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# A liquid chromatographic method for simultaneous determination of amoxicillin sodium and sulbactam sodium in a combination formulation

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**Abstract**

An isocratic liquid chromatographic method with UV detection at 210 nm is described for simultaneous determination of amoxicillin sodium and sulbactam sodium in a new combination formulation. Chromatographic separation of the two drugs was achieved on a Hypersil $C_{18}$ column using a mobile phase consisting of a binary mixture of methanol and 0.01 mol/l sodium acetate (5:95, v/v). The commonly used paired-ion aqueous mobile phase for the determination of penicillins was avoided in this study. The developed LC method offers symmetric peak shape, good resolution and reasonable retention time for both drugs. Linearity, accuracy and precision were found to be acceptable over the concentration ranges of 155.3–1553.0 μg/ml for amoxicillin sodium and 45.0–450.0 μg/ml for sulbactam sodium. The proposed LC method can be used for the quality control of formulated products containing these two drugs.


*Keywords:* Liquid chromatography; Amoxicillin sodium; Sulbactam sodium

## 1. Introduction

Amoxicillin sodium is a semi-synthetic penicillin with activity against both gram-positive and gram-negative bacteria. Sulbactam sodium is a competitive, irreversible beta-lactamase inhibitor and has good inhibitory activity against the clinically important plasmid mediated beta-lactamases most frequently responsible for transferred drug resistance. Both drugs have been listed in the current Pharmacopoeias such as USP, EP and ChP. Combinations of beta-lactamase inhibitors with penicillins, especially aminopenicillins, have broad-spectrum antibacterial activity against most of the common pathogens of the respiratory and urinary tracts [1–4]. To meet the clinical needs, a new combination formulation containing both drugs was developed and then an analytical method was required for the quality control of this combination formulation.

The methods available for the determination of amoxicillin sodium include spectrophotometry [5,6], capillary electrophoresis [7,8] and liquid chromatography (LC) with different ways of detection such as amperometry [9], fluorometry [10], mass spectrometry [11,13] and UV [12,14–18,23]. The methods published for the determination of sulbactam sodium are LC [19–21], enzymatic method [22] and spectrophotometry [24]. Most of the LC methods with UV detection were used for the individual determination of the two drugs in biological samples and complex chromatographic conditions were involved such as chiral stationary phase [12], on-line postcolumn [13] or precolumn [21] derivatisation, column switching [14] or coupled column [15], paired-ion aqueous mobile phase [16–18] and gradient elution [17,18]. Two LC methods have been reported for simultaneous determination of amoxicillin sodium

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and sulbactam sodium in pharmaceutical preparations [25,26].

LC with UV detection is often preferred in ordinary laboratories because of its wide availability and suitability. To simplify the analytical process for the quality control of this combination formulation, an isocratic LC method without using paired-ion aqueous mobile phase became the target of our work. To our knowledge, aqueous solution of tetrabutylammonium hydroxide (TBAH) is often used in mobile phase for the analysis of penicillins. But from our previous experience [19], TBAH produced some negative effects on the seal of the pump, sometimes even causing leak.

The present study describes an isocratic LC method with UV detection using a mobile phase of a binary mixture without TBAH for the simultaneous determination of amoxicillin sodium and sulbactam sodium in this new formulation product, which can be used for the quality control of this product in ordinary laboratories. This study achieved satisfactory results in terms of selectivity, linearity, precision and accuracy under simple chromatographic conditions.

## 2. Experimental

### 2.1. Chemicals and reagents

Amoxicillin sodium and sulbactam sodium reference standards were from the National Institute for Control of Pharmaceutical and Biological Products (NICPBP) (Beijing, China). Amoxicillin sodium and sulbactam sodium sterile powder for injection was from Shenyang Pharmtech Institute of Pharmaceuticals (Shenyang, China). Each ampoule contains 1.0 g amoxicillin and 0.25 g sulbactam. Methanol was of chromatographic grade from Concord Technologies Reagent (Tianjin, China). All other chemicals and reagents used were of analytical grade unless indicated otherwise.

### 2.2. Apparatus

Chromatographic separation was performed on an HP 1100 series liquid chromatographic system equipped with a G1310A iso pump, an HP variable UV–vis detector and a G1328A manual injector with a 20 μl loop (Agilent, USA). Echrom 98 chromatography workstation was employed for data collecting and processing (Elite, China). A Shimadzu UV-2201 UV–vis double-beam spectrophotometer (Shimadzu, Japan) was used for scanning and selecting the detection wavelength.

### 2.3. Chromatographic conditions

Chromatographic separation was performed on a Hypersil $C_{18}$ column (250 mm × 4.6 mm, 5 μm). The mobile phase consisting of a binary mixture of methanol and 0.01 mol/l sodium acetate adjusted to pH 4.0 with acetic acid (5:95, v/v) 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 prior to use. Separation was performed at ambient temperature and detection was made at 210 nm. The injection volume was 20 μl.

### 2.4. Preparation of stock and standard solutions

A stock solution with amoxicillin sodium at 1553.0 μg/ml and sulbactam sodium at 450.0 μg/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 sodium and sulbactam sodium in the concentration ranges of 155.3–1553.0 μg/ml and 45.0–450.0 μg/ml, respectively.

### 2.5. Preparation of sample solution

The content of five ampoules were taken and accurately weighed. An accurately weighed portion of the powder equivalent to 60 mg amoxicillin was transferred to a 100 ml volumetric flask and made to the mark with mobile phase and filtered. The resulting solution was used as the sample solution for chromatographic analysis.

## 3. Results and discussion

### 3.1. Method development

Taking into consideration the instability of amoxicillin sodium and sulbactam sodium in strong alkaline and acidic conditions, the pH value of the mobile phase should be limited within the range from 3 to 7. Since mild acidic pH favors the retention and separation of two drugs on $C_{18}$ column, acetate buffer is preferred to phosphate buffer in our work. After some trials, sodium acetate buffer with pH 4.0 was finally selected. Methanol is the most common solvent used for LC analysis and often is the first choice for many researchers. Therefore, a binary mixture of methanol and acetate buffer became the initial mobile phase for the determination of the two drugs. Firstly, various concentrations of acetate buffer were tried to find the proper one to achieve our purpose. As a result, 0.01 mol/l acetate buffer was found to be ideal for our work. Then, the proportion of methanol and 0.01 mol/l acetate buffer in mobile phase was determined by varying the proportion of methanol and acetate buffer from 20:80 to 5:95. Finally, the mixture of methanol and 0.01 mol/l acetate buffer (5:95) was employed for the simultaneous determination of the two drugs, for this system produced symmetric peak shape, good resolution and reasonable retention time for both drugs. Since sulbactam sodium has no significant UV maximum absorption but only end absorption, detection was performed at 210 nm where amoxicillin also has reasonable absorption. The retention time of amoxicillin sodium and sulbactam sodium was 8.1 and 5.5 min, respectively. The

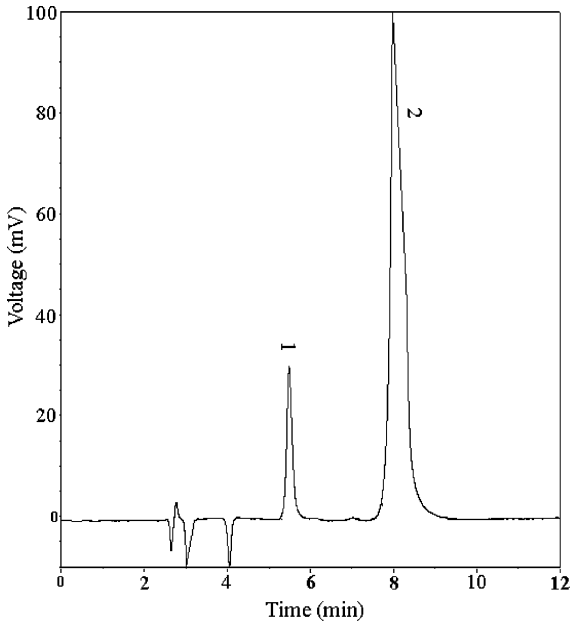


Fig. 1. A typical LC chromatogram of a sample solution. Peaks: (1) sulbactam sodium ($t_R$ = 5.5 min); (2) amoxicillin sodium ($t_R$ = 8.1 min).

run time is less than 10 min. A typical chromatogram of a sample solution is shown in Fig. 1.

To our knowledge, aqueous solution of tetrabutylammonium hydroxide (TBAH) is often used in mobile phase for the analysis of penicillins. But from our previous experiences, TBAH has some negative impact on the seal of pump, sometimes leading to leak. Therefore, we tried to avoid using this reagent in mobile phase for this work. As a result, our purpose was achieved and a mixture of methanol and 0.01 mol/l acetate buffer was found to be excellent both for the simultaneous determination of amoxicillin sodium and sulbactam sodium and for the maintenance of the apparatus. The results of this work can also provide useful information for the analyses of other penicillins.

### 3.2. Selectivity

Selectivity of the described method was determined by analyzing forcedly degraded powder samples. Forced degradation studies were performed to provide an indication of the stability indicating property of the proposed method. Intentional degradation was achieved by exposing the formulation product to stress conditions of light (4500 lx), high temperature (80 °C), acid (3 mol/l HCl) and base (3 mol/l NaOH) in order to test the ability of the proposed method to separate the active components from degradation products. Samples were degraded to levels where the content of amoxicillin sodium and sulbactam sodium in the sample was lowered to less than 90% of the original level. Chromatograms for photo-degradation, thermal degradation, acid degradation and base degradation were individually shown in Fig. 2A–D. Under the given stress conditions, two drugs are unstable and significant degraded peaks appear. Two major peaks corresponding two parent drugs can be found in Fig. 2A and B, but disappear in Fig. 2C and D. This suggests the poor stability of the two drugs under acidic and base conditions. Fig. 2A–D shows that most of the degradation products could be well resolved from the active components and the proposed method displays satisfactory selectivity to amoxicillin sodium and sulbactam sodium and their degradation products.

In addition, placebo formulations were also determined to see if any interference from the excipients existed. A clean chromatogram was obtained, indicating no interferences from the powder excipients. The ability of the method to separate the drugs from their degradation products and the non-interference from the matrix indicates the good selectivity of the developed method.

### 3.3. Linearity

Linearity was determined by building three calibration curves with five concentration levels each. Peak area ($A$) and concentration ($C$) of each drug substance was subjected to regression analysis to calculate the calibration equation and correlation coefficients. The regression equations obtained for the two drugs were $A = 9322.2 + 256.7C$ ($r = 0.9991$, $n = 5$) for amoxicillin sodium and $A = 264.6 + 55.2C$ ($r = 0.9996$, $n = 5$) for sulbactam sodium, respectively. The individual linear range was 155.3–1553.0 μg/ml for amoxicillin sodium and 45.0–450.0 μg/ml for sulbactam sodium. The results show that within the concentration range tested, there was an excellent correlation existed between peak area and concentration of each drug.

### 3.4. 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. LOQ of each drug was experimentally verified by six injections of each drug at its LOQ concentration. The LOQ of amoxicillin sodium and sulbactam sodium were found to be 1.28 and 3.06 μg/ml, respectively.

### 3.5. 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 as the percentage of analyte recovered by the assay. Mean recoveries (mean ± S.D.) for amoxicillin sodium and sulbactam sodium from the combination formulation are 99.3 ± 1.2% ($n = 9$) and 100.7 ± 0.83% ($n = 9$), respectively. The results indicate satisfactory accuracy of the method for simultaneous determination of the two drugs in the formulation.

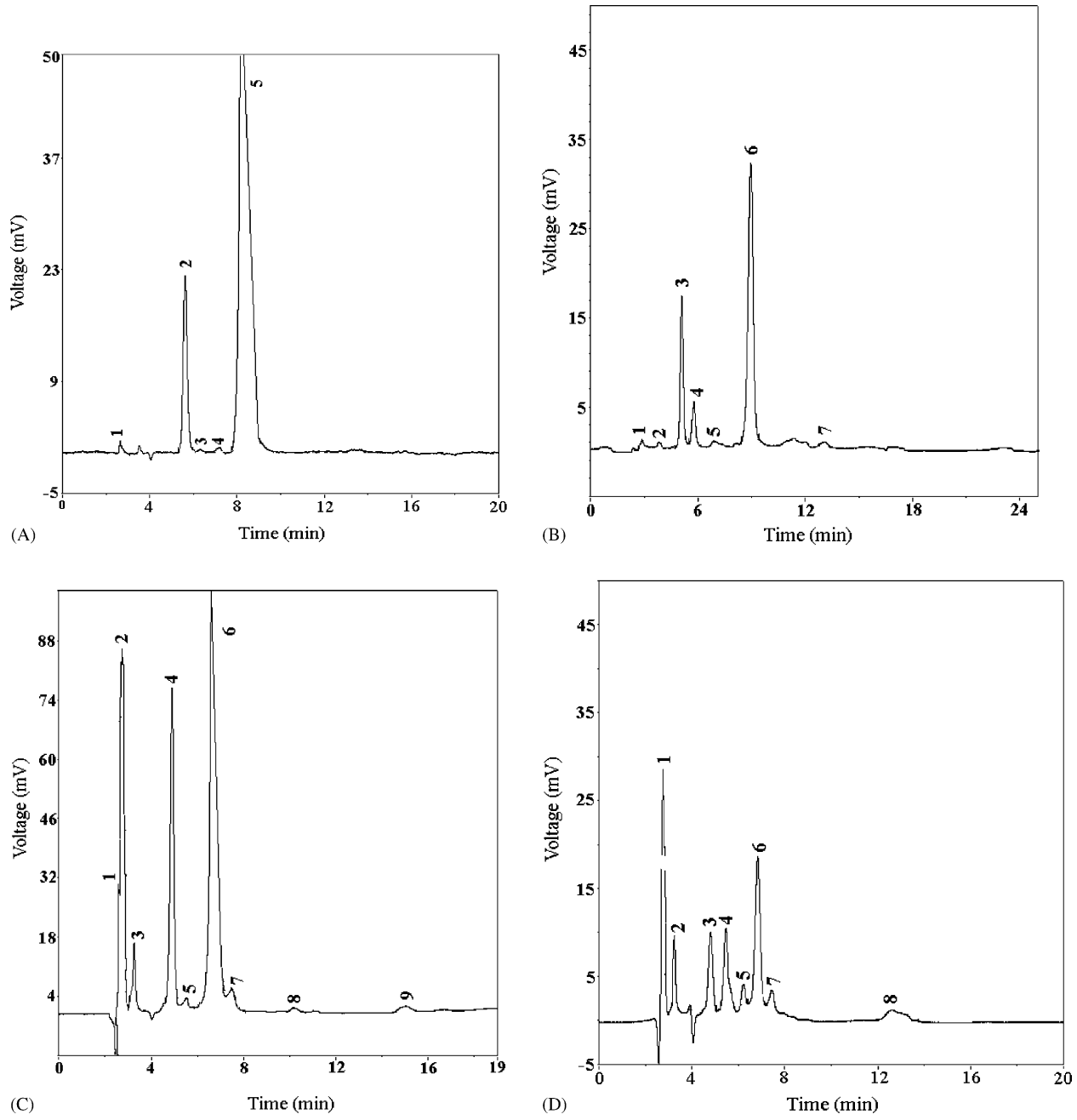


Fig. 2. LC chromatograms of amoxicillin sodium and sulbactam sodium and their degraded products: (A) photodegradation; (B) thermal degradation; (C) acid degradation; (D) base degradation.

### *3.6. Precision*

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 R.S.D. values for amoxicillin sodium and sulbactam sodium was 0.84 and 1.4%, respectively.

Intra-day precision of the developed LC method was determined by preparing samples of the same batch in nine determinations. The samples were taken in three levels corresponding to 80, 100 and 120% of label claim and three replicate determinations were made at each level. R.S.D. values of the assay results, expressed as a percentage of the label claim, was used to evaluate the precision of the method. The obtained R.S.D. values were 1.1% for amoxicillin sodium and 1.1% for sulbactam sodium. Inter-day precision was determined by assaying the powder samples for consecutive 6 days, which was found to be 1.9 and 2.0% for amoxicillin sodium and sulbactam sodium, respectively. The above results indicated the sufficient precision of the developed LC method.

Table 1
Assay results for amoxicillin sodium and sulbactam sodium sterile powder for injection (mean ± S.D., %)

| Batch no. | Amoxicillin | *Sulbactam* |
|---|---|---|
| 1 | 98.7 ± 1.1 | 101.8 ± 1.2 |
| 2 | 101.3 ± 1.0 | 104.0 ± 1.1 |
| 3 | 100.3 ± 1.2 | 100.5 ± 1.5 |

### *3.7. Solution stability*

The stability of both standard and sample solutions was determined by monitoring the peak area responses of the standard solution of amoxicillin sodium and sulbactam sodium, and a sample solution over a period of 4 h at room temperature. The results showed that the retention times and peak areas of amoxicillin sodium and sulbactam sodium remained almost unchanged and no significant degradation was observed within 2 h, indicating that both solutions should be freshly prepared for the analyses.

### *3.8. Method application*

The validated LC method was applied to the simultaneous determination of amoxicillin sodium and sulbactam sodium in sterile powder for injection. Three batches of the samples were assayed and the assay results, 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.

## 4. Conclusion

The developed isocratic LC method with UV detection offers simplicity, selectivity, precision and accuracy. In the absence of such paired-ion reagent as TBAH, the proposed LC method produced symmetric peak shape, good resolution and reasonable retention time for both drugs. It can be used for the simultaneous determination of amoxicillin sodium and sulbactam sodium in pharmaceutical formulations in ordinary laboratories.

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