Simultaneous Analysis of Cefoperazone and Its Degradation Products: Optimized HPLC approach

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Abstract: The insight into the chromatographic methods revealed a limited number of highly sensitive procedures providing the selective determination of both cefoperazone and its degradation products with straightforward isocratic elution. Therefore, this research is focused on the optimization and determination of cefoperazone and its degradation products using HPLC–DAD (High Pressure Liquid Chromatography Diode Array Detection). During the HPLC method optimization, maximum separation was achieved using ACN : H_3PO_4 (30:70, v/v) at a flow rate of 0.8 cm^3/min, which resulted in sharp and well-resolved peaks. The retention time was 6.7 ± 0.1 min. The hydrolysis of cefoperazone was performed at 25 ± 1 °C and 4 ± 1 °C for 7 days. At 25 °C, cefoperazone was hydrolyzed to its P1–P4 intermediates. This study illustrates the capability of optimizing the HPLC method for precise monitoring of cefoperazone and its degradation products with a suitable analysis time.

Keywords: method optimization; isocratic elution; chromatographic separation; β-lactam antibiotics; pharmaceutical analysis

1. Introduction

The simultaneous analysis of the antibiotic cefoperazone and its degradation products attracted attention in the area of analytical chemistry. The analysis is directed toward the enhancement of chromatographic methods and analytical strategies in the analysis and detection of cefoperazone [1]. Cefoperazone is a new third-generation cephalosporin. It is a semisynthetic injectable cephalosporin with a broad spectrum of activity [2]. It has bactericidal activity and is mainly used for the treatment of bacterial infections caused by Gram-positive and Gram-negative microorganisms [3]. Administered intravenously or intramuscularly, cefoperazone acts against a wide variety of diseases, including infections of the biliary tract, and in many anaerobic infections [4]. There are no severe side effects, the most common include diarrhea and skin rashes [5].

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High-performance thin-layer chromatography was reported to be used in the detection of cefoperazone. The optimized method was reported as selective, simple, with a precise workflow that supports stability assessment, and routine quality control [6]. Vaghela and Rao developed a stability-indicating HPLC (High Pressure Liquid Chromatography) method. An optimized method was used for the observation of the degradation behavior of cefoperazone. Using this method, they attempt to separate the peak of cefoperazone from the peaks of degradation products. However, challenges in achieving complete resolution of certain degradation products were present under selected conditions [7]. Zhou et al. developed a highly sensitive and rapid liquid chromatography-tandem mass spectrometric (LC–MS/MS) method for the detection of cefoperazone sodium in human plasma. This study showed that LC–MS/MS can deliver targeted and robust analysis in complex matrices. It can also detect degradation products, which are relevant for use in environmental or formulation contexts [8]. Alanazi et al. developed RP-HPLC and multivariate curve resolution (MCR) methods using Box–Behnken design and Six Sigma approaches. They reported that the developed HPLC method enables the detection of cefoperazone and most of its degradation products [9].

Collectively, this research is focused on the optimization and determination of cefoperazone and its degradation products using HPLC–DAD (High Pressure Liquid Chromatography Diode Array Detection). An isocratic elution of acetonitrile (ACN) : 0.1% H_3PO_4 of different compositions at a different flow rate was examined. During the HPLC method optimization, the mobile phase composition as well as the flow rate were adjusted. Maximum separation and sharp, well-resolved peaks were the goal to achieve. Further, the hydrolysis of cefoperazone was performed at 25 ± 1 °C and 4 ± 1 °C for 7 days. At 25 °C, cefoperazone was hydrolyzed to its P1–P4 intermediates. At lower temperatures, hydrolysis was much slower, leading to the formation of mainly P1 and P4 intermediates. This study illustrates the capability of optimizing the HPLC method for precise monitoring of cefoperazone and its degradation products with a suitable analysis time.

In contrast to previously reported methods that often rely on gradient elution or advanced mass spectrometric detection, this study proposes a simple, isocratic HPLC–DAD approach that enables complete and reproducible separation of cefoperazone and its degradation products within a short analysis time.

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2. Experimental

2.1. Chemicals and solutions

Cefoperazone sodium salt ($C_{25}H_{26}N_9NaO_8S_2$, M = 667.65 g/mol, (6R,7R)-7-[[(2R)-2-[(4-ethyl-2,3-dioxopiperazine-1-carbonyl)amino]-2-(4-hydroxyphenyl)acetyl]amino]-3-[(1-methyltetrazol-5-yl)sulfanylmethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid) was used without additional purification. The standard solutions of cefoperazone (0.05 mmol/dm^3) were prepared using ultrapure water (pH = 6.56, κ = 0.055 μS/cm, total organic carbon TOC < LOD). The solution was protected from light and stored at temperatures of 4 °C and 25 °C. The other chemicals used as components of the mobile phase for liquid chromatography were also used without further purification. They were of p.a. (pro analysis) grade: 99.9% ACN, C_3H_3NO (Sigma-Aldrich, Massachusetts, United States), and 85% orthophosphoric acid, H_3PO_4 (Lachema, Neratovice, Czech Republic). ZnO (Sigma-Aldrich, Massachusetts, 99.9% hexagonal wurtzite structure, particle size around 41 nm, specific surface area 6.5 m2/g) was used as the catalyst.

2.2. Degradation procedures

For method optimization solutions of cefoperazone after 20 min of photocatalytic degradation were used. The photocatalytic degradation of cefoperazone was carried out using a Pyrex glass cell equipped with a plain window, onto which the light beam was focused. The total volume of the cell was approximately 40 cm^3. Experiments were performed using 20 cm^3 of 0.05 mmol/dm^3 cefoperazone solution containing 1.0 mg/cm^3 of ZnO. Before irradiation, the reaction mixture was sonicated for 15 min in the dark to establish adsorption/desorption equilibrium and maintained at a constant temperature of 25 °C using a water circulating jacket while exposed to a stream of O_2 at a 3.0 cm^3/min rate. The solution was stirred with a magnetic stirring bar throughout the experiments under a continuous gas flow. A high-pressure mercury lamp (125 W, Philips, HPL-N, emission bands at 290, 293, 296, 304, 314, 335, and 366 nm, with maximum emission at 366 nm) was used as the UV irradiation source.

These degradation conditions were selected to generate representative intermediates for chromatographic method optimization and validation.

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2.3. Analytical procedures

To monitor the cefoperazone and its degradation products in water using HPLC–DAD, 0.5 cm^3 aliquots of the solution were taken. The samples were analyzed on a Shimadzu HPLC system equipped with a UV/VIS DAD detector. An Eclipse XDB-C18 column (150 mm × 4.6 mm, 5 μm particle size, 30 °C) was used for the analysis, and 10 μL of each sample was injected. The mobile phase consisted of ACN and water, with water acidified using 85% H_3PO_4. Chromatograms were recorded using isocratic elution (ACN : H_3PO_4, 30:70, v/v, pH = 2.42) at a flow rate of 0.8 cm^3/min, except during method optimization. The detection was performed at the optimal wavelength of absorbance of cefoperazone (λ = 205 nm).

pH of solutions was measured using a combined glass electrode (pH-Electrode SenTix 20, WTW) connected to a pH meter (pH/Cond 340i, WTW).

3. Results and discussion

3.1. Absorption maximum

Prior to the investigation and monitoring of the hydrolysis of cefoperazone, optimization of the experimental conditions for liquid chromatographic separation of the cefoperazone from the products formed during the degradation was performed. In order to select an appropriate working wavelength, the first step in the optimization was to record an absorption spectrum to determine the absorption maximum of cefoperazone. As can be seen in Figure 1, the absorption maximum of cefoperazone is at 194 nm (Figure 1). Therefore, the chromatograms were recorded at the appropriate absorbance wavelength of 205 nm because the UV detector shows reduced sensitivity and increased baseline noise at wavelengths below 200 nm due to solvent absorption and instrumental limitations [10].

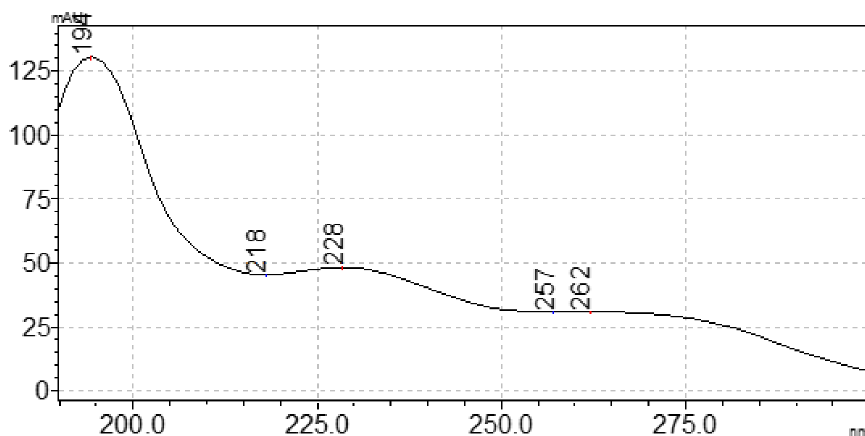

Figure 1. Absorption spectrum of cefoperazone (0.05 $mmol/dm^3$).

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Figure 2 shows a 3D view of the chromatogram of cefoperazone, where its absorption maximum can be clearly seen.

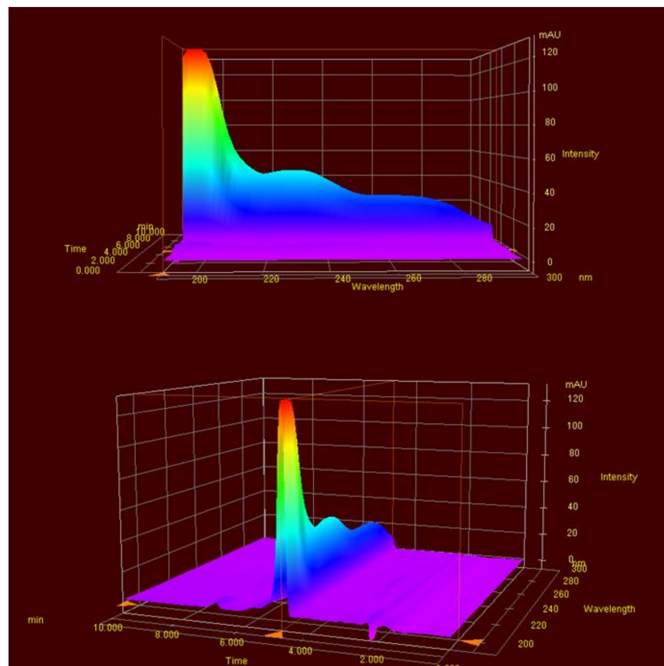

Figure 2. 3D view of the chromatogram of cefoperazone (0.05 mmol/dm^3).

3.2. Optimization of the chromatographic method

Literature overview showed that several methods were employed for determining cefoperazone in the pharmaceutical formulations [11]. These methods include spectrophotometric methods [12], derivative UV-spectrophotometry [13], as well as near infrared reflectance spectroscopy [14]. Also, chromatographic methods [15] were reported [16] as widely used for the determination of cefoperazone [17]. Additionally, cefoperazone was determined in plasma by the LC–MS-MS method [18]. Voltammetric determination and electrochemical behavior of cefoperazone were also studied [19]. However, there is no reported analytical method for the quantitative determination of cefoperazone in the presence of its degradation products. Therefore, we aimed to optimize the chromatographic method for the determination of cefoperazone and its degradation products.

The influence of mobile phase composition on the HPLC separation of cefoperazone from its degradation products was systematically investigated. Using ratios of 50:50 and 40:60 (v/v) ACN : 0.1% H_3PO_4, cefoperazone peaks were at 2.2 and 2.8 $\pm$ 0.1 min, respectively, and the separation from degradation products was insufficient, leading to overlapping peaks and less reliable quantification. The optimal ratio of 30:70 (v/v) ACN :

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0.1% H_3PO_4 provided a sharp, well-defined cefoperazone peak at 4.9 ± 0.1 min, achieving precise and reproducible separation from all degradation products (Figure 3).

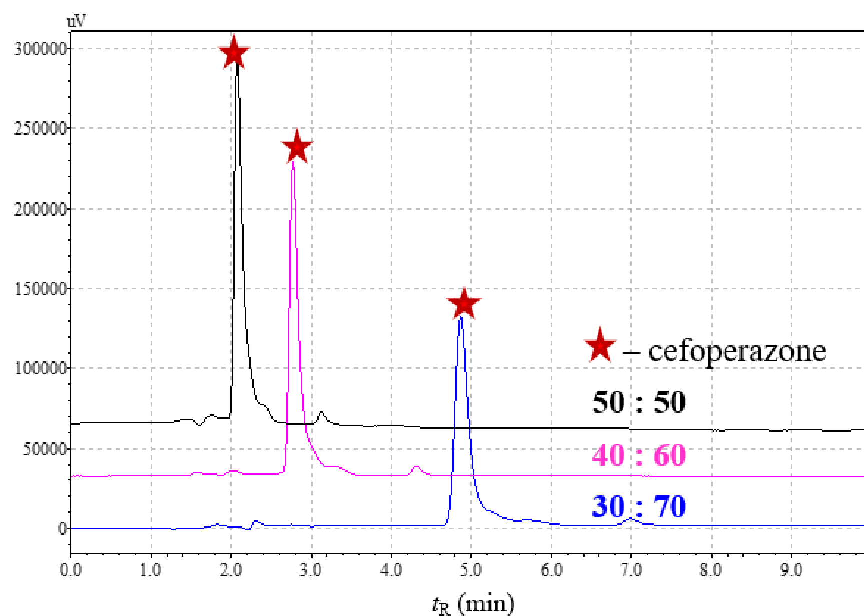

Figure 3. The influence of the composition of the mobile phase on the appearance of the chromatogram of cefoperazone.

Further, the influence of flow rate was investigated. As shown in Figure 4, a decrease in the flow rate to 0.8 cm^3/min leads to better separation of the peaks compared to the separation obtained at a flow rate of 1.0 cm^3/min, and shifts the retention time of cefoperazone to 6.7 ± 0.1 min. Increasing the flow rate to 1.2 cm^3/min causes the cefoperazone peak to shift to a shorter retention time, resulting in poorer separation. A flow rate of 0.8 cm^3/min was selected as optimal, providing satisfactory separation and analysis time.

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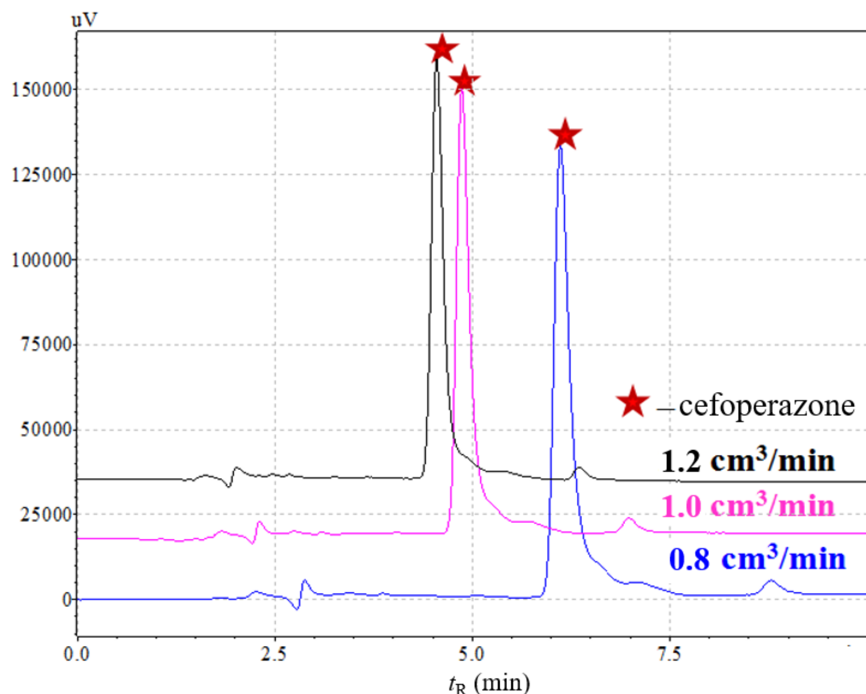

Figure 4. The influence of the composition of the mobile phase on the appearance of the chromatogram of cefoperazone.

The optimal operating conditions were determined: mobile phase ratio ACN : H_3PO_4 (v/v) 30:70, flow rate 0.8 cm^3/min, and λ_{det} = 205 nm. Subsequently, cefoperazone hydrolysis was monitored under the optimized conditions.

The selected chromatographic conditions ensured complete baseline separation of cefoperazone from all detected degradation products, enabling reliable qualitative and quantitative monitoring using a straightforward isocratic protocol.

3.3. Cefoperazone hydrolysis

The stability of cefoperazone at different temperatures, namely 25 ± 1 °C and 4 ± 1 °C, was also examined over 7 days. From the obtained results (Figure 5), it can be concluded that cefoperazone is unstable at 25 °C, leading to the formation of intermediates P1, P2, P3, and P4. In contrast, it is more stable at 4 °C, with slower hydrolysis at lower temperatures. In this case, only intermediates P1 and P4 were formed after 7 days of hydrolysis. Based on these results, it can be concluded that hydrolysis of cefoperazone is one possible way to remove it from the environment. These results show that temperature affects the hydrolysis rate, indicating a potential environmental removal pathway.

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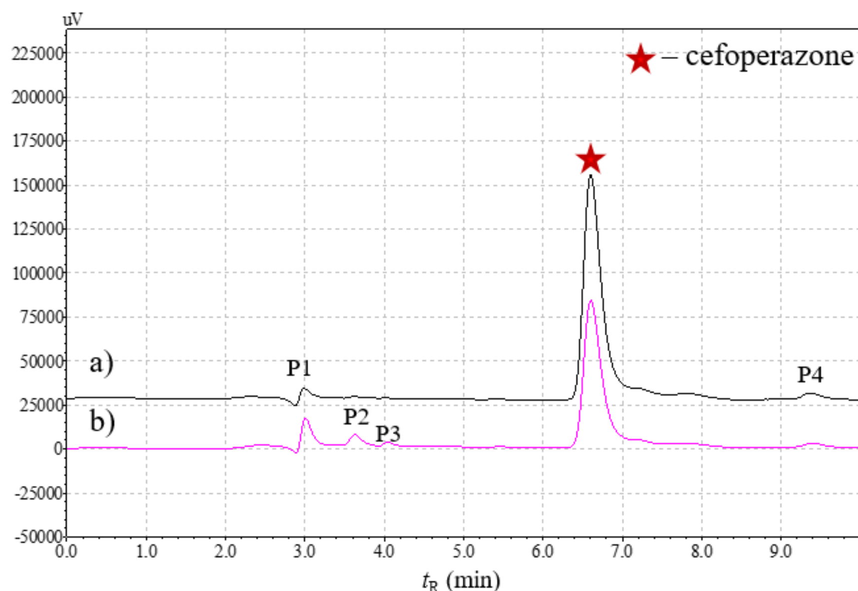

Figure 5. Stability test of cefoperazone at (a) 4 ± 1 °C and (b) 25 ± 1 °C.

From an environmental perspective, these results suggest that hydrolysis may represent one of the pathways contributing to the degradation of cefoperazone in aqueous systems.

4. Conclusions

The focus of this research was on the optimization and determination of cefoperazone and its degradation products using HPLC–DAD. An isocratic elution of ACN: 0.1% H_3PO_4 of different compositions at a different flow rate was used, and cefoperazone was monitored at 205 nm, an appropriate wavelength of absorption of cefoperazone. Maximum separation was achieved using ACN : H_3PO_4 (30:70, v/v) at a flow rate of 0.8 cm^3/min, which resulted in sharp and well-resolved peaks. The optimized HPLC–DAD method provides a reliable and efficient approach for the separation and monitoring of cefoperazone and its degradation products, allowing precise tracking of hydrolysis kinetics. The study demonstrated that both mobile phase composition and flow rate are critical parameters for achieving sharp, well-resolved peaks and reproducible results. The simplicity, robustness, and reproducibility of the proposed HPLC–DAD method make it suitable for routine pharmaceutical analysis, stability studies, and monitoring of cefoperazone degradation.

The hydrolysis of cefoperazone was performed at 25 ± 1 °C and 4 ± 1 °C for 7 days. At 25 °C, cefoperazone was hydrolyzed to its P1–P4 intermediates. At lower temperatures, hydrolysis was much slower, leading to the formation of mainly P1 and P4 intermediates. From these observations, it can be concluded that hydrolysis is a factor in the environmental

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degradation of cefoperazone. Overall, this approach ensures accurate, fast, and reproducible analysis, making it a valuable tool for studying the stability and degradation behavior of cefoperazone.

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