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Stability-indicating LC-analytical method development and validation for the simultaneous estimation of flucloxacillin and amoxicillin in pharmaceutical dosage form

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Abstract

A simple and rapid stability-indicating LC-analytical method was developed for the simultaneous determination of flucloxacillin (fluc) and amoxicillin (amox) in bulk and pharmaceutical dosage form. A chromatographic separation of the two drugs was achieved with a Thermosil C_{18} (4.6 mm × 250 mm, 5 μm) analytical column using potassium dihydrogen phosphate buffer (adjusted to pH 3 by ortho phosphoric acid):methanol (70:30%, v/v) in isocratic mode at a flow rate of 1 mL/min and column at ambient temperature. The detection was monitored at 225 nm using a PDA detector. The stressed samples were analyzed for the degradation study in acid, base, peroxide, thermal, photolytic and validated as per ICH guideline. This proposed method was found to be specific and stability-indicating as no interfering peaks of degradation compounds and excipients were noticed. The described method shows excellent linearity over a range of 20–100 μg/mL for both drugs. The correlation coefficient for fluc and amox was 0.9992 and 0.9993, respectively. The mean recovery value for fluc and amox was 99.9% and 99.7%, respectively. The limit of detection for fluc and amox was 0.018 and 0.009 μg/mL and the limit of quantification was 0.06 and 0.03 μg/mL, respectively. The retention time was observed at 2.582 and 3.407 min for amox and fluc, respectively. The robustness study and percentage of assay of the formulation were found within limit as per ICH guidelines. The proposed method was suitable for quantitative determination and it can be applied in quality control department in industries, approved testing laboratories, bio-pharmaceutics and bio-equivalence and clinical pharmacokinetic studies.

Keywords: Flucloxacillin (fluc); Amoxicillin (amox); Reverse phase high performance liquid chromatography (RP-HPLC); ICH Guidelines; Simultaneous

1. Introduction

Flucloxacillin sodium [1] chemically is (2S, 5R, 6R)-6-({[3-(2-chloro-6-fluorophenyl)-5-methylisoxazole-4-yl]carbonyl}amino)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylicacid and amoxicillin trihydrate [2] (2S, 5R, 6R)-6-[[(2*R*)-2-amino-2-(4-hydroxy phenyl)acetyl]amino]-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]

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heptane-2-carboxylicacid tri hydrate. Flumox is a combination of two bactericidal penicillins: amoxycillin (broad-spectrum penicillin) and flucloxacillin (penicillin-resistant penicillin), to produce a wider spectrum of activity. Flucloxacillin exerts a bactericidal action on penicillinase-producing microorganisms including most staphylococci. This combination exhibits bactericidal activity against a wide range of gram-positive and gram-negative microorganisms including penicillinase and non-penicillinase-producing Staphylococci, *Streptococcus pyogenes*, *pneumoniae*, and *faecalis*, *Corynebacterium diphtheriae*, *Clostridia* spp., *Bacillus anthracis*; *Haemophilus influenzae*, *Moraxella* (*Branhamella*) *catarrhalis*, *Neisseria gonorrheae* and *meningitidis*, *Escherichia coli*, *Proteus mirabilis*, *Salmonella*, *Bordetella pertussis* and *Bacteroides melaninogenicus*. Literature review reveals that few spectrophotometric and potentiometric methods have been reported for analysis of flucloxacillin alone [3–5] or method development in combination with ampicillin in LC–MS [6] and in LC [7] or with amoxicillin by liquid chromatography without forced degradation study [8,9] for pharmaceutical injection [10] and ion-pairs along with gradient elution HPLC method [11]. Although two LC simultaneous methods for pharmaceutical dosage and one for injections were reported in the literature review, existing methods were not subjected to forced degradation study. Moreover reported methods were not much cost-effective in terms of solvent consumption and total run time of the analysis, so we decided to perform rapid, selective and precise stability-indicating isocratic RP-HPLC assay method for simultaneous determination of flucloxacillin and amoxicillin in solid dosage form which was not developed so far.

2. Experimental

2.1. Chemicals and reagents

Flucloxacillin standards were obtained from Surya Pharmaceutical Limited and amoxicillin trihydrate from ABL-Life Care Limited. Methanol (HPLC-grade) was obtained from Merck Fine Chemicals (Mumbai, India). Sodium hydroxide (NaOH), hydrochloric acid (HCl) and hydrogen peroxide (H_2O_2) were from SD Fine Chemicals, Finar Chemicals and Alpha Pharma Limited, respectively. The 0.45-μm pump nylon filter was obtained from advanced micro devices (Ambala Cantt, India). The flucamox (Sedico Pharmaceuticals) and flumox (Eipico) capsules of the combination of flucloxacillin and amoxicillin were purchased commercially. Double-distilled water was used throughout the experiment. Other chemicals used were of analytical or HPLC-grade.

2.2. HPLC instrumental condition

Instrument used in the study was Shimadzu-LC equipped with Auto Sampler, DAD or UV detector and Empower 2 software. A chromatographic separation of the two drugs was achieved with a Thermosil C18 (4.6 mm × 250 mm, 5 m) analytical column using potassium dihydrogen phosphate buffer (adjusted to pH 3 by ortho phosphoric acid):methanol (70:30%, v/v) in isocratic mode at a flow rate of 1 mL/min and column at ambient temperature. All the solvents were filtered through 0.45-μm nylon filter and degassed in ultra-sonic bath prior to use. Measurements were made with injection volume 20 μL and detection at 225 nm. For analysis of forced degradation samples, the PDA detector was used in scan mode with a scan range of 200–400 nm. The peak homogeneity was expressed in terms of peak purity and was obtained spectral analysis report using previously mentioned software. Final optimization was performed with pure analytical standard which was obtained from above mentioned pharmaceutical industry (Table 1).

2.3. Standard solution preparation

Accurately weigh and transfer 10 mg of amox and 10 mg of fluc working standard into a 10 mL clean dry volumetric flask. Then, add about 7 mL of diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (stock solution). Further pipette 0.6 mL of amox and fluc in the above stock solution into a 10 mL volumetric flask and dilute up to the mark with mobile phase.

Table 1
Optimized chromatographic parameters.

Equipment	High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector
Column	Symmetry C18 (4.6 mm × 150 mm, 5 μm, make: Thermosil) or equivalent
Flow rate	1.0 mL/min
Wavelength	225 nm
Injection volume	20 μL
Column oven	Ambient temperature
Run time	8 min