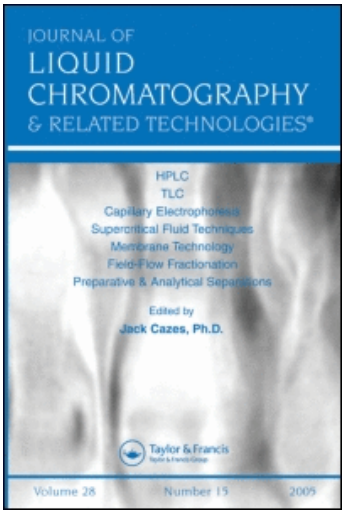

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An LC Method for Simultaneous Determination of Amoxicillin and Sulbactam Pivoxil in a Combination Formulation

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ABSTRACT

A simple and accurate liquid chromatography (LC) method is described for simultaneous determination of amoxicillin and sulbactam pivoxil in a combination formulation. Chromatographic separation of the two drugs was achieved on a Hypersil C18 column (250 mm × 4.6 mm, 5 μm) using a mobile phase consisting of a mixture of methanol, acetonitrile, and water (60 : 1 : 39, v/v/v, pH = 4.5) delivered at a flow rate of 1.0 mL/min, and detection was made at 220 nm. Separation was complete in less than 10 min. The method is linear, precise, accurate, and selective. Linearity, accuracy, and precision were found to be acceptable over the ranges 50.95–509.5 μg/mL for amoxicillin and 46.63–466.3 μg/mL for

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sulbactam pivoxil. Due to its simplicity and accuracy, the method is suitable for routine quality control analysis of amoxicillin and sulbactam pivoxil in the combination formulation.

Key Words: Liquid chromatography (LC); Amoxicillin; Sulbactam pivoxil.

INTRODUCTION

Sulbactam pivoxil[1] is a prodrug of sulbactam,[2] the latter being a beta-lactamase inhibitor with limited oral bioavailability, poorly absorbed in the gastrointestinal tract. In contrast, sulbactam pivoxil has better absorption than the parent drug and significantly improves the oral absorption of sulbactam, and therefore, provides high serum levels after oral administration. Sulbactam pivoxil shows no antibacterial activity itself until transformed into the active drug after absorption. Amoxicillin is a kind of semi-synthetic penicillin widely used in clinical practice as a bactericidal against many gram-positive and gram-negative microorganisms. The structures of amoxicillin and sulbactam pivoxil are shown in Fig. 1.

Co-administration of beta-lactamase inhibitors, together with penicillins, was commonly performed in clinical therapy to improve antibacterial therapy and overcome the bacterial resistance.[3] Based on the clinical requirements, one new formulated product with amoxicillin and sulbactam pivoxil in combination has been developed, and an analytical method is required for simultaneous determination of amoxicillin and sulbactam pivoxil in the new product to control the product quality.

A literature survey found no published methods for simultaneous determination of amoxicillin and sulbactam pivoxil or for individual determination of sulbactam pivoxil, but some methods are available for determination of amoxicillin in bulk substance, pharmaceutical preparations, and biological samples, most methods of which are liquid chromatography (LC) and relatively complex.[4–12]

Simultaneous determination of amoxicillin and sulbactam pivoxil in the new product presents a tough challenge for us because of the great differences of the two drugs in polarity, solubility, and ultraviolet absorption characteristics. The aim of this study is to develop and validate an LC method for simultaneous determination of amoxicillin and sulbactam pivoxil in the new product, which can be used for the routine analysis of the product in ordinary laboratories. This study achieved simultaneous determination of the two drugs of great differences under simple chromatographic conditions.

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Figure 1. Structures of amoxicillin and sulbactam pivoxil.

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EXPERIMENTAL

Chemicals and Reagents

Amoxicillin reference standard was from the National Institute for Control of Pharmaceutical and Biological Products (NICPBP) (Beijing, China). Sulbactam pivoxil reference standard and sulbactam pivoxil dispersible tablets were from Shenyang Pharmtech Institute of Pharmaceuticals (Shenyang, China). Each tablet contains 125 mg amoxicillin and 125 mg sulbactam. HPLC-grade methanol and acetonitrile were from Fisher Scientific (Springfield, USA). Distilled water was prepared by Milli-Q system (Millipore, USA). All other chemicals and reagents used were of analytical grade unless indicated otherwise.

Apparatus

Chromatography was performed on a Hewlett Packard (HP) series 1100 liquid chromatographic system equipped with G1310A Iso Pump, an HP variable UV/VIS detector, G1328A Manual Injector with 20 μL loop (Agilent, USA). Echrom 98 Chromatography Workstation was employed for data collecting and processing (Elete, China). A Shimadzu UV-2201 UV/VIS double-beam spectrophotometer (Shimadzu, Japan) was used for scanning and selecting the detection wavelength.

Chromatographic Conditions

Chromatographic separation was performed on a Hypersil C18 column (250×4.6 mm, 5 μm). The mobile phase comprised of a mixture of methanol, acetonitrile, and water (60 : 1 : 39, v/v/v, pH = 4.5) adjusted to pH 4.5 with acetic acid, was delivered at a flow rate of 1.0 mL/min. The mobile phase was filtered through a 0.45 μm membrane filter and degassed. Separation was performed at ambient temperature and detection was made at 220 nm. The injection volume was 20 μL.

Preparation of Stock and Standard Solutions

A stock solution with both amoxicillin and sulbactam pivoxil at about 1.0 mg/mL was prepared with the mobile phase. Standard solutions were prepared by dilution of the stock solution with mobile phase to give solutions containing amoxicillin and sulbactam pivoxil in the concentration ranges of 50.95–509.5 μg/mL and 46.63–466.3 μg/mL, respectively.

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Preparation of Sample Solution

Twenty tablets were accurately weighed and finely powdered. An accurately weighed portion of the powder, equivalent to 100 mg amoxicillin, was transferred to a 100 mL volumetric flask. After about 20 mL of mobile phase was added to the flask, the mixture was sonicated for 10 min, brought to volume with mobile phase, and filtered. The first 10 mL of the filtrate was rejected, and 2.0 mL of the following filtrate was quantitatively transferred into a 10 mL volumetric flask, and diluted to volume with the mobile phase.

RESULTS AND DISCUSSION

Method Development

The major challenge for the simultaneous determination of amoxicillin and sulbactam pivoxil in this new combination formulation is the large differences in polarity, solubility, and ultraviolet absorption characteristics between the two drugs. Amoxicillin is aqueous, polar, and exhibits significant ultraviolet absorption, while sulbactam pivoxil is non-aqueous, non-polar, and has no maximum absorption in ultraviolet range, except end absorption. Owing to the above reasons, it was once thought impossible to determine the two drugs simultaneously under ordinary conditions of reversed phase LC. To simplify the assay procedure by avoiding individual determination of the two drugs, serious efforts were made in finding an optimal mobile phase to achieve the simultaneous determination of both drugs under the most common LC conditions.

At the beginning of this study, more attention was paid to finding a way to balance the retention behaviors of the two drugs on RP-columns. To achieve simultaneous determination of the two drugs, several reagents such as ion-pairing reagents and beta-cyclodextrin were tried by adding them into mobile phase, but failed because of the short retention of amoxicillin and no elution of sulbactam pivoxil. Then, several other mobile phases were tried step by step. At first, a mixture of methanol, acetonitrile, and 0.01 M phosphate buffer in the volume ratio of 14 : 4 : 10 on a C18 column were used. Owing to the instability of sulbactam pivoxil in alkaline solution, the pH value of the mobile phase was finally fixed at 4.5 throughout this study after testing a series of pH values in the range of pH 2.0–6.0. This system produced reasonable retention time (about 11.6 min) and symmetry peak shape for sulbactam pivoxil, but too short retention of amoxicillin to separate it from the solvent front. Afterwards, the various ratios of the components in the above mobile phase were tested but led to no significant improvement.

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From the above results, phosphate buffer in mobile phase was found to be unfavorable for the separation, which was replaced with water for later study. Taking into account the lipophilicity and solubility of both drugs, it was expected that by reasonably increasing the proportion of methanol and decreasing the concentration of acetonitrile and water might favor the separation of the two drugs. Separation started with the above mixture in the ratios of 60 : 1 : 50 and 60 : 1 : 39 (v/v/v), respectively. The first one (60 : 1 : 50) produced reasonable retention time for sulbactam pivoxil (8.5 min), but slightly short retention time for amoxicillin (2.3 min), which just resolved from the solvent peak. The other one (60 : 1 : 39) achieved the expected results, which produced symmetric peak shape, good resolution, and reasonable retention time of both drugs. The retention time of amoxicillin and sulbactam pivoxil was 3.4 min and 7.2 min, respectively. The run time is less than 10 min. Thus, the mixture of methanol, acetonitrile, and water (60 : 1 : 39) was finally employed for the simultaneous determination of the two drugs. A typical chromatogram for the tablet sample is shown in Fig. 2.

Since sulbactam pivoxil has no significant UV maximum absorption but only end absorption, detection was performed at 220 nm where amoxicillin also has reasonable absorption. The tablet excipients were also determined to see if any interference from them existed. No peaks were observed in the chromatogram of blank sample, which showed no interference from the excipients.

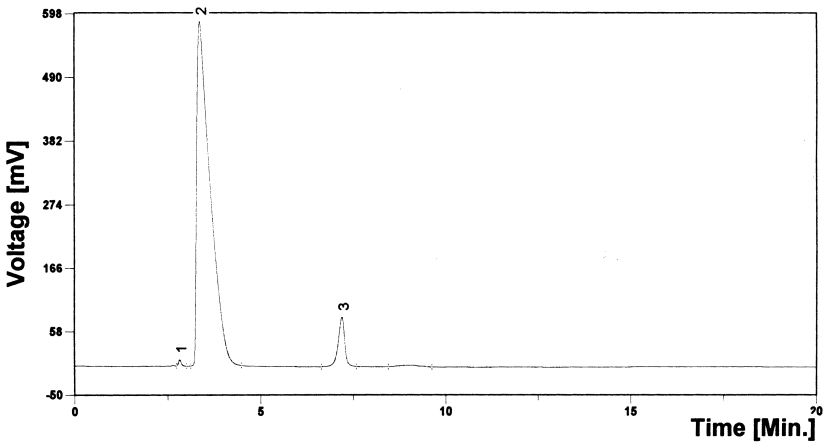

Figure 2. A typical chromatogram of a tablet sample. Peak: 1. solvent; 2. amoxicillin; 3. sulbactam pivoxil. The chromatographic conditions used were: Hypersil C18 column, mobile phase of a mixture of methanol, acetonitrile, and water (60 : 1 : 39, v/v/v, pH = 4.5), flow rate of 1.0 mL/min, detection wavelength of 220 nm, ambient temperature.

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Method Validation

System Suitability Test

To ensure the validity of the analytical procedure, a system suitability test (SST) was established. The SST is an integrated part of the analytical method and it ascertains the suitability and effectiveness of the operating system. Relative standard deviation of the area, tailing factor, and retention time were the chromatographic parameters selected for the SST; they were calculated from six dilutions (working solutions) of the reference solutions, each injected once. The parameters values should meet the following criteria: relative standard deviation (RSD) of the two analytes peak areas is less than 3%, tailing factors for the three analytes (USP Tailing Factor) less than 2, retention time standard deviation (SD) within seconds of the expected retention time. The results have shown that the symmetry factors for the analytes' peaks were less than 2.0, the RSD of the peak areas responses less than 3%, and the migration times within seconds.

Linearity

Linearity was determined by building three calibration curves. For the construction of each calibration curve, five calibration standard solutions were prepared at concentrations ranging from 25 to 200% of the working solution. Each standard solution was injected once. Peak area (A) and concentration (C) of each drug substance was subjected to regression analysis to calculate the calibration equation and correlation coefficients. Linearity is confirmed if the RSD values of the slope and the intercept are less then 3%. The regression equations obtained for the two drugs were: $A = 287.5 + 396.4C$ ($r = 0.9999$, $n = 5$) for amoxicillin and $A = -28.0 + 27.4C$ ($r = 0.9997$, $n = 5$) for sulbactam pivoxil, respectively. The individual linear range was 50.95–509.5 μg/mL for amoxicillin and 46.63–466.3 μg/mL for sulbactam pivoxil. The results show that within the concentration range tested, there was an excellent correlation existing between peak area and concentration of each drug.

Limit of Quantitation

The limit of quantitation (LOQ) was defined as the lowest concentration that can be determined with acceptable accuracy and precision, which can be established at a signal-to-noise ratio of 10. Limit of quantification of each drug was experimentally verified by six injections of each drug at its LOQ concentration. The LOQ of amoxicillin and sulbactam pivoxil were found to be 3.05 μg/mL and 4.03 μg/mL, respectively.

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Accuracy

Accuracy was determined by applying the described method to synthetic mixtures of excipients, to which known amounts of each drug corresponding to 80, 100, and 120% of label claim had been added. The accuracy was then calculated from the test results as the percentage of analyte recovered by the assay. Mean recoveries (Mean ± SD) for amoxicillin and sulbactam pivoxil from the specific formulations are 99.6 ± 0.82 ($n = 9$) and 100.0 ± 1.1 ($n = 9$), respectively. The results indicate good accuracy of the method for simultaneous determination of the two drugs.

Precision

The system precision is a measure of the method variability that can be expected for a given analyst performing the analysis, and was determined by performing five replicate analysis of the same working solution. The obtained RSD for amoxicillin and sulbactam pivoxil was 1.5% and 0.57%, respectively.

The method precision of the developed LC method was determined by preparing the tablet samples of the same lot in nine replicate determinations. RSD of the assay results, expressed as a percentage of the label claim, was used to evaluate the precision of the method. The obtained RSD values were 1.6% for amoxicillin and 1.8% for sulbactam pivoxil. The above results indicated the good precision of the method.

Solution Stability

The stability of both standard and sample solutions was determined by monitoring the peak area responses of solutions of the standard mixture of amoxicillin and sulbactam pivoxil and a tablet sample, over a period of one week. The results showed that for both solutions, the retention times and peak areas of amoxicillin and sulbactam pivoxil remained almost unchanged and no significant degradation was observed within the given period, indicating that both solutions were stable for at least seven days.

Method Application

The validated LC method was applied to determination of amoxicillin and sulbactam pivoxil dispersible tablets. Three batches of tablets were assayed, the assay results of which, expressed as a percentage of the label claim, are shown in Table 1. The results indicate that the amount of each drug in the tablets corresponds to requirements 90–110% of the label claim.

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Table 1. Assay results for amoxicillin and sulbactam pivoxil in dispersible tablets (Mean ± SD, %).

Batch no.	Amoxicillin	Sulbactam
1	100.0 ± 1.0	101.7 ± 0.8
2	99.7 ± 0.7	101.9 ± 0.9
3	100.3 ± 1.1	101.0 ± 1.2

Note: The chromatographic conditions used were: Hypersil C18 column, mobile phase of a mixture of methanol, acetonitrile, and water (60 : 1 : 39, v/v/v, pH = 4.5), flow rate of 1.0 mL/min, detection wavelength of 220 nm, ambient temperature.

CONCLUSIONS

The developed LC method provides a convenient and efficient way for simultaneous determination of amoxicillin and sulbactam pivoxil in the combination formulation. The results show that the proposed LC method has sufficient accuracy, precision, and selectivity, as well as, a short analysis time. It can be used for the routine assay of amoxicillin and sulbactam pivoxil in the combination formulation.

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