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Development and Validation of UV Spectrophotometric Method for the Determination of Cefuroxime Axetil in Bulk and Pharmaceutical Formulation

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Abstract

The present study was undertaken to develop and validate a simple, sensitive, accurate, precise and reproducible UV spectrophotometric method for cefuroxime axetil using methanol as solvent. In this method the simple UV spectrum of cefuroxime axetil in methanol was obtained which exhibits absorption maxima (λ_{max}) at 278 nm. The quantitative determination of the drug was carried out at 278 nm and Beer's law was obeyed in the range of (0.80-3.60) µg/ml. The proposed method was applied to pharmaceutical formulation and percent amount of drug estimated (95.6% and 96%) was found in good agreement with the label claim. The developed method was successfully validated with respect to linearity, specificity, accuracy and precision. The method was shown linear in the mentioned concentrations having line equation $y = 0.05x + 0.048$ with correlation coefficient of 0.995. The recovery values for cefuroxime axetil ranged from 99.85-100.05. The relative standard deviation of six replicates of assay was less than 2%. The percent relative standard deviations of inter-day precision ranged between 1.45-1.92% and intra-day precision of cefuroxime axetil was 0.96-1.51%. Hence, proposed method was precise, accurate and cost effective.

Keywords: UV-Vis spectrophotometer; Method validation; Cefuroxime axetil; Recovery studies.

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1. Introduction

Cefuroxime Axetil is chemically [6R-[6α,7β(Z)]]-3-[[(2-aminocarbonyl)oxy]methyl]-7-[2-furanyl (methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monosodium salt [1,2]. This ester product of cefuroxime increases the lipophilicity of the parent compound and it's oral bioavailability [3]. It is the first oral beta-lactam to combine high intrinsic acitivity with stability to beta–lactamase enzymes

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from most gram-positive and gram-negative organisms [4]. Cefuroxime exerts its bactericidal effect against a range of gram-positive and gram-negative bacteria by inhibiting the synthesis of bacterial cell wall [5-7]. It is a second generation oral cephalosporin antibiotic used for acute otitis media, bone and joint infections, meningitis, pharyngitis and tonsilitis, respiratory tract infections, septicemia, skin and skin structure infections [8]. It is active against some beta lactamase strains that are resistant to cefamandole [9,10].

The estimation of cefuoxime axetil by mercurimetric method [11], high performance liquid chromatography [HPLC] [12-16], electrokinetic [17], high performance thin layer chromatography [HPTLC] [18, 19], and spectroflurimetric [20] method is reported in literature. Although simultaneous UV spectrophotometric estimation of cefuroxime axetil has been reported by many analyst in bulk and in pharmaceutical formulation with 0.1N NaOH, 0.1N HCl and combination of methanol and pH 7.0 phosphate buffer in ratio of (2:8) as solvent [21-23]. But single estimation of this drug with methanol as solvent has not been reported in bulk and in pharmaceutical formulation. Thus the present study was undertaken to develop and validate a simple, sensitive, accurate, precise and reproducible UV spectrophotometric method for cefuroxime axetil in methanol as solvent as per International Conference of Harmonization guidelines [24]. The method has been successfully applied for the determination of the studied cefuroxime axetil in commercial dosage forms. Statistical comparisons of the results with the reference methods show excellent agreement and indicate no significant difference in accuracy and precision. Hence proposed method was precise, accurate and cost effective. This method can be applicable for quantitative determination of the titled drug with respect to assay from their new commercial formulation in quality control laboratories.

2. Experimental

2.1. *Materials and reagents*

Cefuroxime axetil used as working standard was provided by Reneta Pharmaceuticals Ltd. (Bangladesh) and methanol of commercial grade used throughout the experiment was obtained from the local market.

2.2. *Instrumental condition*

The instrument used was an UV-Vis double beam spectrophotometer, Shimadzu UV spectrophotometer UV-1800 with matched pair quartz cell for this study.

2.3. *Method development*

2.3.1. *Selection of media*

Main criteria for media selection are solubility and stability, i.e. drug should be soluble as well as stable for sufficient time in selected media. Solubility test of the drug cefuroxime

axetil was performed by using various solvents. The solvents include ethanol, methanol, chloroform, 0.1 N NaOH and 0.1 N HCl. However, the drug is freely soluble in methanol. Hence, methanol was chosen as a solvent for developing the method and cost of methanol is low as compared to other solvents.

2.3.2. *Preparation of standard stock solution*

Weighed and transferred accurately, equivalent to 10 mg of cefuroxime axetil as working standard into 100 mL volumetric flask. Dissolved and diluted up to the mark with distilled methanol and prepared a stock solution. Transferred 2.5 mL solution from the stock to 50 mL volumetric flask with distilled methanol to produce a concentration of 5 µg/mL and used as standard stock solution.

2.3.3. *Determination of λ_{max}*

From the stock solution 0 mL, 0.8 mL, 1.60 mL, 3.2 mL, and 6.4 mL were pipetted into 10 mL volumetric flask and the volume was made up with distilled methanol to produce concentration of 0 µg/mL, 0.4 µg/mL, 0.8 µg/mL, 1.6 µg/mL and 3.2 µg/mL. The solutions were scanned in UV-VIS spectrophotometer in the range 400-200 nm using methanol as a blank. The wavelength corresponding to maximum absorbance (λ_{max}) was determined as 278 nm (Fig. 1 A).

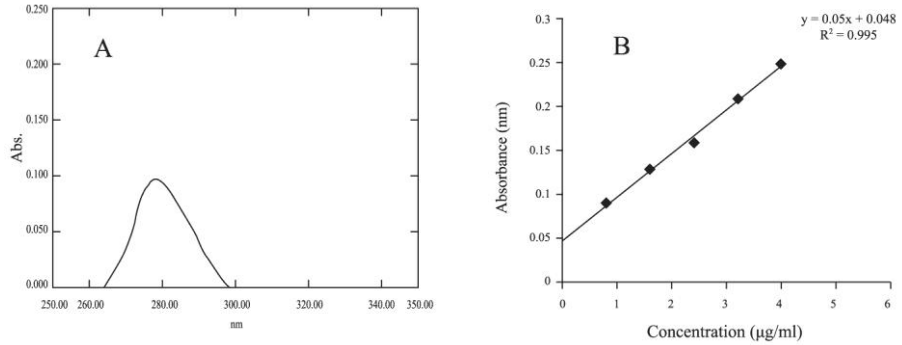

Fig. 1. UV spectrum of cefuroxime axetil (λ_{max} determination) (A), and calibration curve of cefuroxime axetil (B).

2.3.4. *Preparation of calibration curve*

The stock solution (5 µg/mL) was diluted to prepare 0 µg/mL, 0.4 µg/mL, 0.8 µg/mL, 1.2 µg/mL, 1.6 µg/mL, 2 µg/mL, 2.4 µg/mL, 2.8 µg/mL, 3.2 µg/mL, 3.6 µg/mL, 4.0 µg/mL, and 6.0 µg/mL, respectively and absorbance was recorded at $\lambda_{max.}$ The calibration curve was constructed by taking the solution concentration ranged from 0 to 4.0 µg/mL.

against the absorbance. The curve showed linearity in the concentration range of 0.8-3.6 µg/mL (Fig. 1 B).

2.4. *Method validation*

2.4.1. *Linearity study*

Various aliquots of efuroxime axetil were prepared from the stock solution (5 µg/mL) ranging from 0.0 to 4.0 µg/mL. Linearity of the method for cefuroxime axetil was tested from 0.0-40 % of the targeted level of the assay concentration in triplicate. The solutions were scanned using distilled methanol as blank and the result showed the similarity as shown in Fig.1 B.

2.4.2. *Specificity*

Various aliquots of cefuroxime axetil were prepared from the stock solution (5 µg/mL) ranging from 0.0-3.6 µg/mL**.** The solutions were scanned in UV-VIS spectrophotometer in the range 400-200 nm using methanol as a blank. The data showed specificity as absorbance maximum (Fig. 2).

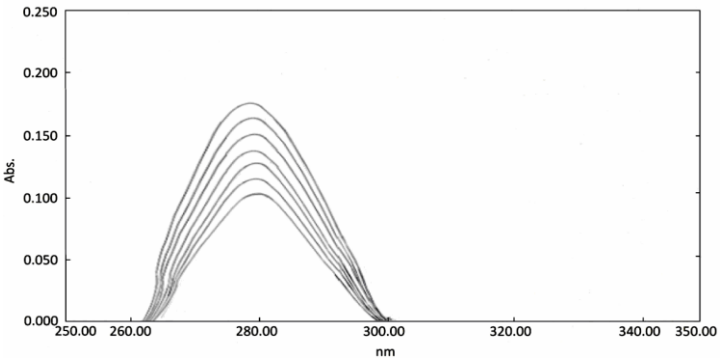

Fig. 2. Specificity of the method.

2.4.3. *Accuracy*

Accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts to tablet. The recovery was performed at three levels, 80, 100 and 120% of cefuroxime axetil standard concentration. The recovered samples were prepared in aforementioned procedure. Three samples were prepared for each recovery level. The solutions were then analyzed, and the percentage recoveries were calculated from the calibration curve. The result is shown in Table 1.

Table 1. Accuracy of the developed UV-Vis spectrophotometric method.

% Recovery	Formulation (µg/ml)	Drug added (µg/ml)	Drug found (µg/ml)	% Recovery	Avg. recovery	% RSD
80	1.6	1.28	1.270	99.2		
80	1.6	1.28	1.281	100.1	99.90	0.479
80	1.6	1.28	1.285	100.4		
100	1.6	1.60	1.630	101.9		
100	1.6	1.60	1.590	99.3	100.05	1.371
100	1.6	1.60	1.580	98.7		
120	1.6	1.92	1.924	100.3		
120	1.6	1.92	1.926	100.3	99.85	0.012
120	1.6	1.92	1.900	98.9		

2.4.4. *Precision*

Precision of the method was demonstrated by inter-day and intra-day variation studies**.** In inter-day variation study cefuroxime axetil solution of same concentration, 2.8 µg/mL of five replicates was analysed for three times in a day i.e. zero hour, four hours and eight hours. From the absorbance obtained, % RSD was calculated**.** In the inter-day precision analysis, solution of same concentration, 2.8 µg/mL in five replicates was analysed for three different days and % RSD was calculated. The result is shown in Table 2.

Table 2. Precision of the developed UV-VIS spectrophotometric method.

Inter-day Precision			
Parameter	0 hour	4 hour	8 hour
Mean conc. (µg/mL)	2.68	2.78	2.81
% RSD	1.62	1.45	1.92
Intra-day precision			
Mean conc. (µg/mL)	2.84	2.78	2.69
% RSD	1.18	0.96	1.51

2.5. ***Analysis of marketed formulation***

10 tablets were weighed accurately and powdered. Each containing of 250 mg quantity of cefuroxime axetil. Equivalent to 10 mg of cefuroxime axetil was weighed and dissolved in 100 mL methanol. The solution was filtered through whatman filter paper. Transferred 2.5 mL solution from this to 50 mL volumetric flask with distilled methanol to produce a

concentration of 5 µg/mL. From this stock an aliquot was pipetted into a 10 mL volumetric flask and diluted with distilled methanol to set the theoretical concentration of the drug at 2.8 µg/mL and the concentration of the drug was determined. The result is shown in Table 3.

Table 3. Application of the developed method in the analysis of marketed formulation containing 250 mg cefuroxime axetil in each tablet.

Brand	Amount of drug labelled (mg)	Amount of drug estimated (mg)	% Labelled claim
Brand A	250	239	95.6
Brand B	250	240	96

3. Results and Discussions

A UV- spectrophotometric method has been developed and validated for determination of cefuroxime axetil in bulk and pharmaceutical formulation using methanol as solvent. The spectral data for the determination of λ_{max} is shown in Fig.1A. The wavelength 278 nm was selected as λ_{max} because the drug in methanol showed maximum absorbance at this wavelength. The method was validated to ensure linearity, selectivity, accuracy, and precision. When absorbance was plotted against concentration, a good correlation coefficient was obtained in the concentration range of 0.8-3.6 µg/mL. The developed method was linear in the mentioned concentration range with correlation coefficient of 0.995 as shown Fig. 1B.

To observe the specificity of the methods various aliquots were prepared and scanned using methanol as blank as described under materials and methods. It was found that various concentration of cefuroxime axetil, showed maximum absorbance at 278 nm. (Fig. 2), which indicated the specificity of the developed methods.

The recovery assay was performed to ensure the accuracy of the developed method using thee concentration levels, 80%, 100% and 120% of cefuroxime axetil in triplicates. The test result is shown in Table 1. Recovery values ranged from 99.2 to 100.4% at 80% recovery, 98.7 to 101.9% at 100% recovery and 98.9 to 100.3% at 120% recovery. The average recoveries at three levels were 99.9%, 100.05%, and 99.85%, respectively. The recovery results showed that the proposed method had acceptable level of accuracy for cefuroxime axetil.

The inter-day precision of the method was also evaluated at three different times of the day, while intra-day precision was evaluated by carrying out three independent assays of cefuroxime axetil at three concentration levels using five replicates of each concentration. The results are summarized in Table 2. In case of interday precision the calculated relative standard deviation (RSD) was ranged between 1.45-1.92 % and for intraday assay RSD was ranged between 0.96- 1.51. In both cases the values were less than the maximum allowed limit [23, 24].

To apply the developed method in the analysis of marketed formulation, two brands were collected from the local market and assayed using the developed method as described before. The concentrations of the drug were calculated from linear regression equation. The potency was found to be 95.6% to 96.0% as shown in Table 3 which was found in good agreement with the label claimed. These data indicate that, the proposed method based on UV-VIS spectrophotometry is precise, accurate and simple to perform analysis of cefuroxime axetil in dosage form and economy in practice.

Table 4. Comparison of the result with the existing standard method of estimation [21-23].

Parameter	Result				
	Methanol	0.1N HCl	2:8 (methanol: pH 7.0 phosphate buffer)	0.1 N HCl	0.1N NaOH 1st derivative method
Absorption maxima (nm)	278	281	281	281	266
Linearity range (µg/ml)	0.8-3.6	0.4-2	4-28	2-30	4-30
Standard regression eq.	$Y = 0.05x+0.048$	$Y = 0.453x+0.078$	$Y =0.0346x+0.0566$	$Y =0.044x+0.004$	$Y =0.00714x+0.0014$
Correlation coefficient (r^2)	0.995	0.998	0.999	0.999	0.99
Accuracy (% recovery)	99.93	98.54-99.98	99.97	99.95	99.34
Precision (% RSD)	Interday (1.45-1.92)% Intraday (0.96-1.51)%	Interday (0.61-1.43)% Intraday (0.54-1.47)%	Interday 99.60% Intraday 99.50%	-	-

Although, several methods have been developed to determine cefuroxime axetil using other solvents such as (0.1 N NaOH, 0.1N HCl, combination of methanol and pH 7.0 phosphate buffer in ratio of 2:8) as shown in Table 4. [20-22], this is for the first time we used methanol, a cheap and most available solvent to develop the method. It does not require expensive or sophisticated instrument and chemicals in contrast with other chromatographic methods such as HPLC. Hence, it can be used for routine analysis of bulk and solid dosage form. Moreover, compared to other methods as reported previously (Table 4), the present proposed method using methanol as solvent was reproducible and simple. The linearity, accuracy and precision of the developed method might add values in the determination of cefuroxime axetil with simplicity and less expensive in terms of money and time.

4. Conclusion

This UV-VIS spectrophotometric technique is quite simple, accurate, precise, reproducible and sensitive. The UV method has been developed for quantification of cefuroxime axetil in pharmaceutical dosage form. The validation procedure confirms that this mehod can be appropriately used for the quantification of cefuroxime axetil in the raw

materials and in formulations. It may also be used in routine quality control of the raw materials and formulations in pharmaceutical industry.

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References

1. United States Pharmacopoeia (XXIV) and National Formulary (XIX), Asian Edition (US Pharmacopieial Convention, Inc., Rockville, MD, 2000)
2. P. A. Insel, Analgesic-antipyretin and antiinflammatory agents and drugs employed in the treatment of gout. *In* Goodman and Gilman's The Pharmacological Basis of Therapeutics, 9th ed. J.G. Hardman, L.E. Limbird, P.B. Molinoff, R.W. Ruddon, and A.G. Gilman, editors. McGraw Hill, New York, 996) pp. 617-657.
3. A. Finn, A. Straughn, and M. Meyer, Biopharm. Drug Dispos. **8**, 519 (1987). http://dx.doi.org/10.1002/bdd.2510080604
4. P. Williams and S. M. Harding, J. Antimicrob. Chemother. **13**, 191 (1984). http://dx.doi.org/10.1093/jac/13.2.191
5. K. Deguchi, N. Yokota, M. Koguchi, Y. Suzuki, S. Fukayama, R. Ishihara, S. Oda, S. Tanaka, Y. Nakane, and T. Fukumoto, Jpn. J. Antibiot. **47**, 365 (1994).
6. L. J. Scott, D. Ormrod, and K. L. Goa, Drugs; **61**, 1455 (2001). http://dx.doi.org/10.2165/00003495-200161100-00008
7. P. Dellamonica, Int. J. Antimicrob. Agents; **4**, 23 (1994). http://dx.doi.org/10.1016/0924-8579(94)90061-2
8. C. Dollery, Therapeutic Drugs, 2nd Edition (Churchill Livingstone, London UK.1999).
9. United States of Pharmacopoeia and National Formulary, 27th edn. (US Pharmacopieal Convention Inc., Rockville, MD, 2000).
10. P. A. Insel, J. G. Hardman, Goodman and Gilman's the Pharmacological basis of Therapeutics, 9th edn. (New York, Mc-Graw Hill, New York, 1996) p. 617.
11. B. Pospisilova and J. Kubes, Pharmazie. **43** (4), 246 (1998).
12. L. Zivanovic, I. Ivanovic, S. Vladimirov, and M. Zeceric, J. Chromatogr. B **800**, 175 (2004). http://dx.doi.org/10.1016/j.jchromb.2003.08.046
13. M. Zajac, L. Dobrowolski, and I. Osczaprwicz, J. Pharm. Biomed. Anal. **32**, 1181 (2003). http://dx.doi.org/10.1016/S0731-7085(03)00237-1
14. R. Peleman, H. Hoorekebe, and R. Pauwels, J Chromatogr. B Biomed. Sci. Appl. **689**, 438 (1997). http://dx.doi.org/10.1016/S0378-4347(96)00329-5
15. K. D. Altria, C. J. Campbell, and M. M. Rogan, J. Pharm. Biomed. Anal. **8**, 1005 (1990). http://dx.doi.org/10.1016/0731-7085(90)80160-Q
16. P. N. Patil and S. Jacob, Int. J. Pharm. Sci. Res. **3(1),** 1 (2011).
17. C. J. Sciacchitano and B. Mopper, J. Chromatogr. B Biomed. Appl. **657**, 395 (1994). http://dx.doi.org/10.1016/0378-4347(94)00355-6
18. S. R. Dhaneshwar, S. S. Kadam, and D. V. Sirisha, Ind. J. Pharm. Sci. **66**, 278 (2004).
19. N. J. Shaha, S. K. Shaha, V. F. Patel, and N. M. Patel, Ind. J. Pharm. Sci. **69** (1), 140 (2007). http://dx.doi.org/10.4103/0250-474X.32131
20. J. A. Murillo, J. M. Lemus, and L. F. Garcia, J. Pharm. Biomed. Anal. **12** (7), 875 (1994). http://dx.doi.org/10.1016/0731-7085(94)E0018-V
21. S. Shelke, S. Dongre, A. Rathi, D. Dhameeha, S. Maria, M. Hassan, and G. Dehghan, Asian J. Res. Chem. **2** (2), 222 (2009).

22. M. D. Game, D. M. Sakarkar, K. B. Gabhane, and K. K. Tapar, Int. J. Chem. Tech. Res. **2** (2), 1259 (2010).
23. J. Pritam, M. P.Sanjay, and S. Sanjay, Int. J. Drug Dev. Res. **3** (4), 318 (2011).
24. ICH-Guidelines Q2 (R1), Validation of Analytical Procedures: Text and Methodology (2005). http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf