective and efficient method for preconcentration [8]

But the limitation of this method is revealed when the concentration of the target compound in the sample is very low. Compared with the traditional solid phase extraction method, magnetic solid phase extraction can be effectively used to purify small amounts of the target compound. The magnetic solid phase extraction method is a new method for preconcentration of the target analyte in large volumes, based on the use of magnetic adsorbents. The separation process can be done directly in the sample solution containing the magnetic solid adsorbent, which is collected and separated from the liquid phase with the help of a magnetic field; with this technique, there is no need for a filtration or centrifugation process. Diffusion solid phase extraction is a solid phase extraction in which the solid adsorbent is dispersed in the sample solution. In this type of extraction, the contact surface is increased compared to traditional solid phase extraction. Therefore, to preconcentrate small amounts of the drug, diffusion solid phase extraction technique using magnetic adsorbent is preferable and superior compared to traditional solid phase extraction [9].

Recently, nanotechnology is widely used in the field of analytical chemistry; the unique properties of nanomaterials, such as excellent distribution in solution and its wide surface area, have made it possible to design new methods for chemical analysis. Nanoparticles such as magnetic iron oxide have many applications in drug delivery, wastewater treatment and chemical separation. The main reason for using magnetic iron oxide nanoparticles as adsorbent in the solid phase dispersion extraction method is that these particles are easily collected by the magnetic field. Therefore, combining the magnetic field with the surface adsorption

characteristics can create an excellent material for the separation of chemicals in the solid phase extraction method [8].

Now, considering the need to measure the blood level of theophylline drug and the advantages mentioned for the diffusion solid phase extraction method using magnetic nano-adsorbent, which is a fast, easy and low-cost method, in this article, biodegradable, safe, cheap and innovative adsorption and Using magnetite, crystalline nanocellulose and beta-cyclodextrin were synthesized so that small amounts of theophylline drug could be preconcentrated and extracted and then measured in a wider linear range.

Experimental

Chemicals

All of the chemicals and reagents were prepared from‹Merck‹ Zibo Senlos Chem., Shandong and Sigma-Aldrich companies.

Apparatus

Analytical balance (Bosch, SAE200), Ben-Marie (Memmert, WNB 14), pH meter (AZ, 86502), UV-Vis (RIGOL, Ultra-3660), FTIR (Shimadzu, 8400S), XRD (Panalytical, X'Pert Pro) and FESEM (ZEISS, Sigma VP) were from Germany, England, Japan, China and Taiwan.

Preparation of magnetite/nanocellulose/betacyclodextrin nanoparticles

In this research, co-precipitation method was used to make magnetite nanoparticles. The advantage of this method is that it is cheap and not time-consuming. In this method, Fe^{2+} and Fe^{3+} ions in the presence of OH^- create magnetite precipitation under the following reaction:

$$2Fe^{3+} + Fe^{2+} + 8OH^- \rightleftarrows 2Fe(OH)_3 + Fe(OH)_2 \rightarrow Fe_3O_4\downarrow + 4H_2O$$

In this research, by adding ammonia to iron salts, the pH of the solution increases to about 11, at this pH, Fe^{2+} and Fe^{3+} ions are converted into iron hydroxides, which, according to the following reaction, with dehydration, magnetite is obtained.

$$2FeOOH + Fe(OH)_2 \rightleftarrows Fe_3O_4\downarrow + 2H_2O$$

$$2Fe(OH)_3 + Fe(OH)_2 \rightleftarrows Fe_3O_4\downarrow + 4H_2O$$

Synthesis of magnetite/nanocellulose nanoparticles

0.08 g of nanocellulose, 0.2 g of ferric chloride salt 6 water and 0.432 g of ferrous chloride salt 4 water were weighed and dissolved in 40 ml of deionized water. The mentioned solution is immediately placed in a bain-marie bath at a temperature of 50°C for 20 minutes. After removing the container from the bain-marie and cooling it, the solution is dispersed for 10 minutes by an ultrasonic device with a probe. In the next step, 3 ml of ammonia is added to the obtained solution, which will immediately cause a black precipitate to form. Then the container is placed in a bain-marie at a temperature of 50°C for 40 minutes. In the last step, after cooling the container, the magnetic sediment is washed and decanted several times by applying an external magnetic field by a magnet and using deionized water until the supernatant solution becomes completely clear. Then the sediment is transferred to a porcelain crucible and dried in an oven at a temperature of 35°C.

Synthesis of β-cyclodextrin modified magnetite/nanocellulose nanoparticles

0.8 g of beta-cyclodextrin is weighed and dissolved in some deionized water. Then this solution is made up to 100 ml with deionized water in a volumetric flask to obtain a beta-cyclodextrin solution with a concentration of 8 mg/mL. The sediment obtained from the previous step is rubbed in the mortar and turned into powder. Then, 100 ml of 8 mg/mL beta-cyclodextrin solution is added to it and dispersed for 5 minutes under an ultrasonic device with a probe. After this step, the container containing the mixture of beta-cyclodextrin and magnetite/nanocellulose is placed in a bain-marie at a temperature of 50°C for 3.5 hours. After this time has passed and the container has cooled down, the resulting sediment is washed and decanted several times by applying an external magnetic field by a magnet and using deionized water until the supernatant solution becomes completely transparent. Then the sediment is transferred to a porcelain crucible and dried in an oven at a temperature of 35°C. After the sediment is completely dried and ground in a mortar until it becomes a powder with fine and uniform particles, the nanocellulose magnetite/betacyclodextrin magnetic adsorbent is ready for use.

Preparation of required solutions

Theophylline stock solution

To prepare a stock solution with a concentration of 600 ppm of the drug, 0.06 grams of theophylline is weighed and made up to volume in a 100 ml volumetric flask with deionized water. This solution is kept at refrigerator temperature.The required solutions of acetic,

phosphoric, hydrochloric acid, sodium hydroxide, ammonia, buffer with different pH were also prepared according to standard methods.

Optimizing test conditions

Magnetite/nanocellulose/betacyclodextrin nanoparticles absorb theophylline with the highest efficiency when the influencing factors on the absorption process are optimized. Therefore, it was necessary to optimize the variables affecting absorption in this research. Variables affecting the absorption process of theophylline by the mentioned magnetic nano adsorbent which were investigated and optimized in this article are respectively: pH, ionic strength by adding NaCl, adsorbent amount, contact time of adsorbent and sample, temperature, type of desorption solvent, volume of desorption solvent and Volume limit. The univariate method was used to optimize these variables; this means that only one of the effective factors was changed and the rest of the factors were kept constant. In this way, in each step, the optimized factor value was used in the next tests. UV-Vis analysis was used to analyze the samples and measure the amount of theophylline.

Wavelength for measuring drugs by UV-Vis

For this purpose, a dilute solution with a concentration of 17 ppm is prepared from the stock solution of theophylline with a concentration of 600 ppm and UV-Vis absorption in the range of 200-700 nm for the target sample. According to the absorption values, λ max of the drug is obtained.

Results

Spectra and confirmatory tests of the synthesized adsorbent and the binding of the drug to it, in three stages, from samples of magnetite/nanocellulose adsorbent (before the addition of beta-cyclodextrin), magnetite/nanocellulose/betacyclodextrin adsorbent and adsorbent with theophylline drug attached to it, FTIR, XRD, EDS and FESEM image were taken and the desired results were observed. Also, two samples, i.e. magnetite alone and magnetite/nanocellulose/betacyclodextrin, were subjected to VSM analysis.

XRD

Peaks 30, 35, 43, 57, and 63 prove the presence of Fe3O4 magnetic nanoparticles, which are stable in all three phases and prove that its phase does not change during the processes of

modification with beta-cyclodextrin and drug binding to the nanosorbent. Diffraction peaks around 16 and 22 indicate the presence of nanocellulose. Spectrum (b) shows the diffraction pattern of magnetic nanocellulose functionalized with beta-cyclodextrin, the increase in oscillations and the number of very small peaks on the background line of diffraction in the functionalized nano-absorbent shows that the absorption of beta-cyclodextrin has been done on the nano-absorbent. In other words, FTIR analysis confirms that beta-cyclodextrin groups are successfully placed on the magnetic nanoparticle substrate, and XRD analysis confirms that the Fe_3O_4 phase is preserved during this process. In spectrum (c), peaks 7, 8 and 13 indicate the addition of anhydrous theophylline (Figure 1).

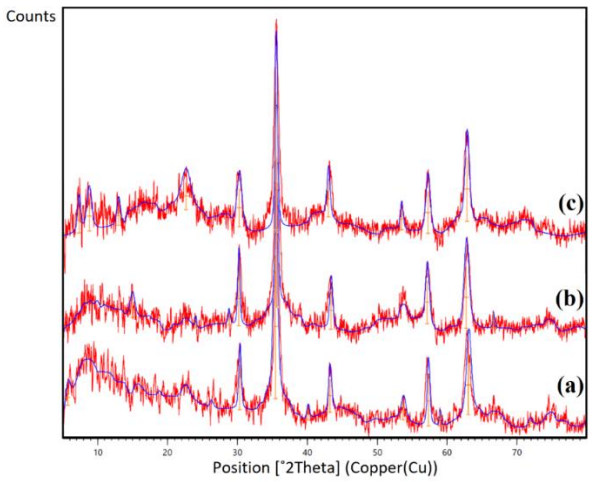

Figure 1. a) XRD spectrum of magnetite/nanocellulose b) XRD spectrum of magnetite/nanocellulose/betacyclodextrin c) XRD spectrum of magnetic nanoabsorbent with theophylline drug.

Field Emission Scanning Electron Microscopy (FESEM)

The synthesized magnetic nanoparticles were examined by field emission scanning electron microscope to determine the morphology, shape and estimation of particle size. The first image is of the magnetite/nanocellulose adsorbent before the addition of beta-cyclodextrin, which shows that the particles are spherical in shape and their average diameter is approximately 42.9 nm. After adding beta-cyclodextrin, as can be seen, a dense layer was placed on the particles, which increased the average diameter of the particles to about 49 nm. It was mentioned earlier in the definition of nano particles that one of their dimensions is on the nanometer scale (below 100 nm). According to the diameter of the particles, the synthesized adsorbent is placed in the category of nanoparticles. (Figures 2, 3)

Figure 4 relates to the binding of theophylline drug to magnetite/ nanocellulose/ betacyclodextrin nanosorbent; the average diameter of the particles in this image is about 53

nm. In addition to the increase in the average diameter of the particles, the change in the appearance of the particles is also evident, which have changed from spherical particles with a polished surface to particles with rough surfaces.

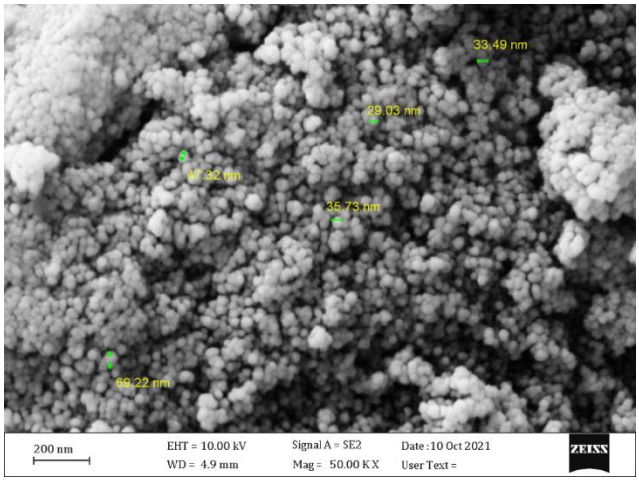

Figure 2. FESEM image of magnetite/nanocellulose particles.

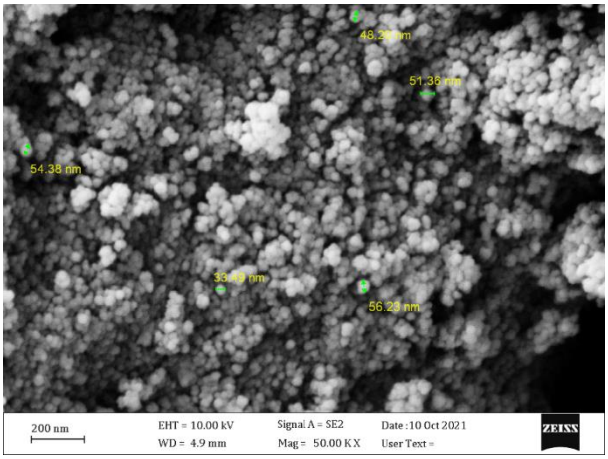

Figure 3. FESEM image of magnetite/nanocellulose/betacyclodextrin particles.

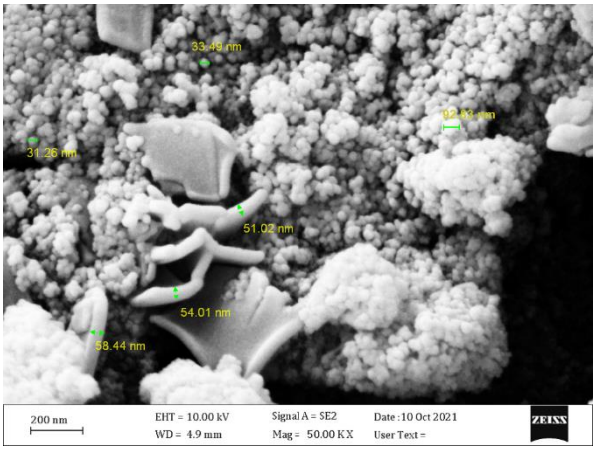

Figure 4. FESEM image of magnetic nanoparticles with theophylline drug.

X-ray Energy Diffraction Spectroscopy (EDS)

EDAX analysis or EDS (X-ray Energy Diffraction Spectroscopy) is an add-on to SEM devices to determine the percentage of elements in solid samples. This analysis can determine the type of element and its weight or atomic percentage by using the unique X-ray energy emitted from the sample. EDAX analysis has unique capabilities compared to other elemental determination methods such as XRF and ICP. This method can detect light elements such as oxygen and carbon or halogens. As a result, it has a great advantage for estimating these elements in especially organic samples.

According to the chemical formula of Fe_3O_4 and nanocellulose ($x(C_6H_{10}O_5)$), iron, oxygen and carbon elements should be present in the EDS analysis of magnetite/nanocellulose nanoabsorbent. There are also carbon, oxygen and hydrogen elements in the structure of beta-cyclodextrin. As can be seen in the analysis of magnetite/nanocellulose nanosorbent, before and after the addition of beta-cyclodextrin, the presence of iron, oxygen and carbon elements in the samples has been confirmed. After connecting the theophylline drug to the magnetic nanoabsorbent, the nitrogen present in the theophylline structure should be added to the list of elements in the sample. According to the EDS analysis of the third sample, the addition of theophylline to the magnetite/nanocellulose/betacyclodextrin adsorbent is confirmed (Figures 5-7).

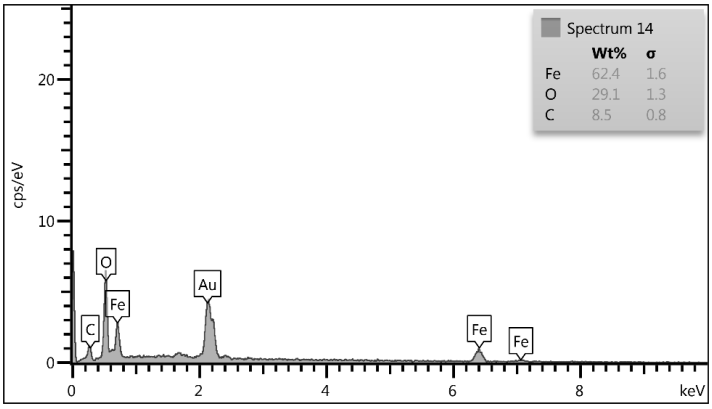

Figure 5. EDS analysis of magnetite/nanocellulose sample.

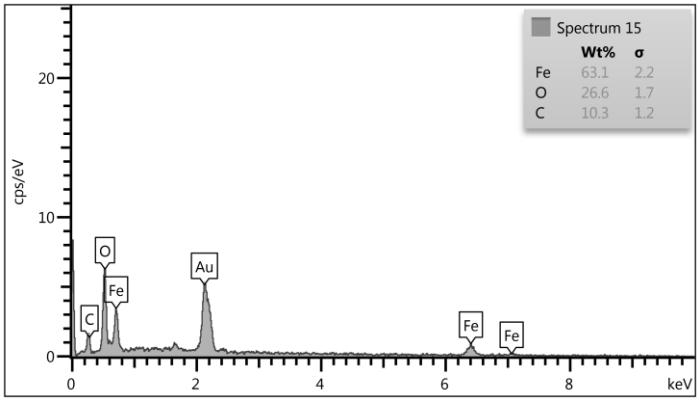

Figure 6. EDS analysis of magnetite/nanocellulose/betacyclodextrin sample.

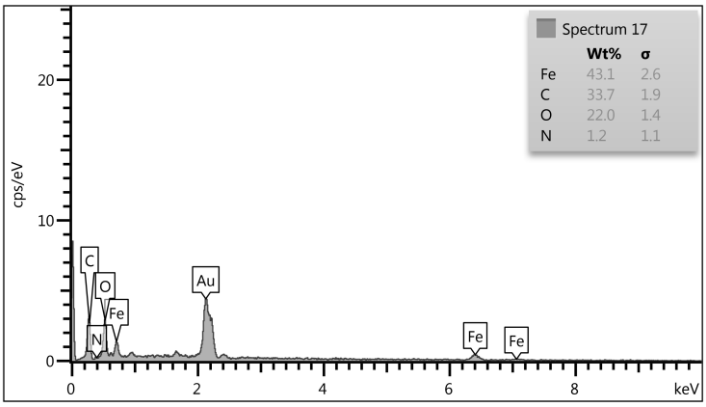

Figure 7. EDS analysis of magnetite/nanocellulose/betacyclodextrin magnetic nanoabsorbent sample with theophylline drug.

VSM

Figure 8 shows the magnetic properties of magnetite and magnetite/nanocellulose/betacyclodextrin nanoparticles obtained by applying a magnetic field at a temperature of 298 degrees Kelvin. The saturation magnetization of magnetite nanoparticles is equal to 42 emu/g and for magnetite/nanocellulose beta-cyclodextrin magnetic nanoabsorbent is equal to 12 emu/g. The reduction of saturation magnetization in the synthesized magnetic nanoabsorbent is due to the coating of nanocellulose and beta-cyclodextrin around the magnetite. All samples show very small hysteresis loop and low coercivity. These results state that the superparamagnetic structure of magnetite has not changed in the magnetite/betacyclodextrin nanocellulose magnetic nanoabsorbent and the synthesized magnetic nanoabsorbent has a favorable behavior due to the application of an external magnetic field.

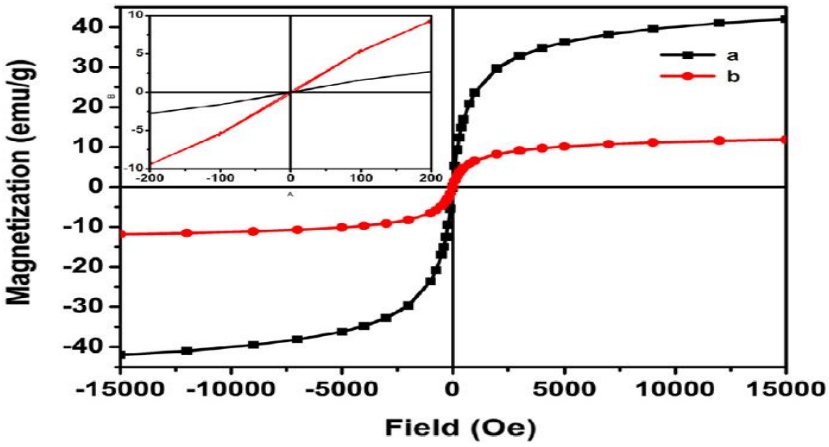

Figure 8. a) VSM diagram of magnetite b) VSM diagram of magnetite/nanocellulose/betacyclodextrin.

Optimization of diffusion solid phase extraction method for preconcentration and measurement of theophylline drug in aqueous samples by magnetic nanocellulose adsorbent

Wavelength for measuring drugs by UV-Vis

According to the result of the UV-Vis spectrum of the theophylline solution with a concentration of 17 ppm in the region of 200-700 nm, the maximum absorption was seen at the wavelength of 270 nm, which corresponds to the λ max of the drug in the USP pharmacopoeia monograph (Figure 9).

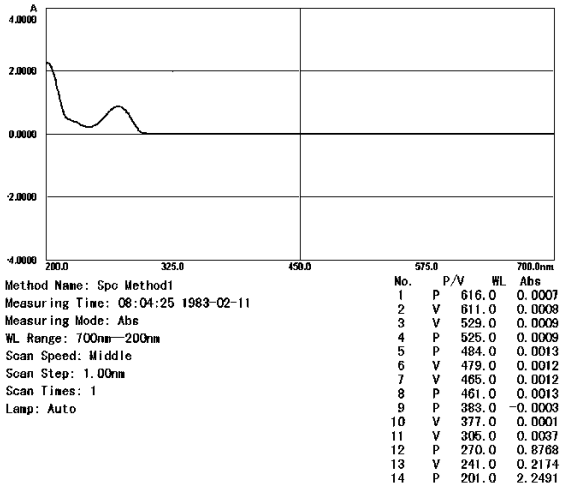

Figure 9. UV-Vis spectrum of theophylline drug.

Effect of pH

In order to check the effect of pH, the pH of the solution in the range of 3 to 11 is checked and the results are given in the graph. As can be seen, the adsorption efficiency reached the maximum at pH = 5 and decreases before and after this pH. Therefore, pH = 5 was chosen for further studies (Figure 10).

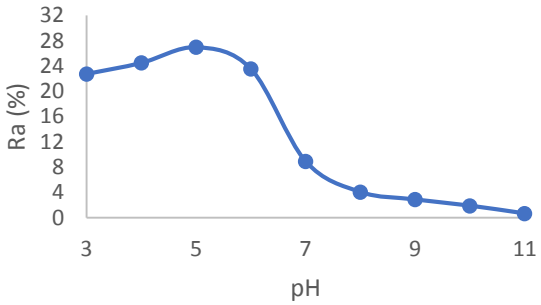

Figure 10. Optimization of pH effect.

Effect of ionic strength

In order to investigate the effect of ionic strength, the absorption efficiency for an aqueous solution containing theophylline drug with a concentration of 17 ppm at pH=5, by adding NaCl in amounts of 0.5, 0.75, 1, 1.25, 1.5, 2 and 5 mg was measured. As shown in the graph, absorption is maximized with the addition of 1 mg of NaCl (Figure 11).

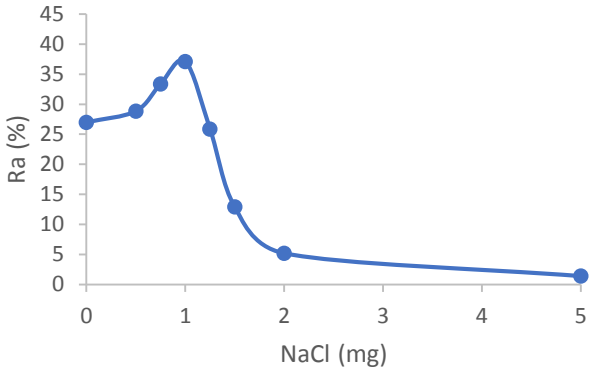

Figure 11. Optimizing the effect of ionic strength by adding sodium chloride salt.

Effect of amount of absorbent

In order to investigate the effect of the amount of adsorbent, the absorption efficiency for 8 samples of aqueous solution containing theophylline drug with a concentration of 17 ppm at pH=5 and with 1 mg of NaCl, by adding the adsorbent in amounts of 0.02, 0.05, 0.07, 1 0.0, 0.15, 0.2, 0.3 and 0.5 grams were measured. As can be seen in the graph, the absorption

efficiency gradually increased with the increase of the amount of adsorbent from 0.02 to 0.2 grams and then remained constant in the range of 0.2 to 0.5 grams. To ensure the extraction of small amounts of analyte from the sample solution, the amount of 0.2 grams of adsorbent was chosen as the optimal amount. At this stage, the capacity of the adsorbent was also calculated according to the amount of adsorbent, and the maximum capacity in the amount of 200 mg of adsorbent was equal to 0.53 mg/g (Figures 12 , 13).

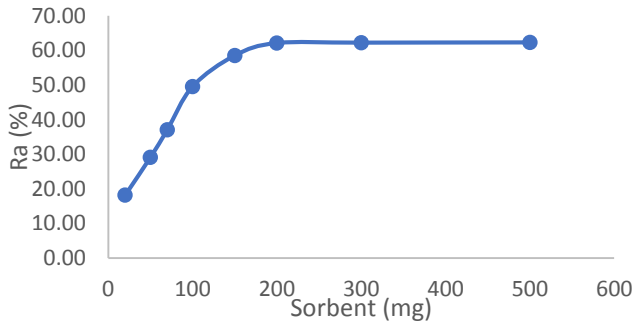

Figure 12. Optimizing the effect of absorber amount.

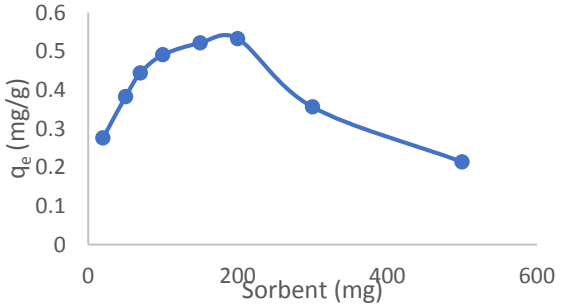

Figure 13. Absorbent capacity according to the amount of absorbent.

Effect of temperature

In order to investigate the effect of temperature, 4 samples of aqueous solution containing theophylline drug with a concentration of 17 ppm at pH=5 and 1 mg of NaCl were prepared and 0.2 g of magnetic nanoabsorbent was added to each. Then, the absorption efficiency of each sample was measured by exposure to temperatures of 15, 25, 30 and 40 degrees Celsius. The graph drawn using the results of this experiment shows that the absorption efficiency increases from 61.65% to 62.84% from 15 to 40 °C. To increase the efficiency of drug absorption and ensure the extraction efficiency is maximized, the temperature of 40 °C was chosen as the optimal temperature. It should be noted that due to the possibility of destruction of the magnetic nanoabsorbent modified with nanocellulose and beta-cyclodextrin and the

structure of the drug at temperatures higher than 40 degrees Celsius, increasing the temperature up to this point was limited (Figure 14).

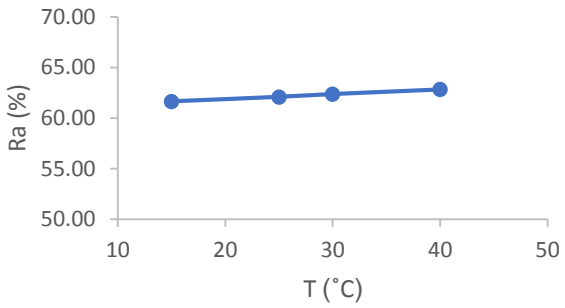

Figure 14. Optimizing the effect of temperature.

Examining the adsorption isotherm model

To find the adsorption isotherm model, the adsorption process for 7 aqueous samples containing concentrations of 5, 7, 9, 11, 13, 15 and 17 ppm of theophylline, with pH = 5 and 1 mg of NaCl, at 40 °C and with 2/ 0 g of adsorbent was done. According to R2 in each of the above graphs, which indicates the degree of correlation, the adsorption isotherm model was more consistent with the Freundlich isotherm (Figures 15-17 and Table 1).

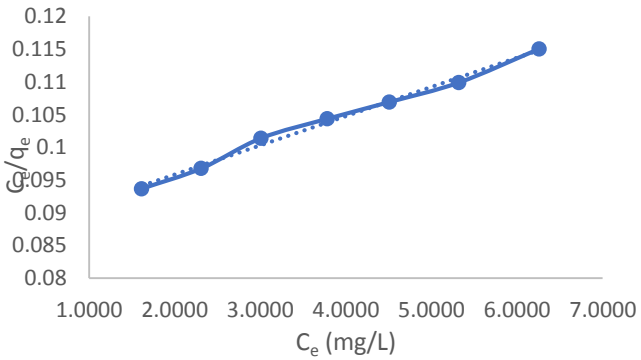

Figure 15. Investigation of the Langmuir adsorption isotherm.

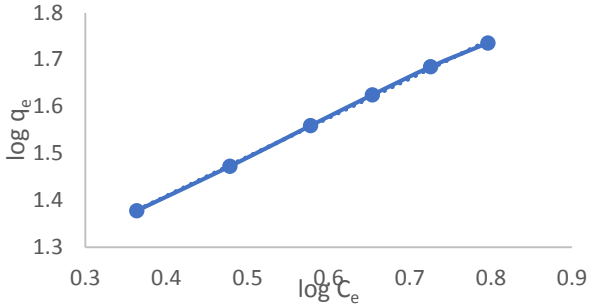

Figure 16. Examination of the Freundlich adsorption isotherm.

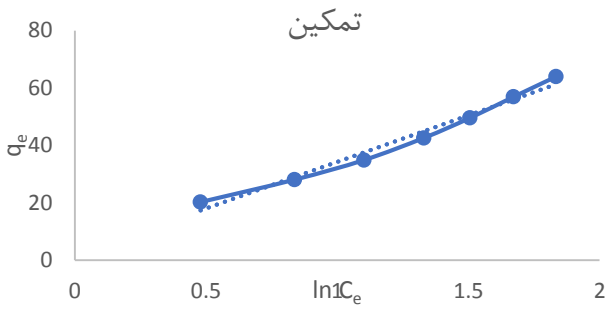

Figure 17. Examination of Temkin adsorption isotherm.

Table 1. Parameters of adsorption isotherm models.

222.22	**q_{max}**	**Langmuir isotherm parameters**
0.05	K_L	
0.79-0.53	R_L	
0.9926	R^2	
11.87	K_F	**Freundlich isotherm parameters**
1.20	n_F	
0.9994	R^2	
0.001	A_T	**Temkin isotherm parameters**
0.09	B_T	
0.9791	R^2	

Effect of sample and absorbent contact time

In order to investigate the effect of the duration of contact between the magnetic nanosorbent and the analyte on the absorption efficiency, the absorption efficiency for 8 10 ml water samples containing theophylline with a concentration of 17 ppm, with pH = 5 and 1 mg of

NaCl, at a temperature of 40 degrees Celsius and with 0.2 g The absorbance was calculated while each of the samples were in contact with the magnetic nano-absorbent for 2, 5, 7, 10, 15, 20, 25 and 30 minutes, respectively. As shown in the graph, the absorption efficiency increased gradually from 2 minutes to 20 minutes and reached from 11.61% to 62.84% and then remained almost constant, which indicates that the contact time of more than 20 minutes has an effect. It has no absorption efficiency. Therefore, the contact time of 20 minutes was determined as the optimal time for contact between the magnetic nanoabsorbent and theophylline drug.

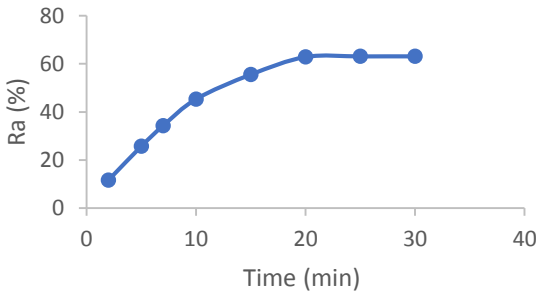

Figure 18. Time effect optimization.

Type of solvent and absorption

The proper selection of solvent and adsorbent is an important factor that affects the MSPE process. A suitable solvent can effectively wash the surface adsorbed analyte with the smallest volume of solvent. In order to choose the appropriate desorption solvent, desorption process was carried out under the influence of 5 different solvents.

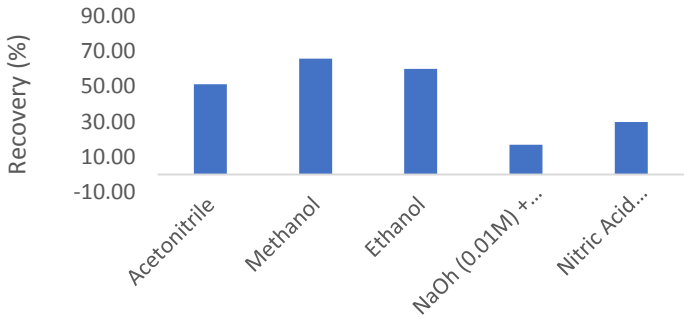

Figure 19. Examining the effect of different types of solvent and absorption.

According to the graph drawn based on the recovery percentage of theophylline drug in each solvent, methanol with 62.65% recovery was chosen as the suitable solvent for theophylline absorption from the magnetic nanosorbent. After that, ethanol and pure acetonitrile respectively showed the highest recovery percentages (Figure 19).

Effect of solvent volume and absorption

It is important to find the minimum necessary volume of the desorption solvent to carry out the drug desorption process from the surface of the magnetic nanosorbent. In order to investigate the effect of desorption solvent volume on recovery percentage, the desorption process was performed with 6 volumes of 2, 5, 8, 10, 12 and 18 ml of methanol. According to the obtained results, the recovery percentage increases gradually up to the volume of 5 ml of the solvent and remains constant after that. So, the volume of 5 ml was determined as the optimal volume of methanol for desorption of theophylline from the magnetic nanoadsorbent (Figure 20).

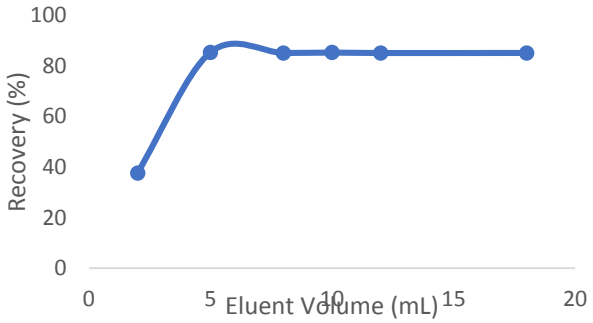

Figure 20. Optimizing the effect of solvent volume and desorption.

Limit volume and concentration factor

The process of absorption and desorption under optimal conditions was carried out for 7 samples containing a certain amount of theophylline (g170µ) which were diluted with different amounts of 10, 30, 50, 60, 70, 90 and 120 ml of deionized water, and the following diagram was obtained.

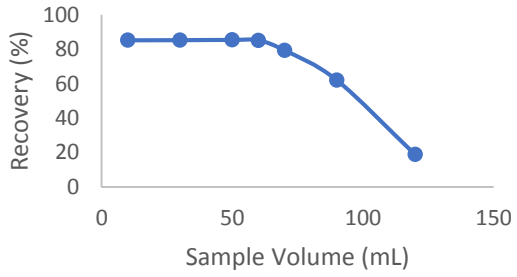

Figure 21. Limit volume.

According to the obtained results, the limit volume is equal to 60 ml and thus the concentration factor will be equal to 12 (Figure 21).

Validation of the proposed method

In order to draw the calibration curve, under optimal conditions, the calibration curve was drawn in the form of UV-Vis absorption of the drug in the absorption solvent (methanol)

according to different concentrations of theophylline drug. The calibration curve included 5 different concentrations of theophylline drug in the concentration range of 50-50 ppm, which was obtained with a good correlation coefficient of R2=0.9991 (Figure 22).

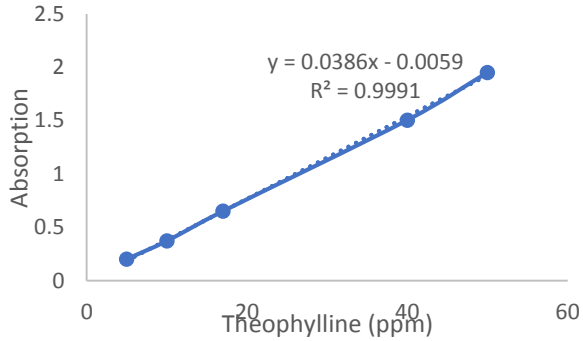

Figure 22. Calibration curve.

The results of the detection limit and accuracy of the method are presented in Tables 2 and 3.

Table 2. Characteristics of the calibration curve of the proposed method.

Calibration line Eq.		(ppm) Line limit	R^2	Analyte
Width from the origin	**Slope**			
0.0059	0.0386	5-50	0.9991	**Theophylline**

Table 3. Analytical characteristics of the proposed method.

%RSD Accuracy	LOD (ppm) Detection Limit	Analyte
1.03	0.17	**Theophylline**

The accuracy of the method

200 µL of the 600 ppm solution of theophylline drug, equivalent to 120 µg of theophylline, was spiked into real samples of distilled water, tap water and urine, and the absorption and extraction process was carried out with the synthesized magnetic nano-absorbent according to the proposed method. This test was repeated 3 times for each sample and the following results were obtained (Table 4).

Table 4. The results obtained by performing the proposed method on the real sample.

Measured drug by standard (µg) method USP	%RSD	Average drug (µg) شده measurement	Added (µg) medicine	Real Sample
-	-	N.D[1]	0	**Distilled water**
-	2.86	122.23	120	
-	-	N.D	0	**Tap water**
-	2.80	123.43	120	
N.D	-	N.D	0	**Urine**
122.07	1.48	121.83	120	

200 µL of 600 ppm solution of theophylline drug was spiked into 10 ml of urine sample and the chromatogram obtained by applying the USP standard method was obtained (Figure 23 and Table 5).

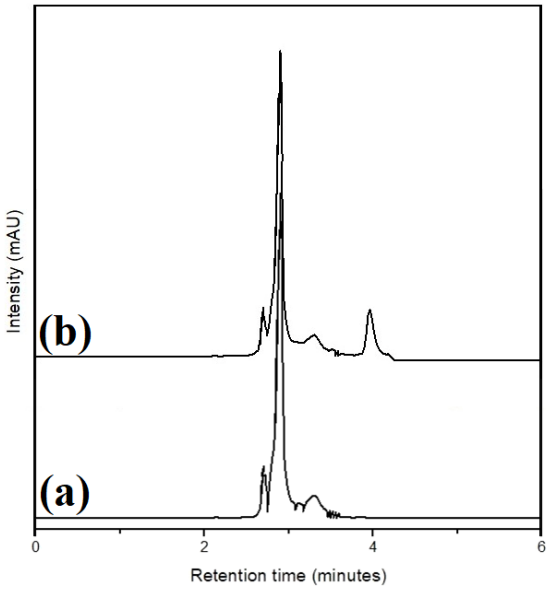

Figure 23. a) Chromatogram of control urine sample b) Chromatogram of urine sample spiked with theophylline.

Table 5. The result obtained by performing the USP standard method on a real urine sample.

(µg) Measured medicine	(min) Ret. time	Analyte	Real Sample
122.07	3.96	Theophylline	Urine

[1] Not Detected

According to the amount of drug measured by the standard method and the proposed method in the urine sample, the T test was performed and the following results were obtained. Results showed that p-value is greater than 0.05; So, with a probability of 96%, there will be no significant difference between the results obtained from these two methods, and the presented method is reliable.

Discussion

In this research, several experiments were conducted to optimize the factors affecting the absorption of theophylline. In the study of the adsorption process, the effect of variables such as pH, ionic strength (adding NaCl), amount of adsorbent, temperature, duration of contact between the adsorbent and the sample, type of desorption solvent and its volume were investigated. In order to optimize the conditions, the univariate method was used and in all stages of the experiment, 6 variables were fixed and by changing the desired variable, its effect was evaluated. In this method, the effect of pH on absorption efficiency was investigated and its optimal value was determined. Then the effect of ionic strength was investigated by adding NaCl and the optimal amount of sodium chloride was determined; after that, other variables were optimized in the mentioned order.

Theophylline drug is a weak base with pKa=8.81. According to the corresponding figure, in the range of pH=3-11, the absorption efficiency reaches the maximum at pH=5. At pHs higher than this value, the absorption efficiency decreases due to the reduction of the cationic form of the drug molecule; At lower pHs, due to the increased competition of H+ with the cationic form of the drug to bind to the surface of the nanoabsorbent particles, the absorption efficiency decreases again (8). pHs lower than 3 were not studied due to the dissolution of magnetite particles in strong acidic environments and the destruction of the magnetic nanoabsorbent structure [11]. Therefore, pH = 5 was chosen as the optimal pH for further studies.

Examining the effect of salt in the range of 0 to 5 mg showed that by increasing the amount of salt up to 1 mg, the absorption efficiency increases and reaches a maximum at 1 mg. At this point, the solubility of the analyte in the aqueous solution decreases and the surface absorption on the adsorbent increases with the phenomenon of salting out. At values higher than 1 mg, the increase in the viscosity of the aqueous solution overcomes the salting-out phenomenon and makes mass transfer difficult and the extraction efficiency decreases. It can also be said that since the interaction between the analyte and the adsorbent is electrostatic, the increase in salt

causes an electrostatic interaction between the salt and the adsorbent and the active sites for the interaction between the drug and the adsorbent are occupied [12].

Regarding the amount of adsorbent, its increase can be associated with an increase in the absorption level. As can be seen in the corresponding figure, the adsorption efficiency gradually increased with the increase of the amount of adsorbent from 0.02 to 0.2 g and then remained almost constant in the range of 0.2 to 0.5 g. This problem can be due to the fact that with 0.2 grams of magnetic nanosorbent, the amount of analyte absorbed on the surface of the nanosorbent and the analyte in the aqueous solution reach equilibrium, and by increasing the amount of the adsorbent more than 0.2 grams, more drug will be on the surface of the nanosorbent. It is not absorbed [12]. In other words, at the optimal point, the maximum possible amount of analyte in the aqueous solution is attached to a certain amount of nanosorbent, and adding more nanosorbent does not affect the absorption efficiency [13].

About the effect of temperature, as can be seen in the corresponding figure, increasing the temperature from 15 to 40 °C only increased the absorption efficiency from 61.65% to 62.84%, which is not very impressive. But in order to increase the drug absorption efficiency and ensure the extraction efficiency is maximized, the temperature of 40 degrees Celsius was chosen as the optimal temperature. This slight increase in adsorption efficiency can be due to the increase in the movement of analyte particles due to the increase in temperature and the increase in the chance of encountering active sites on the surface of the magnetic nanoabsorbent.

In order for drug absorption to reach its maximum value at the equilibrium point, it is necessary to have enough time. For this reason, the contact time between the absorbent and the sample was checked from 2 to 30 minutes. The maximum absorption occurs in 20 minutes and after that it reaches an equilibrium state and remains almost constant.

After optimizing the extraction conditions, the desorption conditions were also investigated. First, the effect of various solvents such as acetonitrile, ethanol, methanol, a mixture of acetonitrile and nitric acid 0.01 M with a ratio of 9:1 and also a mixture of acetonitrile and sodium hydroxide 0.01 M with a ratio of 9:1 was studied on the desorption efficiency. According to the results obtained in the corresponding figure, the highest percentage of recovery was obtained using methanol compared to other solvents. Analytes are usually eluted with polar and protic solvents [14]. Methanol has suitable polarity and protic nature, which works very well in disrupting and disrupting the electrostatic interaction between theophylline and beta-cyclodextrin molecules [15]. Therefore, theophylline molecules are separated from the magnetic nanoabsorbent and enter the solvent and are absorbed.

In addition to the type of desorbing solvent, the volume of desorbing solvent was also measured in the range of 2 to 18 ml. According to the results obtained in the corresponding figure, the recovery percentage increases gradually up to the volume of 5 ml and remains almost constant after that. So, the volume of 5 ml was determined as the optimal volume of methanol for desorption of theophylline from the magnetic nanoadsorbent. In smaller volumes, it seems that the amount of detergent is not enough to desorb the drug from the surface of nanoparticles; because in this case, the adsorbent is not soaked by the solvent and adsorbs well. From the volume of 5 ml onwards, the maximum amount of theophylline drug enters the solvent and is absorbed, and adding more amounts of solvent will not significantly affect the recovery percentage**.**

Conclusion

In this study, a method based on the diffusion solid phase extraction process for preconcentration and measurement of theophylline from aqueous samples was investigated. UV-Vis spectrophotometer was used for analysis, detection and quantitative measurement of the extracted analyte. Magnetic nanoparticles of magnetite were prepared by co-precipitation method and coated with nanocellulose and then modified by beta-cyclodextrin, which formed a safe, non-toxic and green structure. In this method, these nanoparticles were used as adsorbents that were easily separated without the need for filtration or centrifugation, only by an external magnetic field; less time is spent on separation and preconcentration due to the lack of special equipment used in conventional extraction methods. The proposed method in this study was simple, fast, and efficient, and according to the results obtained, i.e., detection limit, linear range, accuracy, and precision, it was determined that the proposed method is suitable, efficient, and usable for measuring theophylline drug.

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