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# SHORT COMMUNICATION Chromatographic analysis of immobilized cefotaxime

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*Abstract*: The aim of the present work was to widen the application of an in-house developed fast, flexible and sensitive high performance liquid chromatography (HPLC) method to the assessment of cefotaxime sodium from aqueous samples. The method was applied to establish the release profile of cefotaxime sodium immobilised in MCM-41 nanoparticles using pH-controlled release in an aqueous medium. The analytical method proved to be sensitive, repeatable (*RSD* < 1.5 %) and reproducible (*RSD* < 1 %) in the studied concentration range (0.01–10  $\mu$ g·mL<sup>-1</sup>). The limit of detection and limit of quantification were 0.036 and 0.12  $\mu$ g·mL<sup>-1</sup>, respectively, suitable for the analysis of the release of a single active ingredient, and the analysis time was short (10 min).

Keywords: cefotaxime sodium; cephalosporins; controlled release; HPLC.

## INTRODUCTION

Cefotaxime sodium (Fig. 1) is a third generation cephalosporin antibiotic that has wide clinical applications for treatment of infections of the respiratory tract, gynaecologic, skin, bone and joint, urinary tract, septicaemia, and documented or suspected meningitis.<sup>1</sup>

For the analysis of cefotaxime sodium, several methods have been employed: electrochemical (cyclic voltammetry, square wave voltammetry),<sup>2–4</sup> spectrofluorimetric and chemiluminescent,<sup>5,6</sup> and high performance liquid chromatographic methods.<sup>7,8</sup> Some performance characteristics of methods applied in the determination cefotaxime or its metabolites are presented in Table I.

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The aim of this study was the development and application of a sensitive chromatographic method with dual detection (diode array and mass spectrometry, HPLC-DAD–MS) for the determination of cefotaxime immobilized on the mesoporous material MCM-41, which is an ordered mesoporous silicate that displays cylindrical mesopores having a unit cell size of 4.6–4.8 nm. In recent years, it is the most used support for the controlled release of drugs.<sup>9–14</sup> Cefotaxime sodium was used as the model drug, a dual detection mode being chosen to ensure a better selectivity of the method. According to the British Pharmacopoeia (2009), cefotaxime sodium is an easily degradable antibiotic. Any detectable impurities might affect the efficacy of the bioactive compound (*e.g.*, cefotaxime dimers if present at a sufficient level. Therefore, the employment of MS analysis was justified in order to provide exact information on the nature and form in which the analyte occurred.



Fig. 1. Chemical structure of cefotaxime sodium.

TABLE I. Comparison between the performance characteristics of previous methods applied in cefotaxime determination

Analyte	Sample matrix	Method	$LOD / \mu g m L^{-1}$	Reference
Cefotaxime, desacetylcefotaxime,	Ocular aqueous	HPLC	0.08	7
	humour			
Cefotaxime, desacetylcefotaxime,	Plasma	HPLC-UV	0.31	7
Cefotaxime	Plasma	HPLC-UV	1.0	8

### EXPERIMENTAL

### Chemicals and reagents

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The cefotaxime sodium standard (MW 477.5) used in this study was purchased from Sigma–Aldrich (Steinheim, Germany). HPLC grade methanol was from Merck (Darmstadt, Germany). All stock solutions and samples were prepared with ultrapure water.

## Instrumentation and procedure

The HPLC analysis was realised on a Shimadzu HPLC system (SCL-10A VP, Kyoto, Japan), consisting of a degasser (DGU-20A5), two pumps (LC-10AD VP) for the delivery of the mobile phase, a thermostatic system (CTO-10AS), and a system controller (SCL – 10A VP). Detection was achieved using a diode array detector (SPD – M20A). A C18 analytical column (150 mm×4.6 mm, 5  $\mu$ m i.d., Fortis) was used in the analysis. The oven temperature was set at 25 °C. A CyberScan PCD6500 pH/ion/conductivity/DO meter from Eutech Instruments was used for pH measurements of the mobile phase.



A Shimadzu mass spectrometer, model 2010, was employed, using an electro-spray ionization interface, in positive mode. The tuning of the detector was accomplished prior each set of determinations, using an appropriate tuning mixture. The detector settings were ESI (+) CDL temperature 250 °C, nebulizing gas flow 1.5 L min<sup>-1</sup>, heat block 200 °C.

*Chromatographic procedure.* The chromatographic analysis was performed in isocratic mode, using as mobile phase a mixture of methanol and water (30:70, *V/V*), adjusted at pH 4.0 with acetic acid. The flow rate was 0.8 mL min<sup>-1</sup>. The chromatographic conditions were as follows: the injection volume was 20  $\mu$ L, column temperature 25 °C, detection was performed at 235 nm and the time of analysis was 10 minutes.

The calibration curve for HPLC-DAD analysis was obtained in a range between 0.01 to  $10 \ \mu g \cdot m L^{-1}$ , using the average area from triplicate analysis.

## Preparation of the standard solution

A stock standard solution of cefotaxime sodium (2000  $\mu$ g·mL<sup>-1</sup>) was prepared in ultrapure water. The solution was kept in the refrigerator to prevent degradation. Working standard solutions were freshly prepared before analysis in water at concentrations ranging from 0.01 to 10  $\mu$ g·mL<sup>-1</sup>. Before use, the solutions were filtered through a 0.20  $\mu$ m filter.

### Drug immobilisation

Cefotaxime sodium was immobilised in MCM-41by mixing 0.32 g of active substance with 0.07 g of MCM-41 in 10 mL of ultrapure water. The mixture was left to settle for 24 h, filtered and dried in a vacuum desiccator for 24 h. The amount of cefotaxime immobilised was 9.19 % (0.098 g, calculated from the difference in initial concentration of cefotaxime solution and final concentration of the supernatant (determined by HPLC).

## Conditions of release

The release profile for cefotaxime immobilized in MCM-41 was adjusted considering the BP releasing tests appropriate for  $\beta$ -lactam antibiotics: 20 mg of the composite (cefotaxime sodium and MCM-41) was mixed with 20 mL of phosphate buffer (to simulate body fluid) at pH 4.80 and kept at a constant mixing rate of 200 rpm. The total release time was 6 h. Samples of 1 mL were collected, centrifuged for 10 min at 9000 rpm, two times, and afterwards the supernatants were filtered through a 0.20  $\mu$ m PTCE membrane and injected into the HPLC system for analysis.

## RESULTS AND DISCUSSIONS

In the present work, the development and partial validation (in terms of linearity, limit of detection, limit of quantification, repeatability and reproducibility) of the HPLC-DAD–MS method was performed and the releasing process of immobilized cefotaxime sodium in mesoporous supports was studied. Different mobile phase compositions were tested and it was found that a mobile phase of methanol:water 30:70 (V/V) (pH 4.00, buffered with acetic acid) resulted in an acceptable resolution (retention times), peak shape (Gaussian) and time of analysis at a column temperature of 25 °C for the studied antibiotic. Various stationary phases were tested, a Fortis C18 analytical column was successfully used being more efficient than a classical silica-based packing. The detection wavelength was 235 nm.

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The peak specific for cefotaxime sodium was characterized by a retention time of 4.6 min. A typical chromatogram for the standard compound, obtained under the described optimal experimental conditions, is shown in Fig. 2. The chromatographic peak corresponding to the studied drug eluted at a retention time of 4.6 min and presented a well-shaped Gaussian form, separated from the solvent front. A total analysis time of around 10 min was employed to ensure adequate column equilibration between two subsequent analyses. No significant interfering chromatographic peaks were observed in the HPLC chromatograms during the analysis of cefotaxime sodium samples released from the support nanoparticles. No interferences from the sample solvent or from impurities were observed at the employed detection wavelength.



Fig. 2. HPLC-DAD chromatogram for cefotaxime sodium standard (1 µg·mL<sup>-1</sup>).

# Partial validation of the method

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The linear response ranged from 0.01 to 10  $\mu$ g·mL<sup>-1</sup>. The *LOD* and *LOQ* values were calculated from the slope and the standard deviation of a blank signal. For repeatability and reproducibility, every level of concentration was injected three times into the chromatographic system and three injections from three different solutions with the same concentration of standard working solution (0.5  $\mu$ g·mL<sup>-1</sup>) were analysed. All results are presented in Table II.

The presence of the studied compound was confirmed by HPLC-DAD–MS analysis. The previously developed and validated method was adjusted to the MS conditions. The column and the mobile phase were the same as those used for the HPLC-DAD analysis. The only adjustment was in the flow rate (0.2 mL min<sup>-1</sup>), with an analysis time of 10 minutes and injection volume of 10  $\mu$ L.

The MS spectrum of cefotaxime sodium is given in Fig. 3.



TABLE II. Several performance characteristics of the HPLC-DAD method applied to cefotaxime sodium determination

Parameter	Reference solution, cefotaxime sodium 0.05–10	
Linearity range, µg·mL <sup>-1</sup>		
Retention time, min	4.6	
Slope	1443423.72	
Intercept	32855.62	
Correlation coefficient	0.9988	
$LOD / \mu g \cdot m L^{-1}$	0.036	
$LOQ / \mu g \cdot m L^{-1}$	0.12	
Repeatability, RSD / %	1.28	
Reproducibility, RSD / %	0.70	



Fig. 3. MS (ESI+) spectrum of cefotaxime sodium.

## Application of the HPLC method to the release of cefotaxime sodium

One of the applications of HPLC method is the determination of antibiotics in pharmaceuticals. For this purpose, the release of cefotaxime sodium immobilised in MCM-41 nanoparticles was studied.

The release profile of the biologically active compound from the MCM-41 composite and the corresponding chromatograms are given in Figs. 4 and 5, respectively.

The released cephalosporin was identified using the retention time and the MS spectrum, since the obtained coefficient of variation was appropriate. The observed retention time (4.6 min) enabled the rapid determination of the analyte. The method provides better limits of detection (*LOD*) and quantification (*LOQ*) than those of other reported methods (Table I); even compared to those of a method able to discriminate between cefotaxime enantiomers since Wang *et al.* reported the separation and determination of cefotaxime enantiomers in injections by capillary zone electrophoresis with an *LOD* of 0.5 µg mL<sup>-1</sup>.<sup>15</sup>

Thus, the method was successfully applied to *in vitro* release of cefotaxime sodium into an aqueous medium of controlled pH.







Fig. 4. The release profile of cefotaxime sodium (as adsorbed mass) immobilised in mesoporous silica MCM-41.



Fig. 5. HPLC-DAD chromatogram for cefotaxime sodium sample at different release times (a - 20 min, b - 50 min, c - 90 min, d - 120 min, e - 180 min).

## CONCLUSIONS

An HPLC-DAD method was developed and partially validated, in terms of linearity, limit of detection, limit of quantification, repeatability and reproducibility to determine cefotaxime sodium and then applied to the controlled release of the drug immobilised in mesoporous MCM-41 nanoparticles.



The main improvement of the proposed method with respect to published methods is the increase in sensitivity (with two magnitude folds) of the *LOD* and *LOQ* and also the relatively low cost of the chemicals used.

The proposed chromatographic method is a simple (uses common solvents, and there is no necessity for a particular preparation of the samples, standards or mobile phase) and rapid procedure (the total analysis time is only 10 min and the retention time is short) for the determination of cefotaxime sodium in aqueous samples. Moreover, its application in establishing the release profiles was proved.

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### ИЗВОД

## ХРОМАТОГРАФСКА АНАЛИЗА ИМОБИЛИСАНОГ ЦЕФОТАКСИМА

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Циљ овог рада је проширење примене једне, лабораторијски већ развијене, брзе, флексибилне и осетљиве методе високоефикасне течне хроматографије (HPLC), за одређивње натријум-цефотаксима у воденим узорцима. Метода је примењена ради одређивања профила ослобађања натријум-цефотаксима, имобилисаног на MCM-41 наночестицама, применом pH контролисаног ослобађања у воденој средини. Доказано је да је метода осетљива, са добром поновљивошћу (*RSD* < 1,5 %) и репродуктивношћу (*RSD* < 1 %) у испитиваном концентрационом опсегу (0,01–10 µg·mL<sup>-1</sup>), са границама детекције и квантификације 0,036, односно 0.12 µg·mL<sup>-1</sup>, и погодна за анализу ослобађања појединачних активних компоненти са кратким временом анализе (10 min).

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