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New Spectrophotometric Methods For The Determination Of Cefetamet In Pharmaceutical Preparations

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ABSTRACT

Two sensitive and simple spectrophotometric methods for the determination of cefetamet have been described in this paper. The method is based on the interaction of diazotized cefetamet with N-(1-naphthyl) ethylenediamine dihydrochloride(NEDA) in neutral or 1-naphthol(1NPL) in alkaline medium. Absorbance of the resulting chromophores is measured at 553 nm or 556 nm respectively. The two coupling reagents are applied successfully for the determination of cefetamet in tablets. The common excipients used as additives in pharmaceutical preparations do not interfere with the determinations. Results from the analysis of pure cefetamet and its commercial tablet by the proposed methods agree well with the reported method. © 2007 Trade Science Inc. - INDIA

KEYWORDS

Cefetamet;
Diazotization;
NEDA;
1NPL;
Spectrophotometry.

INTRODUCTION

Cefetamet is chemically known as(z)-7-[2-(2-aminothiazol-4-yl)-2-methoxyiminoacetamido]-3-methyl-3-cephem-4-carboxylic acid[1,2]. It is a third generation cephalosporin antibiotic characterized by a beta-lactamase producing organisms in addition to its antimicrobial activity against *streptococci*, *staphylococci*, *pneumococci*, etc[3]. Cephalosporin will be distributed widely into tissues and body fluids, including pleural, pericardial, and synovial fluids. However, while earlier cephalosporins failed to penetrate the central nervous system, but this third-generation cephalosporin enters the central nervous system and reach therapeutic concentrations, there by sufficient for the treatment of *pharyngitis* caused by aerobic gram-negative bacteria[4]. These characteristics are of considerable clinical and hence analytical interest[5]. This new drug is not yet included in USP, BP or IP. The reported analytical procedures are available in the literature for the analysis of cefetamet, via high performance liquid chromatographic[6,7], reversed phase high performance liquid chromatographic[8], liquid chromatographic[9] and liquid chro-

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matographic-tandem mass spectrophotometric[10] methods. To our knowledge, no spectrophotometric method for the determination of cefetamet has yet been reported despite the versatility, simplicity and reliability of the technique in chemical analysis. The requirements of pharmaceutical quality control are more severe than in other fields; drug control requires excelled accuracy, specificity and precision. Further, because active component are often in low amounts in pharmaceutical formulations, the methods must be very sensitive. These requirements are fulfilled by two methods presented in this paper. In the present study we have succeeded in developing new coupling agents for the sensitive and selective spectrophotometric determination of cefetamet based on the interaction of diazotized cefetamet with NEDA or 1NPL and the formation of intense purple colored chromogens in neutral aqueous medium or alkaline medium respectively. This method offers the advantage of sensitivity, selectivity and rapidity without need of extraction or heating.

EXPERIMENTAL

Spectral and absorbance measurements were made with ELICO SL 164 double beam spectrophotometer with 1.0cm quartz cells.

All chemicals and reagents used were of analytical grade and all solutions were prepared in doubly distilled water. Cefetamet was obtained from Alembic, India, NEDA and 1NPL from SD Fine, India.

Solutions

Stock solutions were prepared by accurately weighing 100mg of cefetamet into 100ml calibrated flask, dissolved in 100ml double distilled water and kept in dark to avoid any degradation of the drug. The working standard solution cefetamet containing 100μgml^{-1} was prepared by further dilution. A 0.1% aqueous solution of NEDA and 0.1% 1NPL in 20% sodium hydroxide were prepared and protected from sunlight. A 0.05% aqueous solution of sodium nitrite, 0.5% aqueous solution of ammonium sulfamate and concentrated hydrochloric acid were used.

Procedure

Aliquots of the working standard solution of cefetamet(1.0-5.0ml) (1ml=100μg) were transferred into 10 ml calibrated volumetric flasks; 0.2ml of concentrated hydrochloric acid was added to each. After cooling in an ice-bath, 1.0ml of sodium nitrite solution(0.05%) was added with swirling. The solutions were swirled and allowed to stand for 5 min. Next 1.0ml volume of coupling reagent solution NEDA (0.1%) or 1NPL(0.1%) in alkaline solution was added, after 5min the solutions were diluted to the mark with doubled distilled water. After mixing the solution thoroughly, the absorbance was measured at 553 nm for NEDA or 556 nm for 1NPL against the corresponding reagent blank and calibration graphs were constructed.

Procedure for assay of cefetamet in commercial samples

Tablets

Ten to forty tablets depending on content per tablet were weighed and ground into a fine powder. An amount of the powder equivalent to 100mg of active component was weighed into a 100ml volumetric flask, about 60ml of double distilled water was added and shaken thoroughly for about 20min. the volume was increased to the mark with double distilled water, shaken and filtered using quantitative filter paper. For spectrophotometric determination, the filtrate was diluted sequentially to get 100μgml^{-1} of drug. A suitable portion was then used for analysis.

RESULTS AND DISSCUSION

Spectral characteristics

The cefetamet was diazotized in acidic medium and coupled with NEDA in neutral aqueous medium to form purple colored dye of λmax 553nm or coupled with 1NPL in alkaline medium to form purple colored dye of λmax 556nm. These wavelengths were used for all measurements. The absorption spectra of cefetamet reaction products formed and the reagent blank are shown in figure 1.

Optimum reagents concentrations

For the diazotization-coupling reaction, the concentrated hydrochloric acid in the range of 0.2-0.5ml, a 0.05% solution of sodium nitrite in the range of

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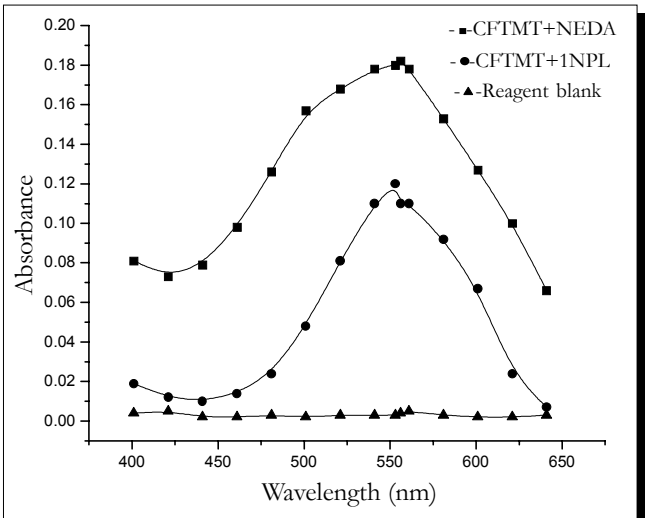

Figure 1: Absorpation spectra of cefetamet drug. Initial concentration of cefetamet 10µg ml^{-1}

0.5-1.5 ml, a 0.5% solution of ammonium sulfamate in the range of 0.5-2.0ml and 0.1% NEDA or 1NPL in the range of 0.5-2.0ml were necessary to achieve maximum color intensity and hence, 0.2ml of hydrochloric acid, 1.0ml sodium nitrite and 0.5 ml ammonium sulfamate, were selected for diazotization. A 1.0ml of NEDA or 1NPL was used as coupling agent to obtain maximum intensity and stability of the color. The color intensity increases slightly in the first three minutes, but then remains constant for more than four hours.

The study of the effect of acidity on the diazotization-coupling of cefetamet showed that the acidity is moderately critical. Higher acidity could not be ascertained because at this very strong acidity the excess nitrate could not be destroyed by ammonium sulfamate and as a result an intense color was produced by the nitrosation of the NEDA(purplish) or 1NPL(purplish). Maximum intensity and stability of the color were obtained with addition of 0.2-0.5ml of concentrated hydrochloric acid and 0.2ml of hydrochloric acid is recommended other mineral acids were tested and found to be unsatisfactory.

When 1.0ml of 0.05% solution of sodium nitrite was used the excess of nitrite could be removed by the addition of 0.5ml of 0.5% ammonium sulfamate solution. An excess of ammonium sulfamate has no effect on color.

In case of NEDA as a coupling agent dilution of the colored solution with different solvents like water, methanol, ethanol, acetic acid and acetonitrile have been tested. Results showed that water gives maximum intensity and stability of the color. In case 1NPL as a coupling agent the purple color is formed in alkaline medium. It was found that 15-25% of sodium hydroxide as diluents was necessary for the achievement of maximum color intensity. Hence 20% sodium hydroxide was selected for all further studies.

It was found that a 0.1% concentration of NEDA or 1NPL in the range 10-50µgml^{-1} was necessary for the maximum intensity and stability of the color. Hence 1.0 ml of NEDA or 1NPL was selected for further studies.

Quantification

Beer's law was obeyed over the cefetamet concentration range 10-50µgml^{-1} for NEDA or 1NPL as coupling agents. The calibration graph are described by the equation:

Y=a + b X

(Where Y=absorbance, a=intercept, b=slope and X=concentration in µgml^{-1}) obtained by the method of least squares. Correlation coefficients, intercepts and slopes for the calibration data are also presented in TABLE 1. Sensitivity parameters such as molar absorptivity and Sandell's sensitivity values and the limits of detection and quantification, are also presented in TABLE 1.

TABLE 1: Parameters for the spectrophotometric determination of cefetamet

Optical character	Values of NEDA	Values of 1NPL
Beer's law range (µg ml^{-1}) (C)	10-50	10-50
Molar absorptivity (l mol^{-1} cm^{-1})	2.2811×10^{3}	2.2326×10^{3}
Sandell's sensitivity (µg cm^{-2})	0.025	0.042
Limit of detection (µg ml^{-1})	1.22	0.88
Limit of quantification (µg ml^{-1})	4.06	2.96
Regression equation(Y)[a]		
Slope (b)	4.1452×10^{-3}	5.0642×10^{-3}
Intercept(a)	4.4865×10^{-2}	3.1035×10^{-2}
Correlation co-efficient (r)	0.9997	0.9998
R.S.D. (%)[b]	1.3573	1.0010
% Range of errors [b]		
(95% confidence level)	±0.0019	±0.0024

[a]**Y=a + b C, where Y is the absorbance, and C concentration in(µg ml^{-1}) for cefetamet**

[b]**Relative standard deviation(n=5)**

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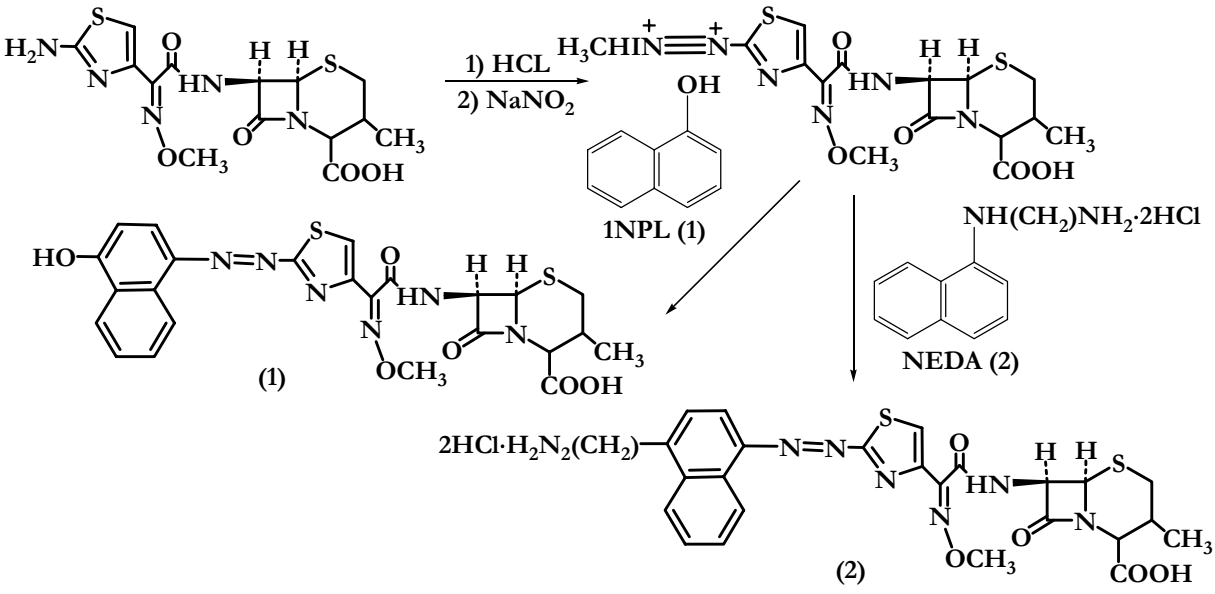

SCHEME 1

Reaction sequence

In an acidic medium nitrite reacts with cefetamet to form diazonium salt. The salt is then coupled with NEDA in neutral and 1NPL in alkaline to yield purple colored azo dyes of λmax 553 nm and 556 nm respectively. The reaction mechanism is shown in SCHEME 1.

Stability

The diazotization of cefetamet is complete in five min. at room temperature. The diazotized salt is then stable for five to ten minutes and then deteriorates slowly. Cooling in ice increased the time needed for diazotization and did not eliminate deterioration. Hence, the reaction is studied at room temperature. The azo products resulting from the suggested method were studied at different temperatures. It was found that the absorbances values remain constant in the temperature range 5-60^{0}C. Hence, the room temperature was recommended for the coupling reaction. The purple colored azo products were stable for more than four hours and results were reproducible.

Interference

Under the diazotization reaction conditions used, other anilines, gave a positive reaction. However, the problem of interferences does not arise in the analysis of the commercially available cefetamet dosage forms. The effects of additives associated with the cefetamet in its formulations were investigated using the developed method. This method does not suffer any interference from common excipients, additives and other substances such as magnesium stearate, glucose, lactose, dextrose, starch, gum acacia, talc, carboxy methyl cellulose and sodium alginate. The results are given in TABLE 2. The effects of additives associated with the cefetamet in its formulations were investigated using the developed method. This method does not suffer any interference from common excipients, additives such as magnesium stearate, talc, starch, lactose, sodium alginate and dexstrose.

TABLE 2: Determination of cefetamet[a] in the presence of excipient and other sustenance's

Material	Amount (mg)	% Recovery of cefetamet ± (R .S .D)[b].
Magnesium stearate	40	99.80 ± 0.37
Glucose	40	99.70 ± 0.39
Lactose	40	99.50 ± 0.52
Dexstrose	40	99.50 ± 0.82
Starch	40	99.30 ± 1.28
Gum acacia	40	99.50 ± 1.05
Talc	40	99.81 ± 0.82
Carboxy methyl cellulose	40	99.60 ± 1.31
Sodium alginate	40	99.40 ± 0.79

[a]10μg ml^{-1} of cefetamet taken
[b]Average of five determinations

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TABLE 3: Results of an assay of cefetamet in pharmaceutical formulations

Preparations*	Label claim mg/tablet	Found Ψ(recovery ± s d)			Students	t-value	F-value Φ	
		Value of NEDA	Value of 1NPL	Reference method	NEDA	1NPL	NEDA	1NPL
Altamet[a]	250	99.88 ± 0.81	99.92 ± 0.89	99.89 ± 1.12	0.08	0.028	1.91	1.58
Cepime O[b]	500	99.56 ± 0.86	99.69 ± 0.56	99.74 ± 0.96	0.39	0.13	1.25	2.93
Ultipime O [c]	250	99.94 ± 0.34	99.74 ± 0.24	99.93 ± 0.42	0.05	1.11	1.52	3.06

***Marketed by: a, Alembic Ltd.; b, Alembic Ltd. and c, Recon Laboratories.**
ΨMean value of eight determinations
Tabulated value at 95% confidence level is 2.365
ΦTabulated value at 95% confidence level is 3.79

TABLE 4: Results of recovery studies by the standard-addition technique

	Method NEDA				Method 1NPL			
Formulations	Amount of drug in formulation/mg	Amount of drug added	Total found/mg	% Recovery of pure drug[a]	Amount of drug in formulation/mg	Amount of drug added	Total found/mg	% Recovery of pure drug[a]
	3.0	3.0	6.02	100.66	3.0	3.0	5.96	98.66
Altamet(250 mg)	3.0	7.0	10.10	101.42	3.0	7.0	10.06	100.85
	3.0	10.0	12.92	99.2	3.0	10.0	13.09	100.90
	3.0	3.0	5.98	99.33	3.0	3.0	6.02	100.66
Cepime O (500 mg)	3.0	7.0	10.04	100.57	3.0	7.0	9.98	99.71
	3.0	10.0	13.10	101.0	3.0	10.0	13.14	100.40
	3.0	3.0	6.08	102.66	3.0	3.0	6.06	102.00
Ultipime O (250mg)	3.0	7.0	9.94	99.14	3.0	7.0	10.08	101.11
	3.0	10.0	12.97	99.97	3.0	10.0	12.98	99.80

a. Average of three determinations

Application

The developed methods were applied to the assay of cefetamet in commercially available tablets; the results are given in TABLE 3. The same batch tablets were also analyzed by the official method[6]. A statistical analysis of the results using the student's t-test and F-test showed no significant differences with regard to the accuracy and precision. The reliability and accuracy of the methods were further confirmed by recovery studies through the standard-addition method. To a fixed and known amount of the drug in a tablet solution(pre-analyzed), pure cefetamet was added at three different levels, and the total amount was found by the proposed methods. Each level was repeated three times using three different market formulations. The percent recoveries of the added pure drug were given in TABLE 4. which indicate that the commonly encountered tablet excipient, such as talc, starch, gum acacia, lactose, sodium alginate, dextrose and magnesium stearate, did not interfere with the determination by the proposed method.

CONCLUSION

Although cefetamet have been determined by a variety of technique, the methods described here are simple, sensitive(spectrophotometric), convenient and do not require special working condition, unlike many other reagents. The statistical parameters and the recovery study data clearly indicate the reproducibility and accuracy of the method. These merits, in addition to the use of simple and inexpensive chemicals and instrument, suggest the use of methods for routine analysis.

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REFERENCES

[1] S.C.Sweetman; 'Martindale-The Complete Drug Reference', 33rd Ed., Pharmaceutical Press.

[2] M.J.O' Niel; 'Merck Index' 13th Ed., Merck and Co. Inc., N.J.Rathway, USA, 1937 **(2001)**.

[3] D.Basseti; 'Chemioterpici, Antiffetive e Loro Impiego Razinole', 4th Ed., Lomardo, Rome, Italy, **(1986)**.

[4] B.G.Kantzung; 'Basic and Clinical Pharmacology', 2nd Ed., Appeleton and Lange, 522-525 **(1988)**.

[5] F.M.Demotes Mainard, G.A.Vincon, C.H.Jarry., C.Albin; J.Pharm.Biomed.Anal., **6**, 07 **(1988)**.

[6] Wang, Jain; Yaowu Fenxi Zazhi., **19**, 264 **(1994)**.

[7] Wang, Jain; Zhongguo Yiyao Gongye Zazhi., **31**, 308 **(2000)**.

[8] L.W.Laichun, Jaing, Xuehua, P.W.Yantang; Huaxi Yaoxue Zazhi., **17**, 196 **(2002)**.

[9] Morch, M.Lisoni, Bitten court, F.Celso; J.Pharm. Biomed.Anal., **30**, 643 **(2002)**.

[10] Chen, Xiaoyan, Defang; J.Chrom.Anal.Techn., **17**, 784 **(2003)**.