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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING UV SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF DICLOXACILLIN SODIUM IN BULK DRUGS

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ABSTRACT

A simple and precise stability-indicating UV Spectrophotometric method has been developed and validated for quantitative analysis of Dicloxacillin Sodium in the bulk drugs. Separation of the drug from its degradation products was achieved by UV spectrophotometric method using distilled water and scanned between 200 to 400 nm. The maximum absorbance was found to be at 273.60nm and found to be linear over the range 100-500 µg/ml with good correlation coefficient (r^2) 0.999. The limits of detection and quantification were 6.9623 and 21.0980 µg/ml, respectively. Forced degradation studies were carried out on Dicloxacillin Sodium by subjecting it to stress conditions [hydrolysis (acid, base), oxidation, photolysis, and thermal degradation] and the degraded samples were further analyzed by using this method. Major degradation was observed in alkaline, thermal and oxidative conditions. Dicloxacillin Sodium was quite stable under the other stress conditions investigated. Thus the method proved to be stability indicating. The proposed method was found to be economical, selective and sensitive for the desirable range.

KEYWORDS

Dicloxacillin Sodium, Forced degradation, Stability Indicating, UV spectrophotometry and Validation.

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INTRODUCTON

Chemically Dicloxacillin sodium is [2S - (2α, 5α, 6β)] - 6 - [[[3 - (2,6 - dichlorophenyl) - 5 - methyl - 4 - isoxazolyl] carbonyl]amino]-3, 3-dimethyl- 7 - oxo - 4 - thia - 1 – azabicyclo [3.2.0] heptane - 2 - carboxylic acid monosodium salt[1] (Figure No.1). It is a semi synthetic antibiotic substance which resists destruction by the enzyme penicillinase (beta-lactamase). Dicloxacillin is used to treat infections caused by bacteria such as bronchitis, pneumonia, or staphylococcal (also called "staph") infections[2].

It is official in United state Pharmacopoeia (USP)[3] and European Pharmacopoeia[4].

The literature survey reveals that the methods available for estimation of Dicloxacillin Sodium include UV Spectrometry[5-8], RP-HPLC[9-11], and HPTLC[12,13] methods. To the best of author's knowledge, there are few UV[14,15] and HPLC[16,17] stability indicating methods in combined dosage form, but no UV stability study using force degradation includes Acid, Alkali, Oxidation, Thermal and Photolysis has been reported independently for the estimation of Dicloxacillin Sodium in bulk. Accordingly the objective was to develop a simple, precise and accurate stability indicating assay method for the estimation of Dicloxacillin Sodium in bulk. This article mainly deals with the forced degradation of Dicloxacillin Sodium under the stress conditions like acid and base hydrolysis, oxidation, heat, and light, and validation of the method for accurate quantification of Dicloxacillin Sodium in bulk drugs.

EXPERIMENTAL

Reagents and Chemicals

Dicloxacillin sodium bulk drug (purity 99.7%) was procured as gift sample from Watson Pharma Ltd (Goa, India). Distilled water used in all experiments was prepared in analytical laboratory by distillation apparatus. Sodium hydroxide (NaOH) and hydrochloric acid (HCl) were from Ozone International (Mumbai) and hydrogen peroxide (H2O2) from DIPA Chemical Industry (Aurangabad).

Preparation of Standard Dicloxacillin Sodium Solution

In the present method distilled water is used as solvent because it has an advantage of being inexpensive, non-volatile and relatively ecofriendly. Moreover the maximum wavelength for drug remains stable by changing the concentration of the drugs.

Standard stock solution of Dicloxacillin Sodium was prepared by dissolving (100 mg) API in 100 ml distilled water to give a stock solution of concentration 1000 mcg / ml. In a 100 ml volumetric flask, pipette out5 ml from the standard stock solution and dilute it up to the mark with distilled water and scanned between 200 to 400 nm and 273.60 nm was found to be maximum wavelength for absorption as shown in Figure No.2.

Forced Degradation Studies[18]

Acid and alkaline hydrolysis

Forced degradation in acidic media was performed by adding 10mg of Dicloxacillin Sodium to 10 ml 0.01 N HCl and refluxing the mixture at 80°C for approximately 2hours. The solution was then left to reach ambient temperature, neutralized to pH 7 by addition of 0.01 M NaOH, then diluted up to 20 ml with 0.01 N HCl so as to get final concentration 500 µg/ml and run the spectrum as shown in Figure No.3.

Forced degradation in alkaline media was performed by adding 10 mg of Dicloxacillin Sodium to 10 ml 0.01 M NaOH and refluxing the mixture at 80°C for approximately 2hours. The solution was then left to reach ambient temperature, neutralized to pH 7 by addition of 0.01 N HCl, then diluted upto 20 ml with 0.01 M NaOH so as to get final concentration 500µg/ml and run the spectrum as shown in Figure No.4.

Oxidative Degradation

To study the effect of oxidizing conditions, an 10 mg of Dicloxacillin Sodium was added to 10 ml 3 % H_2O_2 solution. The solution was then diluted upto 20 ml with distilled water so as to get final concentration 500 µg/ml and run the spectrum as shown in Figure No.5.

Thermal Degradation

To study the effect of temperature, approximately 50 mg Dicloxacillin Sodium was stored at 80°C for 2 days. Then 10 mg taken and was dissolved in 10 ml distilled water and volume was adjusted upto 20 ml with distilled water. To get concentration 500µg/ml.

Photolysis

To study the effect of UV light, approximately 50 mg Dicloxacillin Sodium was exposed to short and long wavelength UV l3333ight (254 and 366 nm, respectively) for 24 hours (shown in Figure No.7), then it dissolved in 50 ml of distilled water and made up volume by distilled water in 100 ml volumetric flask to get final concentration 500

µg/ml. then spectrum were recorded and represented in Figure No.8 and Figure No.9 respectively.

METHODS VALIDATION

The method was validated for linearity, limits of detection (LOD) and quantification (LOQ), precision, accuracy and stability in accordance with ICHGuidelines[19]

Linearity

A standard stock solution of Dicloxacillin Sodium was prepared (1mg/ml). Aliquots of 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5 ml from standard stock solution of Dicloxacillin Sodium were pipette out in to a series of nine, 10 ml volumetric flasks and the volume was made upto 10 ml with distilled water to get concentration 100, 150, 200, 250, 300, 350, 400, 450 and 500µg/ml of Dicloxacillin Sodium respectively The absorbance were measured in triplicate at 274.40 nm against reagent blank. The calibration curve was constructed by plotting absorbance v/s concentration (mcg/ml) as shown in Figure No.8. Linearity was checked over the same concentration range on three consecutive days. % RSD of the slope and Y-intercept of the calibration plot was also calculated.

Detection Limits

The limits of detection (LOD) and quantification (LOQ) for Dicloxacillin sodium were determined as the amounts for which signal-to-noise ratios were 3:1 and 10:1, respectively.

Precision

To determine precision, 7 days measurement (intra-days and interday) were computed with relative standard deviation (RSD %) for replicate samples (n = 5) using concentration 200, 300 and 400µg/ml both the intra-day and interday samples were calibrated with standard curve concurrently prepared in the same day of analysis.

Intraday Precision

Intraday precision of test method is demonstrated by three samples of the same batch (same concentration) at initial, 24 and 48 hours (Table No.1).

Interday Precision

Interday precision of test method is demonstrated by three samples of the same batch (same concentration) on three successive days (Table No.1).

Accuracy

To determine the accuracy of the proposed method, recovery study was carried out by adding different amount (80%, 100%. 120%) of bulk sample of Dicloxacillin Sodium within the linearity range and results obtained are compiled in Table No.2 and show good accuracy for the method.

RESULTS AND DISCUSSION

Degradation behavior

UV spectrophotometry studies on Dicloxacillin Sodium under different stress conditions suggested the following degradation behavior (Table No.3).

Major degradation was observed in alkaline, thermal for 48 hours, photolysis under UV_{254} and oxidative conditions. However there was no appreciable change by thermal degradation for 24 hours and UV_{365} with acidic hydrolysis.

The stability studies indicates that appreciable changes were observed by treating the drug with UV light, thermal stress, oxidation and basic hydrolysis along with their appreciable change in λmax values . Thus the proposed method proved to be stability indicating.

The proposed method for determination of Dicloxacillin Sodium showed molar absorptivity of 0.6055×10^3 Lt. $mole^{-1}$ cm^{-1} and Sandell's sensitivity of 0.7766 mg/cm^2/0.001 absorbance units. Linear regression of absorbance on concentration gave the equation y = 0.001 x-0.010 with a correlation coefficient (r) of 0.999. The average percentage relative standard deviation (%RSD) for intraday and interday analysis was found to be 0.4351 and 0.7977 respectively. Limit of detection and limit of quantitation were found to 6.9623 and 21.0980ug/ml respectively. The higher percentage recovery value indicates that there is no interference of the excipients present in the formulation.

Table No.1: Intra-day and Inter-day Precision of Dicloxacillin Sodium

S.No	Dicloxacillin Sodium taken (µg/ml)	Intraday Accuracy and precision			Inter day Accuracy and precision		
		Dicloxacillin Sodium found (µg/ml)	RE %	RSD %	Dicloxacillin Sodium found (µg/ml)	RE %	RSD %
1	200	200.51	0.1709	0.2085	200.11	0.2959	0.3623
2	300	300.11	0.3239	0.5259	300.72	1.084	0.8836
3	400	400.15	0.4667	0.5711	398.24	1.8654	1.1473

Table No.2: Recovery Data

S.No	Level	Amount of Dicloxacillin sodium added (µg)	Amount of Dicloxacillin sodium found (µg)	% Recovery	% RSD
1	80 %	80	81.16	101.45	1.45
2	100 %	100	100.38	100.38	1.32
3	120 %	120	120.85	100.71	0.88

*An average value ± relative standard deviation of 5 observations

Table No.3: Summary of Forced Degradation Study of Dicloxacillin sodium (500ug/ml)

S.No	Condition	λmax	Absorbance	Concentration Obtained (ug/ml)	(%)	Degraded (%)	Observation
1		273.60	0.655				
2	Acid 2 hours	274.40	0.814	634.3077	126.8615	26.8615	Degraded
3	Alkaline fresh	274.40	0.961	747.3846	149.4769	49.4769	Degraded
4	Alkaline 2 hours	274.40	1.15	895.0759	179.0154	79.0154	Degraded
5	Thermal 24 hours	274.10	0.72	562.0000	112.401	12.401	Degraded
6	Thermal 48 hours	274.10	0.968	752.7692	150.5538	50.5538	Degraded
7	UV_{254}	274.40	1.031	801.2308	160.2462	60.2462	Degraded
8	UV_{365}	274.40	0.729	538.9231	107.7846	7.7846	Degraded
9	3% H_2O_2	274.40	0.892	694.3077	138.8615	38.8615	Degraded

Table No.4: Optical characteristics data and validation parameters of Dicloxacillin Sodium

S.No	Parameter	Analytical data
1	Linearity Range (µg/ml)	100 – 500
2	λ max (nm)	274.40
3	Molar extinction coefficient	6.055×10^4
4	Sandell's sensitivity	0.7766
5	Slope	1.3×10^{-3}
6	Intercept	-0.0106
7	Correlation co-efficient (r)	0.9998
8	Limit of detection (LOD, µg/ml)	6.9623
9	Limit of quantification (LOQ, µg/ml)	21.0980
10	Intra-day Precision (%RSD)	0.4351
11	Inter-day Precision (%RSD)	0.7977
12	Accuracy (% Recovery)	100.85

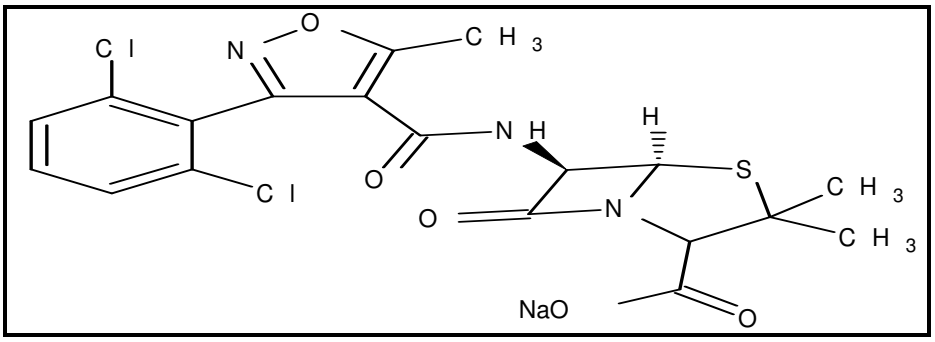

Figure No.1: Chemical structure of Dicloxacillin Sodium

Figure No.2: Standard spectrum of Dicloxacillin Sodium

Figure No.3: Acid degraded spectrum of dicloxacillin sodium

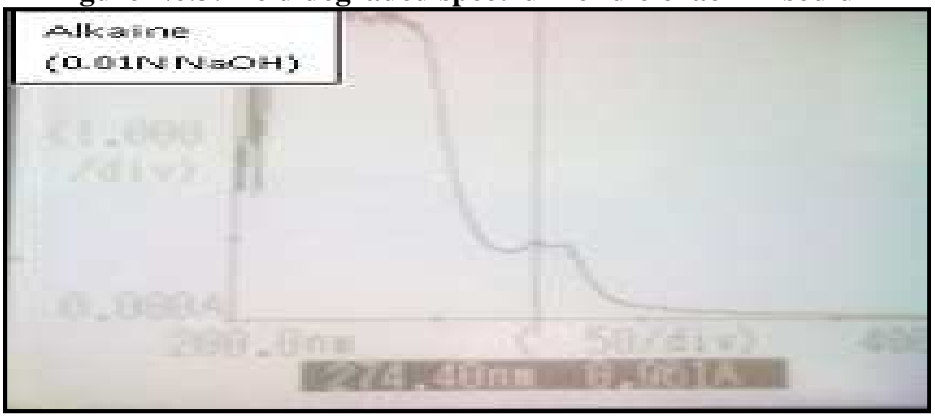

Figure No.4: Alkaline degraded spectrum of dicloxacillin sodium

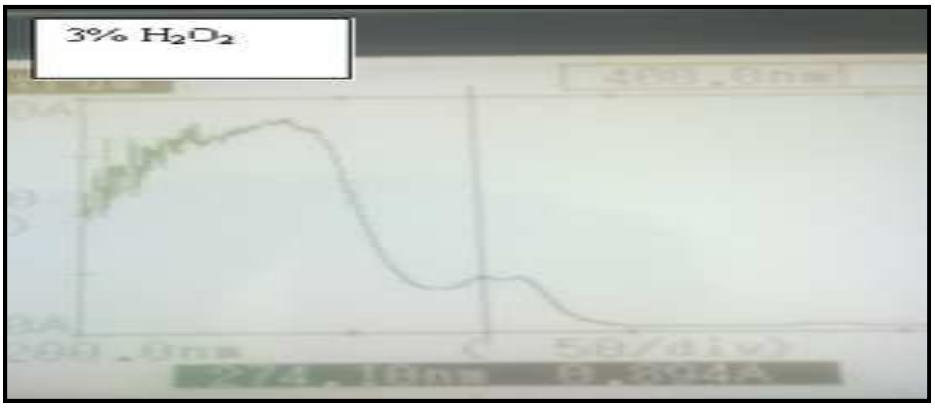

Figure No.5: Oxidative degraded spectrum of Dicloxacillin Sodium

Figure No.6: Thermal Degradation of Dicloxacillin sodium

Figure No.7: Photograph of Photolysis of Dicloxacillin Sodium λ max 254nm and 365nm

Figure No.8: Photolysis of Dicloxacillin Sodium at λ max 254 nm

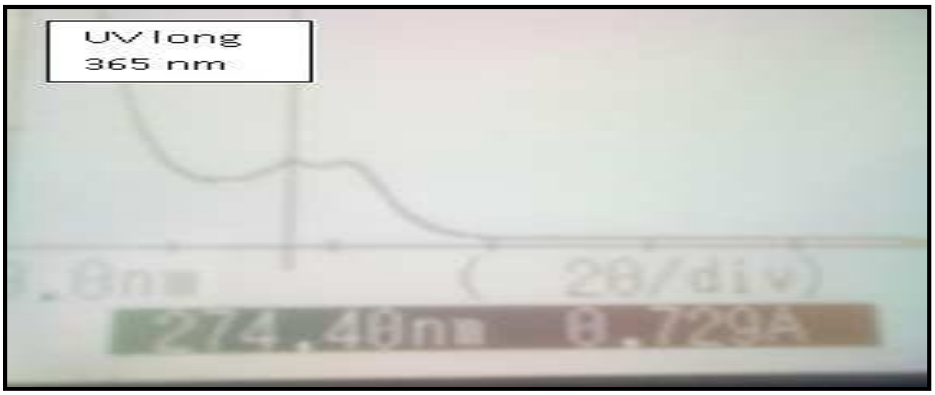

Figure No.9: Photolysis of Dicloxacillin Sodium λ max 365 nm

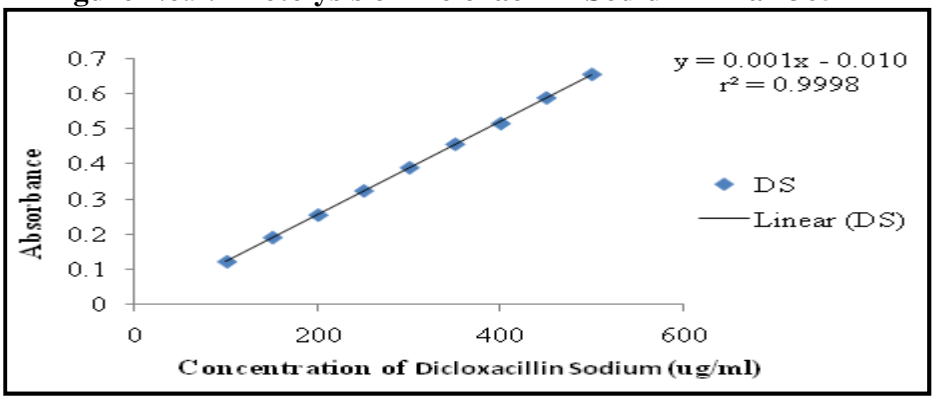

Figure No.10: Calibration curve for Dicloxacillin Sodium at 274.40 nm

CONCLUSION

Most Published stability indicating methods to quantify Dicloxacillin Sodium in bulk and pharmaceutical dosage form use highly sophisticated and costly instruments HPLC and HPTLC methods. The proposed UV method has been evaluated over the linearity, precision and accuracy and proved to be convenient and effective for the quality control of Dicloxacillin Sodium in bulk. The stability studies indicate that appreciable changes were observed by treating the drug with UV light, thermal stress, oxidation and basic hydrolysis. However there was no appreciable with acidic hydrolysis. Thus the proposed methodology is simple, as it requires a sample preparation procedure and easy to understand and apply. It represents a good procedure of Dicloxacillin Sodium estimation in bulk, hence method can be easily and conveniently adapted for routine quality control analysis of Dicloxacillin Sodium in bulk and pharmaceutical formulations.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBILIOGRAPHY

1. Maryadele and Neil J O. The Merck Index, an Encyclopaedia of Chemicals, Drugs and Biological, New Jersey: Published by Merck Research Laboratories, *Division of Merck and Co. Inc. Whitehouse station,* 14th Edition, 2006, 523.
2. Sweet Man S C. Eds: In the Martindale, The Complete Drug Reference, *London:*

Pharmaceutical Press, 35th Edition, 2007. 237.
3. The United State Pharmacopeia. USP30-NF24. *Rockville MD: United State Pharmacopeia Convention, Inc,* 2007, 1924-25.
4. European Pharmacopoeia, 5th Edition, 2005, 1422.
5. Solomon M, Sam W and Venkatnarayanan R. Simultaneous Estimation of Amoxycillin Trihdrate and Dicloxacillin Sodium in Formulation by UV-Spectroscopy, *Asian Journal of Research in Chemistry,* 4(2), 2011, 241-243.
6. Reddy A. UV Spectrophotometric method for Simultaneous Determination of Cefixime and Dicloxacillin in bulk and combined dosage form, *International Journal of Advance Pharmacy and Nanotechnology,* 2(1), 2012.
7. Chaudhari B G and Patel H J. Optimization of simultaneous analysis of Cefixime and Diclixacillin sodium in oral tablets, *International journal of Pharmaceutical science and Nanotechnology,* 7(4), 2014, 2638-2645.
8. Abdelrahman M M, Naguib I A, Elsayed M A and Zaazaa H A. Three Spectrophotometric Methods for Simultaneous Determination of Ampicillin and Dicloxacillin in Presence of Their Major Impurity 6-Aminopenicillanic Acid, *Austin Journal of Analytical and Pharmaceutical Chemistry,* 2(5), 2015, 1-7.
9. El-Wallity A F, El-Anwar F, Eid M A, Awaad H. High-performance liquid chromatographic and derivative ultraviolet spectrometric determination of Amoxicillin and Dicloxacillin mixtures in capsules, *Analyst Journal,* 117(6), 1992, 981-984.
10. Abdel M E, Abounassif M, El-RA, Gad K and Khattab N. Simultaneous Determination of Amoxycillin and Dicloxacillin in capsules by Potentiometric Titrimetry and High-Performance Liquid Chromatography, *Talanta,* 40(6), 1993, 811-817.
11. Karageorgou E, Samanidou V and Papadoyannis L. Ultrasound-assisted matrix solid phase dispersive extraction for the Simultaneous Analysis of β-lactams (Four Penicillins and Eight Cephalosporins) in milk by High Performance Liquid Chromatography with Photodiode Array Detection, Journal of Separation Science, 35(19), 2012, 2599-2607.
12. Tank M, Thumar K and Tanna R. Method Development and Validation for Simultaneous Estimation of Cefixime Trihydrate and Dicloxacillin sodium in combined dosage form by High Performance Thin Layer Chromatography, *Inventi Impact: Pharm Analysis and Quality Assurance,* 2012(3), 2012, 183-186.
13. Prashant U. Tompe, Madhura V. Dhoka, Mrinalini C. Damle and Ashwini R. Madgulkar. Validated HPTLC method for determination of dicloxacillin in simulated urine, *Journal of Chemical and Pharmaceutical Research,* 5(9), 2013, 77-83.
14. Patel S N, Kamlesh R. Prajapati and Dhrubo J S. Development and Validation of Stability Indicating Assay Method for the Estimation of Cefpodoxime Proxetil and Dicloxacillin Sodium in Tablet Dosage Form, *World Journal of Pharmacy and Pharmaceutical Sciences,* 3(5), 2014, 1108-1127.
15. Girona V, Pacareu C, Rjera A, Pouplana R, Castillo M and Bolos J. Spectrophotometric Determination of the Stability of an Ampicillin-Dicloxacillin suspension, *Journal of Pharmaceutical and Biomedical Analysis,* 6(1), 1988, 23-28.
16. Joseph Sunder R T, Bharatia C H, Ranga R K, Satyanarayana R P, Narayana G K A S S, Parikh K. Identification and characterization of degradation products of dicloxacillin in bulk and pharmaceutical dosage forms, *Journal of Pharmaceutical and Biomedical Analysis,* 43(4), 2007, 1470-1475.
17. Bhinge S D and Malipatil S M. Development and validation of a stability-

indicating method for the simultaneous estimation of Cefixime and Dicloxacillin using the RP-HPLC method, *Journal of Tibah university of Science,* 10(5), 2016, 734-744.

18. Charde M S, Kumar J. Welankiwar A S and Chakole R D. Review: Development of forced degradation studies of drugs, *International Journal of Advances in Pharmaceutics,* 2(3), 2013, 34-49.
19. ICH [Stability Testing of New Drug Substances and Products (Q1AR2)], International Conference on Harmonization, *Food and Drug Administration, USA,* November 1996 and February 2003.

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