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DEVELOPMENT AND VALIDATION OF NEW ANALYTICAL METHOD FOR CEFODROXIL MONOHYDRATE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

Kareti Srinivasa Rao[a]*, M.E Bhanoji Rao[a], S.Sreenivas Patro[b], Ajay Kumar Patnaik[c],

[a]Roland Institute of Pharmaceutical Sciences, Berhampur -760010, Orissa (India).

[b]School of Pharmacy, Taylors University, Malaysia

[c]Khallikote College, Berhampur, Orissa (India)

ABSTRACT

A simple, sensitive, accurate and precise LC assay method was developed for the quantitative determination of Cefadroxil monohydrate (CDM) in pharmaceutical dosage form. Chromatographic separation was achieved by use of XTerra RP-18 column (250 × 4.0 mm, 5 µm). The described method was linear over a range of 1.0-120 µg mL^{-1} for determination of CDM (r= 1). F-test and t-test at 95% confidence level were used to check the intermediate precision data obtained under different experimental setups; the calculated value was found to be less than critical value. The developed method was found to be simple, specific, robust, linear, precise, and accurate for the determination of CDM in pharmaceutical formulations.

KEYWORDS Cefadroxil monohydrate, validation, assay, recovery studies.

INTRODUCTION

Cefadroxil chemically a 7-[[2-amino -2-(4-hydroxyphenyl) acetyl] amino]-3-methyl-8-oxo- 5-thia-1-azabicyclo, oct-2-ene-2-carboxylic acid monohydrate is a first generation, oral cephalosporin antimicrobial agent active against Gram-positive organisms. It is official in IP, USP and BP. It is a first generation cephalosporin antibacterial drug that is the para-hydroxy derivative of cefalexin, and is used in the treatment of mild to moderate susceptible infections. It is a broad spectrum bactericidal antibiotic that is effective against many Grampositive and Gram-negative bacteria, including Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus piogenes, Moraxella catarrhalis, Escherichia coli, Klebsiella and Proteus mirabilis.

Literature search reveals a wide variety of analytical methods have been reported for the determination of CDM in pure form, in pharmaceutical preparations and in biological fluids. These methods mainly involve UV-Vis spectroscopy, atomic absorption spectrophotometry, fluorometry, chemiluminescence, polarography, high performance liquid chromatography, and capillary electrophoresis. Few methods have been developed for the simultaneous determination of CDM when combined with other drugs in various dosage forms. CDM and cefotaxime have been determined by flow injection spectrophotometric method. CDM and cefotaxime in binary mixtures have been estimated by derivative spectrophotometry. CDM and cephalexin in combination have been determined simultaneously by coupling technique of synchronous fluorimetry and H-point standard addition methods . Similarly, CDM and cephalexin have also been determined by HPLC method while CDM and cefuroxime determined in urine first derivative spectroscopy and HPLC .

Although the reported HPLC method for estimation of CDM in pharmaceutical formulations present adequate linearity, precision, and recovery, they show a series of limitations including lack of sensitivity, which results in the lower limit of quantification and long chromatographic times. However, this method is relatively non-specific, laborious, time consuming and have long retention times. However, the present study achieved satisfactory results in terms of selectivity, linearity, precision and accuracy under simple chromatographic conditions.

EXPERIMENTAL

Materials

CDM reference standard was obtained from Torrent pharma., India. CDM commercial tablets (Acudrox 500 mg) were purchased from the local market. HPLC grade Acetonitrile was purchased from Rankem, India, and high pure water was prepared by using Millipore Milli Q plus purification system. Dibasic potassium phosphate, monobasic potassium phosphate, Sodium citrate were purchased from Qualigens Fine chemicals, India.

Apparatus and chromatographic conditions

Quantitative HPLC was performed on Shimadzu HPLC with LC 10 AT VP series pumps besides SPD 10 A VP UV-Visible detector. The chromatographic separations were performed using XTerra, C18, RP column (250 mm × 4mm × 5 µm) maintained at ambient temperature, eluted with mobile phase at a flow rate of 1 mL/min for 15 min. The output signal was monitored and integrated using Shimadzu Class-VP version 6.12 SP1 software. The mobile phase consisted of acetonitrile: phosphate buffer (4:96 %v/v). Measurements were made with injection volume 20µl and ultraviolet (UV) detection at 254 nm.

Preparation of Phosphate buffer (pH 5.0)

13.6 g of diabasic potassium phosphate and 4.0 g of monobasic potassium phosphate were dissolved in water to obtain 1000 mL solution. This soloution was adjusted with glacial acetic acid to yield pH 5.0±0.1

Preparation of mobile phase

A mixture of phosphate buffer (pH 5) and acetonitrile (96:4%v/v) are thoroughly mixed and degased in ultrasonic water bath for 5 minutes, the final solution obtained was filtered through 0.45 membrane filter.

Preparation of standard and sample solutions

Stock solution of CDM (1mg/mL) was prepared by dissolving 25 mg of CDM in 25 mL of volumetric flask containing 10 mL of mobile phase. The solution was sonicated for about 30 minutes and then made up to volume with mobile phase. Working standard solutions of CDM were prepared by taking suitable aliquots of CDM stock solution and diluted to 10 mL with mobile phase in a 10 mL volumetric flask to yield the drug concentrations in the range of 5-720 $\mu g\ mL^{-1}$.

To prepare a sample solution, twenty weighed tablets of ACUDROX® (500 mg of CDM) were ground and an amount of powder equivalent to 10 mg of active compound was diluted with mobile phase and then sonicated for 20 min. The sample solution was filtered and the appropriate aliquot was diluted in the mobile phase to obtain a final solution containing 10 $\mu g\ mL^{-1}$ of CDM.

Method validation

The validation procedure for the analysis of CDM by LC method followed the International Conference on Harmonization (ICH) guideline and United States Pharmacopoeia. The performance parameters evaluated in this method were specificity, robustness, linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, and accuracy.

Robustness

Chromatographic parameters (peak retention time, theoretical plates, tailing factor, retention factor, and repeatability) were evaluated using both samples and reference substance solutions (10 $\mu g\ mL^{-1}$) changing wavelength (249 and 259 nm), column temperature (23 and 27 ºC), flow rate (0.8 and 1.2 mL min^{-1}) and acetonitrile concentration (2 and 6%).

Linearity

Linearity was established by least squares linear regression analysis of the calibration curve. The constructed calibration curves (n=3) were linear over the concentration range of 5-720 μg/mL. Peak areas of CDM was plotted against their respective concentrations and linear regression analysis was performed on the resultant curve.

LOD and LOQ

LOD and LOQ were determined by reducing the concentration of a standard solution until the CDM peak response was approximately three or ten times, greater than the noise, respectively.

Precision

The precision of the proposed method was evaluated by carrying out six independent (50µg/mL) assays of test sample. RSD (%) of six assay values obtained was calculated. Intermediate precision was carried out by analyzing the samples by a different analyst on another instrument.

Accuracy

The accuracy of the method was determined through the recovery test of the samples, using known amounts of CDM reference standard. For LC method, aliquots of 0.8, 1.0 and 1.2 mL of a CDM standard solution (100 µg mL^{-1}) were added to three sample solutions containing a fixed amount of CDM (100 µg) in mobile phase, respectively. Therefore, this recovery study was performed at a final concentration solution of 80%, 100% and 120% level of CDM. All solutions were prepared in triplicate and analyzed.

System suitability test

System suitability tests were performed to ensure that the LC system and procedure are capable of providing quality data based on USP 31 requirements. The system suitability parameters include CDM retention time, tailing factor and number of theoretical plates, as well as the peak area relative-standard deviation (RSD, n= 6) of reference standard.

RESULTS AND DISCUSSION

Optimization of LC method

To develop a suitable and robust HPLC method for the determination of CDM in different mobile phases - Phosphate buffer (pH 5.0) : Methanol (98:2v/v), Phosphate buffer (pH 4.5) : Methanol (95:5v/v), Ammonium Acetate buffer (pH 4.5) : Acetonitrile (95:5v/v), Phosphate buffer (pH 4.5) : Acetonitrile (95:5v/v), Ammonium Acetate buffer (pH 5.0) : Acetonitrile (96:4v/v) at different flow rates (0.5, 0.75, 0.8, 1.0, 1.2, 1.5, mL/min) with different detection wavelength. The mobile phase phosphate buffer (pH 5.0) : acetonitrile (96:4 v/v) at a flow rate of 1.0 mL/ min gave peaks with good resolution for CDM are eluted at retention time around 7.46 min and with symmetric peak shape as shown in Figure 1.

Method Validation

Robustness

The robustness of the method was examined by small variations of critical parameters, and percent of CDM, retention time (R_t), number of theoretical plates (N) and tailing factor (T), were evaluated (table 1).

The robustness study has been proved that in every employed condition, the chromatographic parameters agreed with established values and the assay data remained acceptable. A tailing factor of 1.15 refers to a symmetric peak. The calculated values for the tailing factor for each chromatographic condition were in the acceptable range of $0.8 \leq T \leq 1.5$. The number of theoretical plates demonstrated the measure the column efficiency in different conditions. Flow rate (0.8 and 1.2 mL min^{-1}) and percent of acetonitrile (2 and 6%) resulted in changes in the retention time in comparison with the proposed normal condition. However, no significant changes were observed regarding quantification of CDM.

Linearity

The standard curves for CDM were constructed and demonstrated to be linear in the concentration range of 5-720 μg mL^{-1}. The representative linear equation Y= 25614+8602.8, where x is the concentration (μg mL^{-1}) and y is the peak area. The correlation coefficient was r= 1.0. Linearity data were validated by the analysis of variance (ANOVA), which demonstrated significant linear regression and no significant linearity deviation ($p < 0.05$).

LOD and LOQ

The limit of quantitation (LOQ) of the present method was found to be 1.2μg/mL with a resultant %RSD of 0.94% (n = 5). The limit of detection (LOD) was found to be 0.36 μg/mL. This low values obtained were indicative of the high sensitivity of the method.

Precision

Precision values obtained for the determination of CDM in samples with their RSD are shown in table 2. F-test and t-test was applied to the two sets of data at 95% confidence level, and no statistically significant difference was observed.

Accuracy

Accuracy was evaluated by the simultaneous determination of the analyte in solutions prepared by the standard addition method. Three different concentrations of CDM standard were added to Acudrox® solution. The mean recovery was shown (table 3) and this value showed that the method was accurate.

System suitability test

The system suitability parameters evaluated, under the experimental conditions, showed a single peak of the drug around 7.4 min, tailing factor (T= 1.15) and number of theoretical plates (N= 3288), as well as the peak area relative-standard deviation (RSD= 0.92%, n = 6).

Assay

The validated method was applied to the determination of CDM in commercially available Acudrox ® 500mg vials. Figure-2 illustrates a typical HPLC chromatogram obtained from the assay of Acudrox® solution. The results of the assay (n = 9) undertaken yielded 99.76% (%RSD = 1.3%) of label claim for CDM. The observed concentration of CDM was found to be 498.81±11.2 µg/mL (mean±SD). The mean retention time of CDM was 7.6 min. The results of the assay indicate that the method is selective for the analysis of CDM without interference from the excipients used to formulate and produce these tablets.

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of CDM from pure and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of CDM in pure form and its dosage forms and can also be used for dissolution or similar studies.

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Figure legends

Figure 1: Representative chromatogram of CDM in reference standard solution

Figure 2: Representative chromatogram of CDM in pharmaceutical formulation

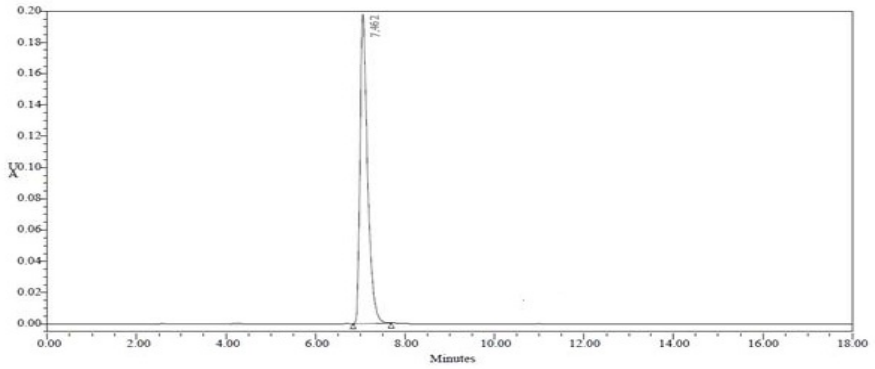

Figure1:

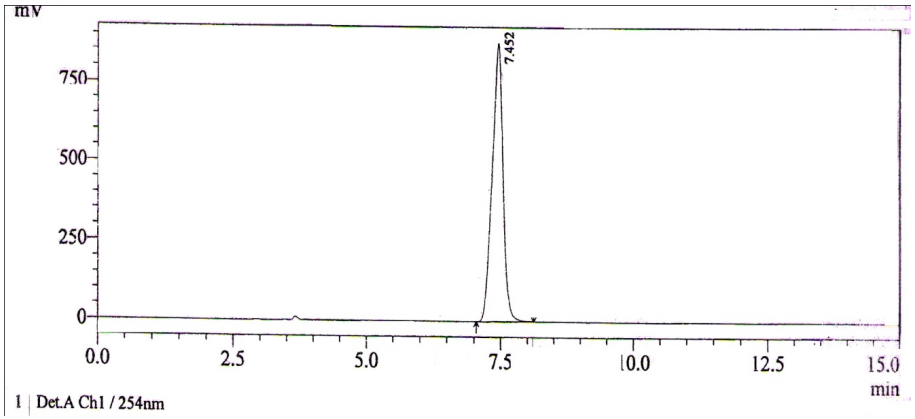

Figure 2:

Chromatographic parameter	Condition	CDM (%)	R_t[a] CDM (min)	N[b]	T[c]
Wavelength (nm)	249	98.19	7.48	2901	1.27
	259	98.73	7.32	3015	1.27
Temperature (ºC)	23	99.09	7.14	3420	1.35
	27	98.47	7.22	3309	1.13
Flow rate (mL min^{-1})	0.8	99.18	7.350	3207	1.41
	1.2	101.05	7.099	3197	1.33
Acetonitrile (%)	2	100.59	7.163	3213	1.34
	6	100.17	7.477	3105	1.21
	Normal[d]	100.09	7.461	3288	1.15

Table 1: Robustness experiments of LC method for determination of CDM.

[a] R_t: retention time

[b] N: number of theoretical plates

[c] T: tailing factor

[d]Normal condition (mobile phase): XTerra, C18, RP column (250 mm × 4mm × 5 µm), acetonitrile: phosphate buffer (4:96 %v/v), flow rate 1.0 mL min^{-1}; UV detection at 254 nm

Precision	CDM
	Mean assay (%)/%R.S.D
Set 1(n=6)	97.8/0.529
Set 2(n=6)	99.6/0.791
	Calculated value/ critical value
F-test	1.832/3.368
t-test	1.608/2.106

Table 2: Precision of CDM by proposed method.

Amount added ($\mu g\ mL^{-1}$)	Amount found ($\mu g\ mL^{-1}$)	% Recovery[a] ± RSD	Mean % Recovery
80.0	79.67	99.58 ± 0.81	
100.0	99.88	99.88 ± 1.02	99.88
120.0	120.24	100.2 ± 1.31	

[a] Each value is a mean hree determinations.

Table 3: Recovery of standard solution added to commercially available sample