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NOTE

HPLC Analysis of Ceftriaxone and Ceftizoxime

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A rapid and simple reverse phase HPLC method has been developed and validated for the estimation of ceftriaxone and ceftizoxime. The cephalosporins were resolved on a reverse-phase C_{18} column (25 × 0.46 cm i.d., 5 μm) utilizing a mobile phase of methanol and water at a flow rate of 1.0 mL min^{-1} and UV detection of 242 nm for ceftriaxone and 310 nm for ceftizoxime. Both cephalosporins demonstrated excellent linearity and the method of standard addition revealed recovery of 97.7–100.2%, with detection limits ranging from 1.0–1.5 μg mL^{-1} for both drugs. Retention time was 2.15 and 2.21 min for ceftriaxone and ceftizoxime respectively. This method was found to be a simple analytical method.

Key Words: HPLC, Analysis, Ceftriaxone, Ceftizoxime.

Ceftriaxone is a β-lactamase-resistant cephalosporin with an extremely long serum half life. It contains a highly acidic heterocyclic system on the 3-thio methyl group. This unusual dioxotriazine ring system is believed to confer the unique pharmacokinetic properties on the drug. Ceftriaxone exhibits excellent broad-spectrum antibacterial activity against both Gram positive and Gram negative organisms.

Ceftizoxime is a third generation cephalosporin which exhibits excellent activity against the enterobacteiaceae, especially *E. coli, K. pneumonia, E. cloacae.* Several chromatographic methods have been reported for the determination of ceftriaxone[1–6] and ceftizoxime[7–10] in biological matrices, including HPLC methods.

Pure samples of ceftriaxone and ceftizoxime were obtained as gift samples from Aristo Pharma, Mumbai, India and GSK Ltd., Nasik, India respectively.

HPLC grade methanol and water packed under nitrogen and filtered through 0.2 μm filter were purchased from Merck, Mumbai.

Preparation of Standard Solution

Standard stock solutions of ceftriaxone and ceftizoxime (1 mg mL^{-1}) were prepared separately in methanol. Working solutions were prepared from these stock solutions by dilution with HPLC grade water.

The chromatographic apparatus consisted of a low-pressure gradient HPLC pump, a UV variable detector from Shimadzu (Tokyo, Japan). The data were acquired and processed by means of Spinchrom CFR-2.3.0.149 chromatography software.

Chromatographic separation was achieved on a reverse-phase column (C_{18}, 25 × 0.46 cm i.d., 5 μm particle size) from Phenomenex supplied by Spincotech, Bangalore.

Solvent used for the separation was methanol : water in the ratio 70 : 30. The total analysis time was 5 min at a flow rate of 1.0 mL min^{-1} at room temperature (25°C). The elute was monitored with a UV detector set at a wavelength of 242 nm and 310 nm for ceftriaxone and ceftizoxime respectively. The compounds were quantified using their peak height.

Sample Preparation

Dry powders for injection of strengths 250 mg and 500 mg procured from the local market were studied for formulation analysis. Samples were prepared to get a final concentration of 1 μg mL^{-1}. The solutions were vortexed for a minute and filtered through Millipore filter.

Preparation of calibration standards

During validation, five point calibration standards were freshly prepared in the mobile phase, covering a concentration range of 1–5 μg mL^{-1}. Calibration data was acquired by plotting the peak height of the analytes against the concentration of the calibration standards, followed by linear regression analysis (Table-1).

TABLE-1

Concentration (μg/mL)	Ceftriaxone peak area	Ceftizoxime peak area
1	21.9200	20.099
2	43.5260	40.163
3	67.0155	59.918
4	88.0700	80.014
5	109.7600	100.057

Various ratios of methanol and water and different flow rates were examined to obtain the best chromatogram. Although peaks where obtained at different ratios, peak splitting and peak broadening were observed except at 70 : 30 ratio of methanol and water, which was selected as the mobile phase at a flow rate of 1 mL/min wherein the peaks were well resolved.

Selectivity was indicated by the absence of any endogenous interference at the retention times of the peak of interest. The retention times for ceftriaxone and ceftizoxime were 2.15 and 2.21 min respectively.

Performing recovery studies tested the reliability and reproducibility of the proposed method. The percentage recovery obtained was between 97.7–99 and 99.7–100.25 ceftriaxone and ceftizoxime respectively which indicates that the proposed method is precise and accurate (Table-2).

TABLE-2
ANALYSIS OF FORMULATION

Formulation	Amount present (mg)	Amount found (mg)	% Label claim
Ceftriaxone I	250	247.50	98.92
Ceftriaxone II	500	496.703	99.34
Ceftizoxime I	1000	997.0	99.7

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