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Quantitative measurement of amoxicillin in Ibuprofen tablets using UPLC

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ABSTRACT

A novel quantitative analytical method for the determination of Penicillin contaminant, Amoxicillin in non-penicillin pharmaceutical drug product (Ibuprofen tablet 400 mg) has been developed and validated using Ultra performance liquid chromatography (UPLC). The extraction of amoxicillin from the drug tablets was carried out with bi-distilled water and the separation was achieved by making use of a BEH C18 column with particle size of 1.7 μm (100 mm × 2.1 mm). The isocratic run accomplished using phosphate buffer (pH 5.0): methanol (95:5, v/v) mixture as mobile phase run at a flow rate of 0.3 mL/min. The rapid, accurate and low cost UPLC method was proven to be suitable within the current good manufacturing practices (cGMP) of pharmaceutical ingredients. In addition, the validation of the developed method was conducted as per the ICH (International conference of harmonization) guidelines Q2 (R1). Further, the method was found to be linear in the range of (0.024–0.096 μg/mL for amoxicillin) with a correlation coefficient, R^2 of 0.999 and net in terms of specificity, linearity, precision, accuracy, detection limit (DL), and quantitation limit (QL) are appeared to be satisfactory. The precision was assessed in terms of injections repeatability with a maximum %RSD of 1.8%, while the intermediate precision Day-1 with % RSD of 0.96 and the intermediate precision Day-2 with %RSD of 1.56 were observed. Thus, from the observation of satisfactory results for amoxicillin detection, the developed UPLC-based method can successfully be applied in the pharmaceutical quality control laboratories to fulfill the regulatory requirements.

1. Introduction

Penicillin is one of the most well-known β-lactam antibiotic drugs used for the treatment of bacterial infections in humans and animals. In addition, it also acts as a sensitizing agent to trigger hypersensitive exaggerated allergic immune responses in some people. The common allergic reactions due to penicillin intoxication cause rashes, hives, itchy eyes, and swollen lips, tongue, or face [1]. The most serious allergic reaction to penicillin is an anaphylactic reaction which can be life threatening. Accordingly, implementing regulatory requirements for preventing cross-contamination with penicillin is a key element of the manufacturing sites [2,3].

In the pharmaceutical manufacturing, one of the general concerns is the cross-contamination of penicillin into non-penicillin drug products, especially when different drugs are manufactured within the same unit. FDA (food and drug administration) strictly regulated the manufacturing of different parts of the 21 CFR 211 (current good manufacturing practice for finished pharmaceuticals). Thus, for all penicillin manufacturers, it is recommended to establish a stringent control including separate facilities, air handling systems and dedicated equipment for penicillin drug products. Furthermore, if some reasonable possibility exists that a non-penicillin drug product has been exposed to penicillin by means of cross-contamination, the non-penicillin product should thoroughly be tested for the presence of penicillin traces and such drug products shall not be marketed if any detectable levels are found [4]. In view of the above mentioned facts, it is essential to establish a quantitative analytical method for the detection of penicillin traces in non-penicillin drug products manufactured in the common facilities. Also, the requirement for the developed

Abbreviations: UPLC, ultra performance liquid chromatography; cGMP, current good manufacturing practices; ICH, international conference of harmonization; RSD, relative standard deviation; FDA, food and drug administration; DOE, design of experiment; DL, detection limit; QL, quantitation limit; LOQ, limit of quantitation; LOD, limit of detection; ppb, parts per billion; mg, milligrams; mL, milliliters.

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method is that, it should have a detection limit not exceeding the safer and allowable tolerance levels set by the FDA which is 10 ppb for amoxicillin [5].

Many different analytical methods were reported for the detection of penicillin traces in milk [6,7], food [8,9], animal tissues [10,11], and in groundwater [12], so as to avoid the adverse impacts of penicillin contamination. On the other hand, the reported method for the detection of penicillin residues in the pharmaceutical sector focusses mainly on the cleaning validation [13]. Hence, there is a huge demand for the development of analytical methods to trace even smaller amounts of penicillin residues in other pharmaceutical products. To the best of authors' knowledge, the only report which deals with studies related to penicillin determination in non-penicillin drug products is the use of HPLC (high pressure liquid chromatography) coupled with Tandem mass spectrometry [14]. Thus, the objective of this report is to introduce a novel approach by making use of UPLC (ultra performance liquid chromatography) for the determination of penicillin traces (amoxicillin) in non-penicillin drug products in pharmaceutical industry. For the developed UPLC method, the mobile phase selected was consists of phosphate buffer (pH 5.0): methanol (95:5, v/v), run at a flow rate of 0.3 mL/min. From the analysis of results, the developed method investigated to be rapid, accurate and low cost, in addition to finding its suitability within the current good manufacturing practices (cGMP) of pharmaceutical ingredients. By making use of the current developed method, the minimum amoxicillin amount detected can be of 0.08 μg/mL, which is far lower than the guidelines set by FDA (10 ppb). Further, the method was validated using the ICH (International conference of harmonization) guidelines Q2 (R1) for amoxicillin determination and found to be linear in the range of 0.024–0.096 μg/mL with a correlation coefficient of 0.999, in addition to generating the satisfactory results in terms of accuracy, precision, specificity, and linearity.

2. Experimental

2.1. Chemicals and reagents

The API Amoxicillin trihydrate (AM-SN-09966) was supplied by ABC pharmaceutical Co., Malaysia. Ortho-phosphoric acid and potassium dihydrogen phosphate of analytical reagent grades were obtained from Fischer scientific, UK. Methanol (HPLC grade) was obtained from BDH, UK. Buffer solutions with accuracy ±0.02 @ 20 °C (pH's 4.0 and 7.0) were obtained from Pancreas, Spain.

2.2. Equipment

Acquity UPLC System (Waters, Milford, MA, USA) was used to run the analysis. The system was equipped with "binary solvent manager" as a pump, "sample manager" as auto-injector, "tunable TUV detector" for UV detection and "empower 3" for controlling and handling the data.

Seven multi pH meters (Mettler Toledo, USA) were used for the pH adjustments, Ultrasonic Bath-MXB6 (Grant, UK) was utilized for the sonication of standards and samples, Analytical balance- XP26 (Mettler Toledo, USA) for weighing, Centrifuge-Z-300 (Hermle, Germany), Mechanical shaker–SM 30 (Edmund Buhler, Germany) and Vortex Mixer-Whirli (Grant, UK) during sample preparations. Minitab 17 Statistical Software was used for the data analysis.

2.3. Chromatographic conditions

The UPLC column used was of Waters Acquity BEH C18 (2.1 × 100 mm × 1.7 μm particle size), and the column temperature maintained at 35 °C. The flow mode of mobile phase was isocratic at a rate of 0.3 mL/min and composition of 95:5 (v/v) of phosphate buffer (pH 5.0): methanol mixture. The mobile phase was filtered using 0.22 μm millipore nylon membrane filter and degassed for 20 min using ultrasonic bath. The injection volume taken of was 5 μL using a partial needle overfill as injection mode. The weak needle wash was limited to 10% methanol, while the strong needle wash was extended to 70% methanol. The peaks detection was monitored at a wavelength of 230 nm and adjusted the filter time constant at 1.25 s.

2.4. Preparation of standard solutions

About 60 mg of amoxicillin trihydrate RS was weighed accurately on an analytical balance and transferred into a 100 mL volumetric flask, added 50 mL bi-distilled water and shaken for 5 min using a mechanical shaker, sonicated for 5 min using ultrasonic water bath, then diluted to the required volume using bi-distilled water and mixed well (stock solution of 0.6 mg/mL). Further dilutions were prepared from the stock solution to have 0.024, 0.036, 0.048, 0.060, 0.072, 0.084 and 0.096 μg/mL of amoxicillin in bi-distilled water (representing 40–160%). Filtered the solutions into 2 mL vials using Acrodisc GHP 0.2 μm syringe filters.

2.5. Preparation of sample solutions

About 10 Ibuprofen tablets (each of 400 mg) were transferred to a 25 mL centrifuge tube. Spiked with 20 mL of amoxicillin trihydrate RS at concentration levels of 100% standard solution and mixed using the vortex mixer for 5 min, then centrifuged at 2000 rpm. Following this, the solution was filtered into a 2 mL vial using Acrodisc GHP 0.2 μm syringe filter (spiked sample solution for precision).

In each of 9 centrifuge tubes (25 mL), 10 Ibuprofen tablets (400 mg) were inserted. Spiked individually 3 × 3 replicates of amoxicillin trihydrate (20 mL) at concentration levels of 80%, 100%, and 120% of working standard solution, mixed using the vortex mixer for 5 min then centrifuged at 2000 rpm. Following this, the solution was filtered into 2 mL vials using the same syringe filters (spiked sample solution for accuracy/recovery).

2.6. Method development

The target is to develop a UPLC method using isocratic elution mode for detecting the drug traces "amoxicillin" in the presence of matrix "Ibuprofen tablets 400 mg" with acceptable resolution and detectable levels. The mobile phase that often used in the separation of the drug in the official compendial method "USP/NF [15] or EP" [16] is the mixture of phosphate buffer "pH = 5.0" and acetonitrile in different composition ratios. The use of acetonitrile leads to noticeable levels of interference but, enough separation was obtained upon replacement with methanol. The drug detection was best found to be at 230 nm wavelength. In addition, the calibration graphs achieved at 230 nm showed a good correlation coefficient. Different pH values of buffer used in the mobile phase preparation reported a good recovery but, the run time seems to be getting longer. To solve this problem, several mobile phases were tested, which was accomplished by varying the pH's and composition. This was performed so as to obtain the desired chromatographic separations. The proposed mobile phase composed with a composition of 95:5 (v/v) phosphate buffer (pH 5.0): methanol where the buffer prepared by weighing 2.72 g potassium dihydrogen phosphate in 1 L bi-distilled water, adjusted to a pH of 5.0 using ortho-phosphoric acid. An UPLC™ BEH C18 (100 × 2.1 mm) 1.7 μm column was selected to achieve good peak shape and symmetry. However, shorter column of 50 mm length did not provide