CHEMICAL ENGINEERING TRANSACTIONS
VOL. 38, 2014
A publication of AIDIC
The Italian Association of Chemical Engineering
www.aidic.it/cet
Guest Editors: Enrico Bardone, Marco Bravi, Taj Keshavarz

ISBN 978-88-95608-29-7; **ISSN** 2283-9216
DOI: 10.3303/CET1438066

Determination of Drugs in Biological Sample by Using Modified Magnetic Nanoparticles and HPLC

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A microextraction method using Ag modified-magnetic nanoparticle (Ag-MNPs) coupled with high performance liquid chromatography (HPLC) has been applied for determination of cefteriaxone in plasma. Magnetic nanoparticles were synthesized via a mild solution route. The prepared nanoparticles were modified with a thin layer of silver and characterized with different methods such as X-ray diffraction (XRD), transmission electron microscopy (TEM), FTIR and ultraviolet-visible(UV-Vis) spectroscopy.
Effect of several parameter such as pH of donor and acceptance phase, amount of magnetic particle and extraction and desorption time were optimized. Under the optimal condition the enrichment factor was obtained 19. The detection limit was 0.02 mg/mLand a wide linear range as 0.06 to 40µg/mL were obtained.

1. Introduction

Ceftriaxone (CFT) is a semisynthetic third-generation cephalosporin antibiotic. It has broad-spectrum activity against Gram-positive and Gram-negative bacteria. New fast methods were developed for extraction and determination of Ceftriaxone as a third-generation cephalosporin antibiotic due to increasing use of ceftriaxone for its high stability to most bacterial β-lactamase. (Shiffman et al. 1990). Due to the low concentration of drugs in blood and several interferences, sample clean up and preconcentration must be carried out before determination of drugs (Zhang et al. 2008, Olszway et al. 2013). Various methods were used for this purpose such as liquid- phase microextraction (LPME) (Bagheri et al. 2008, Mahan et al. 2012), solid phase microextraction (SPME) (Olszowy and Szulthla, 2011), three phase microextraction (TPME) (Mofazzeli, 2013; Freitas et al. 2010). SPME is the most common sample preparation technique for clinical and pharmaceutical analysis because of its advantages (Xie et al. 2009,More and Mundhe,. 2013). It is a solvent free, simple, fast and attractive technique for pre-treatment of complex sample matrices prior to chromatographic (Qing 2013).
We reported an SPME method for pre-concentration of ceftriaxone from plasma samples. Magnetic nanoparticles coated with Ag, was made through a mild solution method. By using magnetic particle, Ag-MNPs could be recovered and used several times for treatments. HPLC was used for treatment monitoring.

2. Material and methods

Phosphoric acid, sodium hydroxide, silver nitrate, sodium citrate tetraheptyl ammonium bromide and potassium dihydrogen phosphate were purchased from commercial sources (Merck, Fluka or Sigma) and used as received, without further purification. The distilled-deionized water was used in all solution preparation (18 MΩ).The stock solutions of the cefteriaxon sodium (20 ppm) were prepared freshly in distillated water. Ag coated magnetic nanoparticles after extraction was separated with magnetic field.

Please cite this article as: Enayati Ahangar L., Movassaghi K., Bahrami F., Enayati M., Chianese A., 2014, Determination of drugs in biological sample by using modified magnetic nanoparticles and hplc, Chemical Engineering Transactions, 38, 391-396
DOI: 10.3303/CET1438066

2.1 Preparation of the Ag coated magnetic nanoparticles (Ag-MNPs)

2.1.1 Preparation of the Fe_3O_4 nanoparticles

Hydrophilic magnetic-nanoparticles were prepared as flowing method, briefly, 3 ml of iron (II) sulphate solution (2 M) and 10 ml of an iron (III) chloride solution (1 M) were mixed under vigorous mechanical stirring at room temperature and 15 ml of HCl solution (2 M) was used to dissolve the iron salts. An aliquot of 50 ml of tetraethylammonium hydroxide was added to the above solution until the solution reached a pH of 13. Immediately a black solution formed.

2.1.2. Preparation of Ag coated Fe_3O_4 (Ag-MNPs)

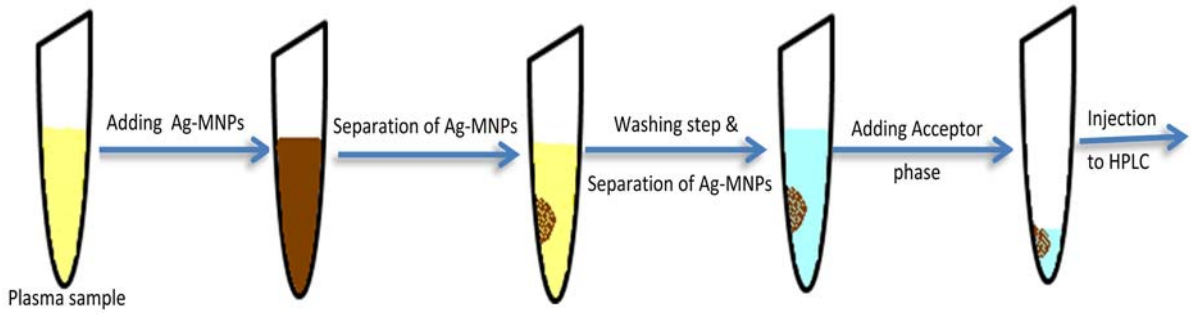
50 mg of magnetite-nanoparticles was dispersed in 20 ml of deionized water contain 1 mM of AgNO3 and 0.1M of sodium citrate salt under stirring for 24 hours. The aggregated magnetic particles were separated and the suspension was transferred to another beaker. The separated Ag /Fe_3O_4 nanoparticles were washed with water (Chen and Mao. 2007) and were stored in dark place in 4 °C.

2.2 Apparatus

The waters 600 HPLC system from Waters Corporation (USA) array detector consisted of waters 600 pump and waters 996 photodiode. The separation was performed on a MZ analytical, perfectsil target C18 (125× 4.0 mm, 3.5 µm) column. The solvents used as mobile phase were contained of 44mL of 0.2 M of buffer pH=7 0f sodium citrate, 4mL of 0.2M of buffer pH=7 dibasic potassium phosphate, 40mL of tetraheptyl-ammonium bromide, 325mL of acetonitril then water was added to make 1000 mL solution. The resulting solution was filtered through a 0.5 µm or finer appropriate filter. For detection the UV detection was set at 270 nm and the flow rate was 1 mLmin-1.

2.3 Extraction procedures:

5ml of donor solution was added to a 10 ml vial and its pH was adjusted with 0.5 ml of 2 M phosphate buffer. 0.1 µL of Ag-NMPs solution was added to vial then the vial was shacked for a definite time. In the next step by applying an external magnetic field, the Ag-NMPs were collected and the solution removed carefully. In washing step, 3 mL of distillate water was added to above Ag-NMPs and the vial was shacked vigorously then the particles was separated by an external magnetic field and washing solution was removed (this step repeat two times). In desorption step, 100 µL of alkaline 0.05 M phosphate buffer was added to resulted nanoparticle and shacked for certained time. In the last step, the nanoparticle was sep01arated and its solution was injected to HPLC system to determine the drug peak. Scheme 1 shows the extraction procedure.



Scheme 1. Schematic plan of extraction procedure.

3. Results and discussion

3.1 Optimization steps:

For accomplishing the high extraction efficiency, important parameter were optimized, such as the pH of donor pH of acceptor phase, extraction time, back-extraction time, amount of nanoparticle and shaking speed. In the last step the method validation and real sample analysis was done in optimized cindition.

3.1.1 The pH of donor and acceptor phase:

The pH of donor and acceptor phase is very effective parameter in extraction efficiency which can improve the transfer of the analyte from donor to acceptor phase. The pH of donor phase should be adjusted to deionise the analyte and produce an effective interaction between nanoparticle and drug. The different pH were used for sample donor phase. Finally pH=3 showed the highest efficiency (Fig.1a). For back-extraction procedure the pH of acceptor phase is important to complete the extraction process, in a good

back-extraction the pH of acceptor phase must be adjusted to ionized analyte. For back-extraction pH 10.5 have the highest extraction efficiency but Ag-MNPs have low stability therefore pH=8.5 selected as optimum pH (Figure 1b).

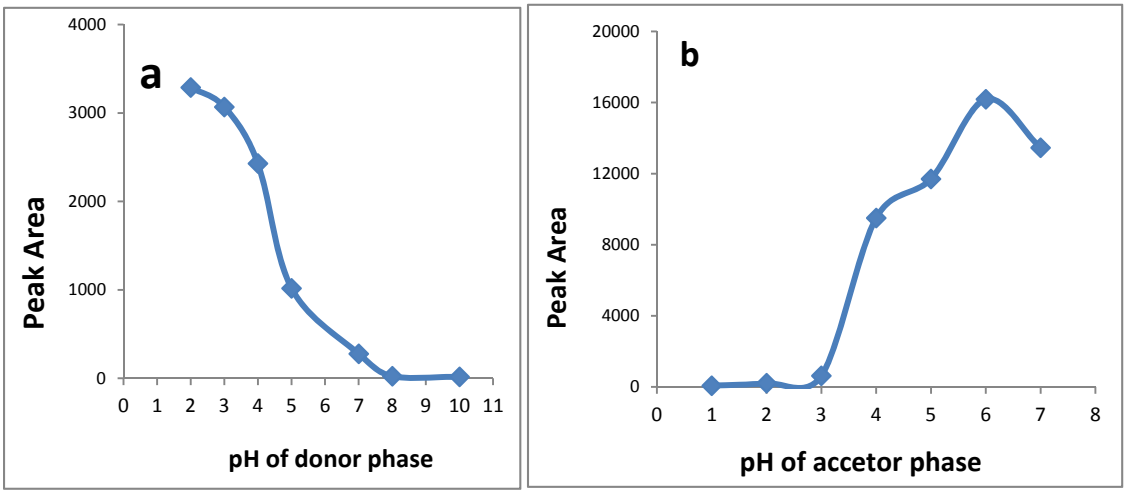


Figure.1. Effect of different a) donor phase pH and b) acceptor phase pH on extraction efficiency.

Other parameter such as amount of nanoparticle and shaking speed was tested and finally 0.1 µL of Ag-NMPs solution contion 0.1 mg of Ag-NMPs show a good efficiency and was selected as optimum nanoparticle amount. High shaking speed showed better efficiency but in very high speed efficiency is constant and reduced little.

3.1.2 Effect of extraction and back-extraction time

Extraction and back extratiion times are important to reach good extraction efficiency. The time of each step were optimized at final step of optimization. In the first step the 40 min show the best result. For back extraction step the 30 min have best extraction efficeincy.

3.2 Analytical performance

The analytical performance of this method was explored using different concentrations of the CFT target, according to the described procedure. Calibration curve was drawn for water samples, for each concentration three replicate extractions were performed under optimum conditions. Calibration curves of the three analytes were obtained by plotting their peak areas vs. their concentrations in the samples. This curve was linear in range of 0.06 to 40 µg/mL. The limit of detection (LOD), and the limit of quantification (LOQ),were 20 and 60 ng/mL, respectively. For evaluating the precision and accuracy of the present method, relative recovery tests were conduced by adding known amounts of the FCT, at, 1.0 mgL, into plasma samples and the results are describe in next section.

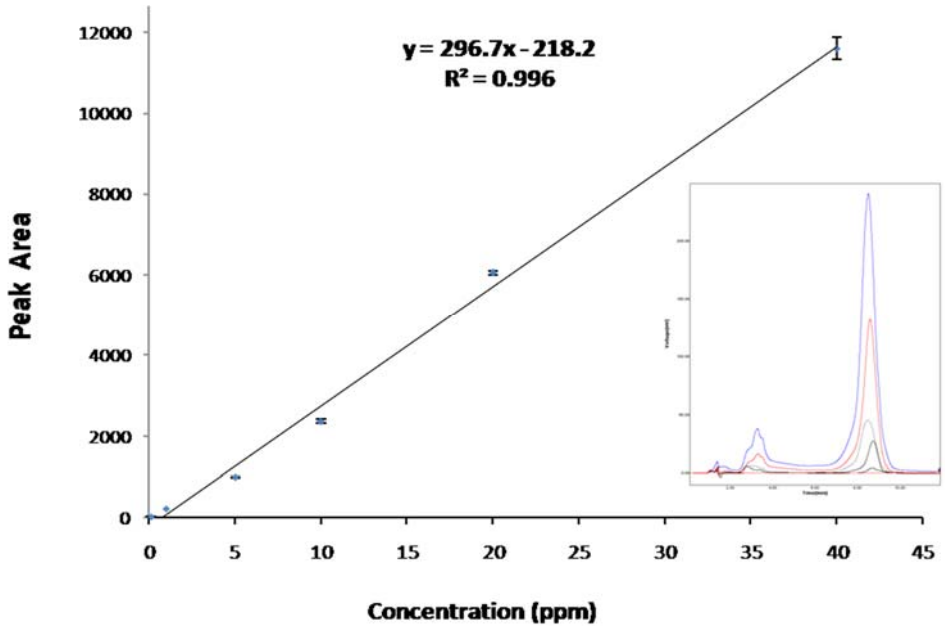


Figure 2. Calibration Curve for different concentration of CFT.

Enrichment Factor was obtained 19 for developed extraction method at optimum condition. Figure 3 shows the peak of direct injection for 10 ppm of CFT solution (small peak at 7.5 min) and after extraction process (large peak at 7.5 min) in optimum condition.

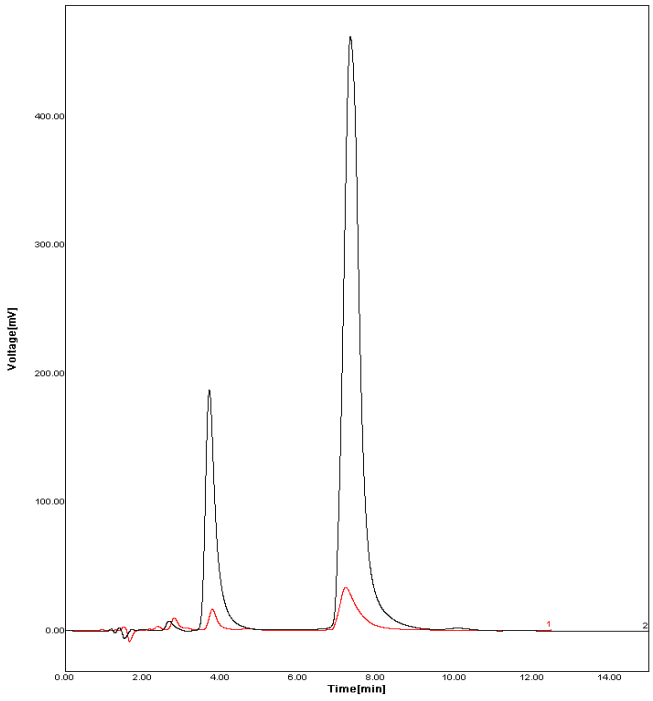


Figure 3. chromatograms of 10 µg/mL of CFT solution before and after extraction

3.3 Real sample analysis

In order to inspect the influence of biological fluid, the HPLC method was applied to analyze a standard solution containing CFT. Drug free sample was spiked with 1 µg/L of CFT and its pH was adjusted with

adding 0.5 mL of 2M phosphate buffer pH=3. Spiked plasma and blank plasma was extracted by Ag-MNPs at optimum condition without anymore purification. For Human plasma relative recovery was calculated 89 %. Also for water sample which was spiked with the same concentration of the analyte. The recovery was 99%. (Figure 4).

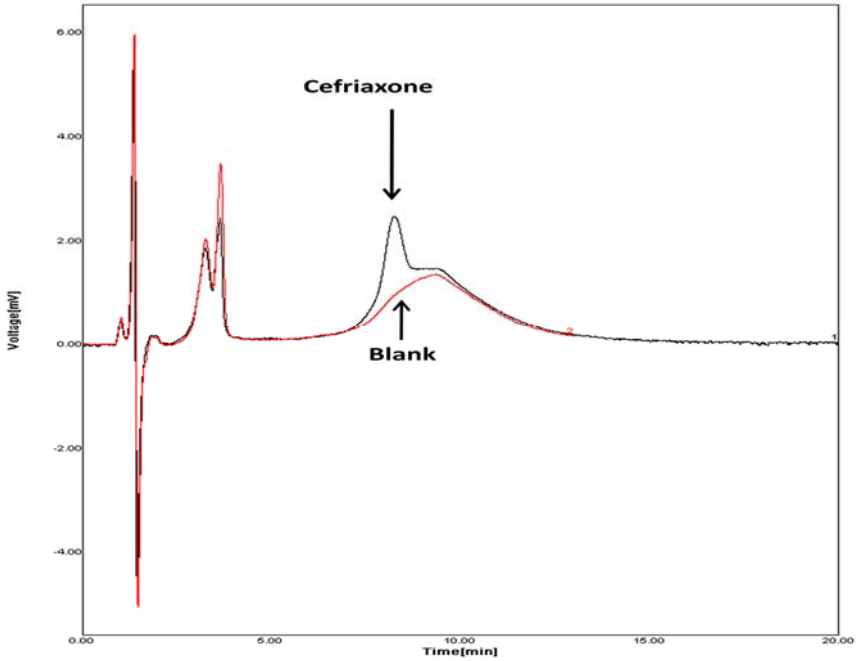


Figure 4. Chromatogram obtained real sample: (Red colour) blank plasma and (Black colour) 1 ppm of spiked plasma ephedrine using developed method under optimized condition.

Table 1. Comparison between the proposed method and other reported techniques.

Method	analyte	LOD	Recovery %	Reference
Dionex Ultimate 3000 UHPLC system	Ceftriaxon	170 ng/mL	98.88	(Shrestha et al. 2013)
Dionex Ultimate 3000 UHPLC system	Tazobactam	580 ng/mL	98.84	(Shrestha et al. 2013)
Three-phase, liquid-phase microextraction	Fluoxetine	5 ng/mL	70.9	(Freitas et al. 2010)
This proposed assay	Ceftriaxon	20 ng/mL	89	Proposed method

4. conclusion:

Ag-MNPs method combined with HPLC-UV was successfully used for the determination of ceftriaxone in human plasma. The proposed procedure exhibited high-quality performance with with a low LOD for the determination of CFT in plasma sample. The proposed method offers a simple,rather easy, rapid, and inexpensive technique with high relative recovery for the analysis of CFT and it is recommended for determination other drugs from biological sample such as urin and blood and plasma and also for doping controls. These modified magnatic nanoparticle could use for treatment of wastewater and air for removing polutant agents. The versatility of this method can also be easily extended to a range of extraction coatings providing analysis of a wide range of analytes.

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