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 a moderate-spectrum beta-lactam antibiotic used to treat infections caused by penicillin-sensitive gram-positive bacteria as well as some gram-negative bacteria.[1] Amoxicillin is resistant to inactivation by gastric acid. It is usually the drug of choice because it is more rapidly and more completely absorbed than other beta-lactam antibiotics when orally administered. To overcome its sensitivity to destruction by beta-lactamases, amoxicillin has been co-administered with clavulanic acid, a potent betalactamase inhibitor [2] in pharmaceutical preparations.

Cloxacillin is a semisynthetic penicillin used as the sodium salt to treat staphylococcal infections due to penicillinase-positive organisms.[1] Unlike amoxicillin, this antibiotic is incompletely absorbed from the gastrointestinal tract, and absorption is reduced by the presence of food in the stomach. To produce a wider spectrum of activity, cloxacillin may be co-formulated with other antibacterials, in particular with amoxicillin (ratio 1:1, w/w) in capsules.

In the literature, the high performance liquid chromatography (HPLC) technique has been reviewed as a valuable tool for the analysis of antibiotics in formulated and unformulated samples.[3] As a result, this technique has been widely used for the simultaneous determination of penicillins such as amoxicillin and cloxacillin in pharmaceuticals, biological fluids, and tissues.[4–8]

On the other hand, amoxicillin was also spectro-
photometrically analyzed without prior separation using UV derivative techniques in the combination with clavulanic acid[9–12] or in antibiotic pharmaceutical mixtures.[13–16]

Albeit HPLC is often an official method for the analysis of antibiotics, the need for other simple, reproducible and accurate analytical methods still exists. This study was carried out to assess the stress degradation effects using HPLC technique.

**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 column grace vydac C-18 column.

**Preparation of standard & sample solution:**

**Preparation of standard stock solution:**
The std. stock solution 1000 μg/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 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. Small portion of sample solution was stirred through 0.45 μ nylon filter and used for injection on HPLC.

**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%H₂O₂ for 1 hr. 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 24 hr. at room temp. The samples were withdrawn periodically and subjected to analysis after suitable dilutions.

**Results and Discussion**

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%H₂O₂:
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%H₂O₂ 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°C for 24hr. The major degradation products resolved at 2.72, 9.63, 9.92 and 10.42 min.

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