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Research Article

STRESS DEGRADATION STUDIES ON AMOXICILLIN AND CLOXACILLIN IN DOSAGE FORM BY HPLC

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Abstract

A simple, rapid and accurate and stability indicating RP-HPLC method was developed for the determination of amoxicillin and cloxacillin in pure and tablet forms. The method was validated with respect to linearity, precision, accuracy, and selectivity. The mean values of slope, intercept and correlation coefficient were 0.9991(r2) respectively. The % COV values for repeatability and intermediate precision studies were < 2 indicates good precision of the method. The recovery of the drug ranged from 99.95-100.72% from a mixture of degradation products. The method was specific to drug and also selective to degradation products. The method showed a linear response for concentrations in the range of 300-1500 µg/ml using 0.01 M potassium dihydrogen phosphate (pH 5.0) buffer: acetonitrile [15:85] as the mobile phase with detection at 225.0 nm and a flow rate of 5 ml/min and retention time 15-20 min. The method was statistically validated for accuracy, precision, linearity, ruggedness, robustness, forced degradation, solution stability and selectivity. Quantitative and recovery studies of the dosage form were also carried out and analyzed; the % RSD from recovery studies was found to be less than 1. Due to simplicity, rapidity and accuracy of the method, we believe that the method will be useful for routine quality control analysis.

Key Words: Amoxicillin, cloxacillin, stability, HPLC, Validation.

Introduction:

Amoxicillin is an strong antibacterial agent & chemically it is 4-thia-1-azabicyclo (3.2.0) heptane-2-carboxylic acid, 6-(amino 4-hydroxyphenyl acetyl) amino-3,3-dimethyl-7-oxo.OR Hydroxyl- ampicillin.Cloxacillin is also from same class as an antibacterial agent & chemically it is Sodium (6R)-6-(3-(2-chlorophenyl)-5-methyl isoxazole-4-carbomido penicillate monohydrate. The combination of antibiotic in the infection of the otitis media (s.pyrogenes) bronchitis, pneumonia, gonorrhea and urinary tract infection. Empirical therapy of infection is probably the most common reason using a combination of antibiotic. The combining use of amoxicillin and cloxacillin vastly improve curve rate by preventing development of resistance. They act by mechanism inhibition of synthesis of the bacterial peptidoglycan cell wall.[13]The combination of antibiotic amoxicillin and cloxacillin is used in the infection of the otitis media (s.pyrogenes) bronchitis, pneumonia, gonorrhea and urinary tract infection.[14]The combination containing amoxicillin and cloxacillin shows synergistic effect for oral absorption of drug and extensively used for the treatment of certain systemic and urinary tract infections for which oral administration is desirable, leading to higher plasma level. The combined drug having same or identical bacterial spectrum as active against the same gram-positive organisms that are susceptible to other penicillin's & more active against some gram-negative bacteria & enterococci. It is particularly useful in the treatment of acute urinary tract infections caused by E.coli or proteus mirabilis it is the agent of choice against H.influenzae infections.[15, 1]

Material and Methods:

Pure amoxicillin and cloxacillin were obtained as gift samples from kiwi laboratories Ltd., vadodara.HPLC grade acetonitrile and buffer were purchased from Rajesh chemicals. All other chemicals were of analytical reagent grade.

The HPLC system consisted of an on-line degasser (DGU-14A),low pressure gradient flow control valve(FCV-10ALvp),Solvent delivery module(LC-10ATvp),auto injector(SIL-10ADvp), Column oven(CTO-10ASvp), uv-visible dual wavelength detector(SPD-10Avp), system controller(SCL-10Avp), CLASS-vp software, version-6.13. The separations were achieved on an intersil ODS_2 column grace vydac C-18 column.

Preparation of standerd&sample solution:

Preparation of standard stock solution:

The std. stock solution 1000 µgm/ml of each were prepared separately by dissolving working in small proportion of methanol and later diluted to volume with mobile phase.

Preparation of std.calibration solution:

The std.calibration solution of amoxicillin and cloxacillin having the conc. in the range of 50-1000 µg/ml were prepared by diluting stock solution with mobile phase.

Preparation of sample stock solution:

Ten capsules were weighted; their mean weight determined and crushed in mortar. An amount of powdered mass equivalent to one capsule was transferred into 50ml volumetric flask containing 10ml of methanol. Mechanically shaken for 10min., ultrasonicated for 5min.and then diluted volume with mobile phase.

Preparation of sample solution:

About 10ml of sample stock solution was centrifuged at 10,000rpm and 5ml of aliquot dilute to 50ml with mobile phase. Small portion of sample solution was filtered through 0.45 µ nylon filter and used for injection on HPLC.

Procedure for analysis of dosage form:

Ten capsules were weighed and their mean weigh determined and crushed in mortar. An amount of powdered mass equivalent to one capsule content was transferred into 50 ml volumetric flask containing 10 ml of methanol, mechanically shaken for 10 min., ultrasonicated for 5 min. and then diluted to volume with mobile phase (sample stock solution) .About 10 ml of sample stock solution was centrifuged at 10000 rpm and 5 ml aliquots diluted to 50 ml with mobile phase (Sample solution). A small portion of sample solution was filtered through 0.45µm nylon filter and used for injection on HPLC.

1) Acid stress-testing:

The combined drug formulation or products were degraded to corresponding reaction according to their labeled claimed on capsule. They were degraded together in 0.1M HCL for 1hr.at room temp. The samples were withdrawn periodically and subjected to analysis after suitable dilutions.

2) Neutral (water) stress testing:

The combined drug formulation or products were degraded to corresponding reaction according to their labeled claimed on capsule. They were hydrolyzed together in water for 6hr.at room temp. The samples were withdrawn periodically and subjected to analysis after suitable dilutions.

3) Alkali stress-testing:

The combined drug formulation or products were degraded to corresponding reaction according to their labeled claimed on capsule. They were degraded together in 0.1M NAOH for 1hr.at room temp. The samples were withdrawn periodically and subjected to analysis after suitable dilutions.

4) Oxidative stress-testing:

The combined drug formulation or products were degraded to corresponding reaction according to their labeled claimed on capsule. They were degraded together in 3%H_2O_2 for 1hr.at room temp. The samples were withdrawn periodically and subjected to analysis after suitable dilutions

5) Solid state/Thermal stress-testing:

The combined drug formulation or products were degraded to corresponding reaction according to their labeled claimed on capsule. They were degraded together in dry heat /hot air oven at 80°c for 24hr.at room temp. The samples were withdrawn

periodically and subjected to analysis after suitable dilutions

Results and Disscussion

A) Result of degradation studies:

Fig: 1. Chromatogram for standard or pure drugs:

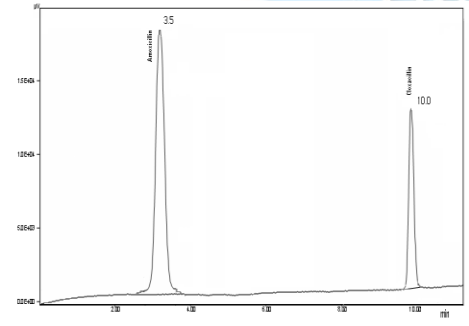


Fig: 2. Chromatogram for sample:

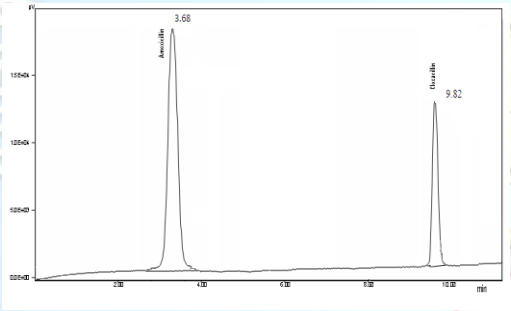


Fig: 3. Degradation in 0.1MHCL

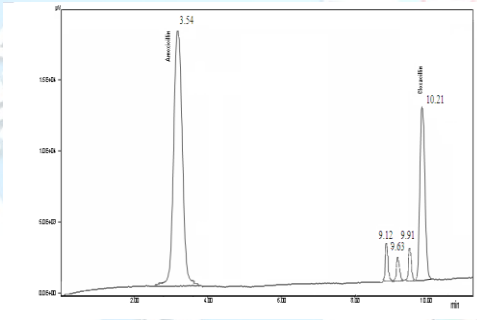


Fig: 4. Degradation in neutral (water) condition:

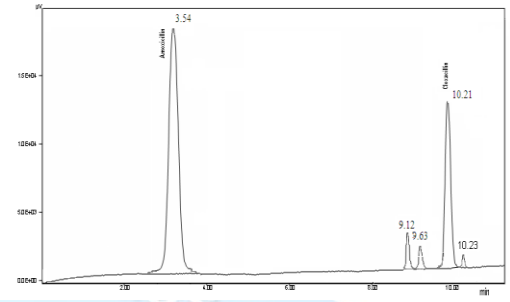


Fig: 5. Degradation in 0.1MNaOH:

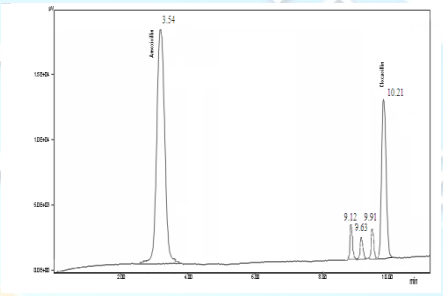


Fig: 6. Degradation in 3%H_2O_2:

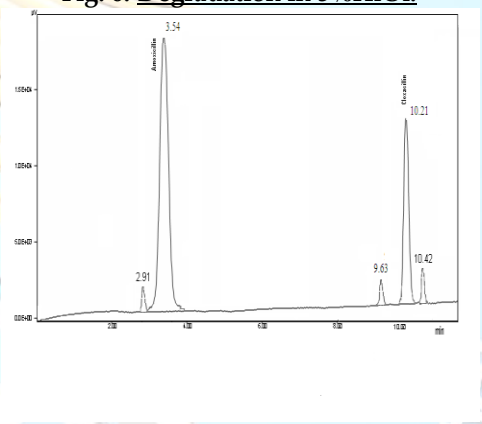
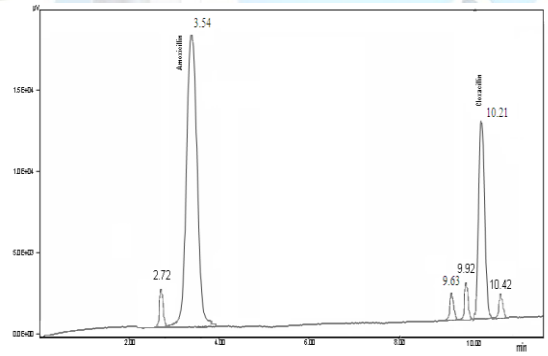


Fig: 7. Degradation in solid state/thermal condition:



DISCUSSION

In acidic degradation studies, combination of drugs showed sufficient degradation within 1hr.at room temp. In 0.1N HCL.The major degradation products were found at retention times (RTs) 9.12, 9.63 AND 9.91 min. In neutral (water) degradation studies, upon refluxing the combination for 6hr.The degradation products appeared at retention times (RTs) 9.12, 9.63, and 10.23min.

In alkali degradation studies, both the drugs are highly liable to hydrolysis in 0.1 N NaoH at room temp.The major products were found at retention times (RTs) 9.12, 9.63 and 10.91min. In oxidative degradation studies drugs showed sufficient degradation in 3%H_2O_2 for 1hr.and the three major degradation products appeared at retention times(RTs) 2.91,9.63,and 10.42 min.

In solid state or thermal degradation studies showed that the combination was unstable. Enough degradation was observed when the combination was exposed to dry heat at 80^{o}c for 24hr.The major degradation products resolved at 2.72, 9.63, 9.92 and 10.42 min.

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Refernces:

1. Raveru higuchi, einar brochmann, Pharmaceutical analysis, 1st edition, 1-7.
2. Willard merrit, Dean settle. Instrumental methods of analysis, 7th edition, 513,625-629
3. Holler Skoog. Principles of instrumental analysis, 6th edition, 829-836.
4. Gary D.Christian.Analytical chemistry, 6th edition, 613.
5. Kenneth A. Connor's, a textbook of pharmaceutical analysis, 3rd edition, 392.
6. David G.watson, Pharmaceutical analysis, 2nd edition, 268-279.
7. S.singh, B.singh, R.sexana *Journal of pharmaceutical biomedical analysis* 41(2006)-1037-1040.
8. A.Mohammadi, I.Haririan, R.B.walker, Journal *of chromatography*, A 1116(2006), 153-157.
9. I.Ivala, Z.Ljiljana, Z.mira, Journal *of chromatography*, A 1119(2006), 209-215.
10. ICH, Q1.A (R2), Stability testing of new drug substances and products in international conference on harmonization. IFPM, Geneva, 2003.
11. G.Grosa, E.D.Grosso, G.Allegro *Journal of pharmaceutical and biomedical analysis*, 41(2006), 798-803.
12. A.Mishal, D.Sober, Journal of pharmaceutical and biomedical analysis, 39(2005), 819-823.
13. Gilbert S.banker, Christopher Rhodes, Modern pharmaceutics 3rd edition, volume-72, 179-208.
14. Sumie Yoshioka, Valentio J.stella, Stability of drugs and dosage forms, 5-24.
15. John.H.Block, John.M.Beale, Wilson and Giswold's, textbook of organic medicinal of pharmaceutical chemistry, 11th edition, 311-313.
16. David A. Williams, Thomas.L.Lemke Foye's principles of medicinal chemistry, 5th edition, 841.
17. Joel. G. Hardman, Lee.e.Limbird, Goodman and Gillman's, the pharmaceutical basis of therapeutics, 10th edition, 1102-1164.
18. H.P Rang &m.m.Dale,Pharmacology,5th edition, 641.
19. Jensten Cartensten &T.Rhodes, Drug Stability 3rd edition, volume-107, 516,580-589.
20. Satindar Abuja &Stephen Scypinski Handbook of modern pharmaceutical analysis, .-09, 95-103.
21. ICH Q2 (R1), Validation of analytical procedures: Text and methodology, IFPMA, Geneva, 2003.
22. Klaus Florey, Analytical profiles of drug substances Volume-07, 21-22, and 34-39.
23. Klaus Flory, Analytical profiles of drug substances, Volume-04, 115,124-126.
24. Martindale The complete drug reference, 13th edition, 192,149-150.
25. The Merck index, 13th edition, 582, 2445.
26. USP (NF)-2003, the official component of standard, Asian edition, 140,498-499.
27. Indian pharmacopoeia (IP)-1996, Volume-I, 199.
28. Vijay kumar, Hemant Bhutani, Saranjit Singh, *Journal of pharmaceutical &Biomedical analysis, july-06.*

29. K.Raghu Naidu, Udhav N.Kale, *Journal of pharmaceutical &biomedical analysis, April-05.*
30. Jalesh Varshosaz, Farid porkoosh, *Journal of pharmaceutical &Biomedical analysis, jan-07.*
31. Sunder Natrajan, Bhanu Raman, *Indian drugs, Feb-07, vol-44 (295-102.*
32. Shah.D.A, Metha R.S., Bhatt.k.k, *Indian drugs, Dec-07, vol-44 (12).*
33. Indian drugs, Volume-43 (5), May-06.
34. Rao B.M.Srinivasu, M.K. Sridhar, G.Reddy, *Indian drugs, volume-41 (1), Jan-06.*
35. Vijaya kumar, M.Muley, *Indian drugs, volume-41 (5), May-04.*
36. Kumar R.S., Reddy R.N., Nagraju B. *Indian drugs, volume-43 (11), November-06.*
37. P.Perez.Lozano, E.Garcia Montoya, M.Minarro, *Journal of pharmaceutical &biomedical analysis,* Volume-42, September-06.
38. ICH Q1A (R2), Stability testing of new drug substances and products.