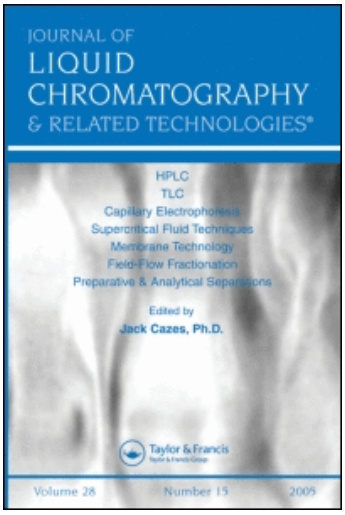

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A Validated Method for Simultaneous Determination of Piperacillin Sodium and Sulbactam Sodium in Sterile Powder for Injection by HPLC

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ABSTRACT

A rapid and accurate high performance liquid chromatography (HPLC) method is described for simultaneous determination of piperacillin sodium and sulbactam sodium in sterile powder for injection. Chromatographic separation of the two drugs was achieved on a Diamonsil C_{18} column (150×4.6 mm, 5 μm) using a mobile phase consisting of a mixture of methanol and 0.01 M tetrabutylammonium hydroxide (TBAH)

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(50 : 50, v/v). The flow rate was 1.0 mL/min and detection was made at 220 nm. Salicylic acid was used as internal standard. Separation was completed in less than 15 min. The method was validated for system suitability, linearity, accuracy, precision, limit of quantitation, and robustness. The linear range for piperacillin sodium and sulbactam sodium was 103.0–1030.0 μg/mL and 24.0–240.0 μg/mL, respectively. Limit of quantitation (LOQ) was 22.05 μg/mL for piperacillin sodium and 5.48 μg/mL for sulbactam sodium. Relative standard deviation (RSD), for piperacillin sodium and sulbactam sodium was less than 1.0 and 1.2%, respectively.

Key Words: Piperacillin sodium; Sulbactam sodium; HPLC.

INTRODUCTION

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. Synergy with beta-lactam antibiotics is most marked in bacterial species in which beta-lactamase is a major mechanism of resistance.[1] Piperacillin sodium is a semisynthetic broadspectrum antibiotic for parenteral use. It exerts its bactericidal activity by inhibiting both septum and cell wall synthesis and is active against a variety of gram-positive and gram-negative aerobic and anaerobic bacteria. The chemical structures of sulbactam sodium and piperacillin sodium are shown in Fig. 1.

Production of beta-lactamases is the most common mechanism by which gram-negative bacteria express resistance to beta-lactam antibiotics. One successful method of circumventing the threat of plasmid-encoded beta-lactamases is to combine inhibitors of these enzymes with penicillins.

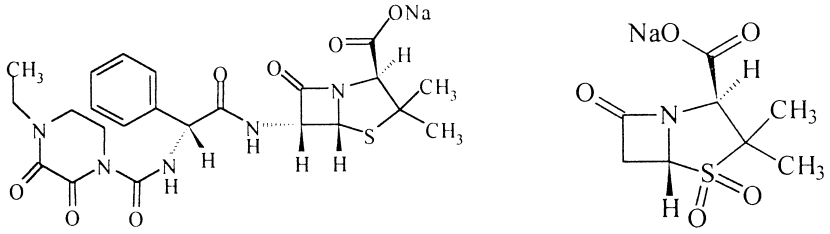

Figure 1. Structures of piperacillin sodium and sulbactam sodium.

The combined use of beta-lactam antibiotics with beta-lactamase inhibitor was effective against beta-lactamase-producing antibiotic-resistant strains.[2]

Sulbactam sodium has been shown to act synergistically with piperacillin sodium and a variety of other beta-lactam antibiotics against a broad spectrum of bacterial pathogens. Based on the clinical effects and requirements, a new combination formulation, piperacillin sodium and sulbactam sodium in sterile powder for injection, has been developed and an analytical method is required for the new combination formulation.

A literature survey revealed that several methods have been used for determination of piperacillin sodium by CE,[3,4] polarography,[5] spectrophotometry,[6] HPLC;[4,7–14] and of sulbactam sodium by CE,[15] UV,[16] enzymatic method,[17] GC–MS and bioassay,[18] and HPLC.[19–25] Among the methods, high performance liquid chromatography (HPLC) is the most often involved method for individual determination of the two drugs. However, no references have been found for simultaneous determination of piperacillin sodium and sulbactam sodium in pharmaceutical preparations.

The present paper describes a rapid and reliable method for simultaneous determination of piperacillin sodium and sulbactam sodium in sterile powder for injection, which can be used for the routine analysis of this combination formulation in ordinary laboratories. In this paper, development, optimization, and validation of such a method are presented.

EXPERIMENTAL

Apparatus

Chromatography was performed on Hewlett Packard (HP) series 1100, which consisted of a G1310A Iso Pump, an HP variable UV/VIS detector, and a G1328A Manual Injector with 20 μL loop (Agilent, USA). An 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.

Chemicals and Reagents

Piperacillin sodium was from Bokang Pharmaceuticals, Ltd. (Taiyuan, China) and sulbactam sodium was from Shenyang Pharmaceuticals, Ltd. (Shenyang, China). Piperacillin sodium and sulbactam sodium in sterile powder for injection was from Shenyang Pharmatech Institute of

Pharmaceuticals (Shenyang, China). One vial contains 1.0 g piperacillin sodium and 0.25 g sulbactam sodium. Mass ratio of piperacillin sodium and sulbactam sodium in the product is 4 : 1. Piperacillin sodium reference standard and sulbactam sodium reference standard were from the National Institute for Control of Pharmaceutical and Biological Products (NICPBP) (Beijing, China). Salicylic acid was used as internal standard. Methanol was of HPLC grade from Tedia Company, Inc. (USA). HPLC-grade water was prepared by using a Milli-Q water purification system from Millipore (Molsheim, France). All other chemicals used were at least of analytical grade.

Chromatographic Conditions

A Diamonsil C_{18} column (150 mm × 4.6 mm, 5 μm) was used. The mobile phase was a binary mixture of methanol and 0.01 M tetrabutylammonium hydroxide (TBAH) (50 : 50, v/v). The aqueous solution of 0.01 M TBAH was prepared by diluting 25 mL of 0.4 M TBAH with 900 mL water, adjusting the pH of the diluted solution to 7.0 with the solution of 1.5 M phosphoric acid and then diluting the solution to 1000 mL with water. The mobile phase was filtered and degassed before use. The flow-rate of the mobile phase was 1.0 mL/min. Injection volume was 20 μL. Experiments took place at room temperature. Absorption was measured at 220 nm.

Preparation of Stock and Working Standard Solutions

Mixed stock solution of piperacillin sodium and sulbactam sodium was prepared in mobile phase with the concentration of piperacillin sodium at 2.06 mg/mL and sulbactam sodium at 0.48 mg/mL, respectively. Stock solution of salicylic acid (used as internal standard) was prepared in mobile phase with the concentration of 0.76 mg/mL. Both solutions were stored at 4°C.

Working standard solutions were prepared by quantitatively transferring 0.5, 1.0, 2.0, 3.0, and 5.0 mL of the mixed stock solution of piperacillin sodium and sulbactam sodium and 1.0 mL of stock solution of salicylic acid to 10 mL of volumetric flasks, and making volume with mobile phase. The concentration range was 103.0–1030.0 μg/mL for piperacillin sodium and 24.0–240.0 μg/mL for sulbactam sodium, respectively. Each solution contained salicylic acid as the internal standard at a concentration of 76.0 μg/mL.

Sample Preparation

An amount of the lyophilized powder equivalent to 200 mg piperacillin sodium was accurately weighed, transferred to a 50 mL volumetric flask, dissolved, and diluted to volume with the mobile phase. Then, 1 mL of the diluted solution and 1.0 mL of the stock solution of salicylic acid were accurately transferred to a 10 mL volumetric flask and diluted to volume with the mobile phase. The prepared solution was injected into the HPLC system and determined by a calibration curve.

Data Analysis

For determination of piperacillin sodium and sulbactam sodium in injection solutions, peak-area ratios of the individual drugs to internal standard were used to construct the calibration curves. Regression equations were obtained through the least-square linear regression analysis, applied to peak-area ratios as a function of their concentrations.

RESULTS AND DISCUSSION

Method Development

For the purpose of achieving simultaneous determination of piperacillin sodium and sulbactam sodium in sterile powder for injection, several eluents based on acetonitrile, methanol, phosphate buffer, and aqueous solution of TBAH were tried in the initial experiments. A mixture of pH 5.8 phosphate buffer, acetonitrile, and water (100 : 20 : 50, v/v/v) was first used, but failed to achieve our purpose because of the short retention time of sulbactam sodium (< 2 min). The proportions of the three solvents in the mobile phase were variously changed later, but the results were not any better. Afterwards, a binary mixture of acetonitrile and 0.005 M TBAH (17 : 83, v/v) produced reasonable separation of the two drugs, with asymmetric peak shapes. Then, acetonitrile was replaced with methanol and the concentration of TBAH was increased from 0.005 M to 0.01 M, which led to better peak shapes and reasonable retention of both drugs. After this, various proportions of methanol and 0.01 M TBAH separately at 45 : 55, 50 : 50, 55 : 50, and 60 : 40 (v/v) were tried. It was found, that increasing the proportion of methanol in the mobile phase had a greater influence in the retention time of piperacillin sodium than that of sulbactam sodium. This may be due to the greater hydrophobicity of piperacillin sodium than sulbactam sodium. The retention time of piperacillin

sodium was significantly shortened from 28.6 to 5.2 min, while the retention time of sulbactam sodium was only slightly changed from 4.1 to 2.5 min, the results of which just met our purpose. Therefore, a binary mixture of methanol and 0.01 M TBAH (50 : 50, v/v) was finally adopted as the mobile phase because it achieved the most reasonable retention, symmetric peak shapes, and good resolution of the two drugs. The retention times of piperacillin sodium and sulbactam sodium were 11.9 min and 3.2 min, respectively. The flow rate was set at 1.0 mL/min.

Since both piperacillin sodium and sulbactam sodium in the mobile phase have no significant UV maximum but end absorption, to ensure the sensitivity of the method, the wavelength of 220 nm was employed for the detection.

Several compounds, such as acetominophen, caffeine, and salicylic acid, were tried to find the right internal standard. Acetominophen and caffeine failed to produce peaks with reasonable retention times and the right position. The retention times were about 2.3 min for acetominophen and 2.8 min for caffeine, respectively. Both the peaks of acetominophen and caffeine were situated before the peak of sulbactam sodium, not between the peaks of piperacillin sodium and sulbactam sodium. As a result, salicylic acid was chosen as the most appropriate one for this study because it had reasonable retention time ($t_R = 6.1$ min), right peak position situated between the peaks of the two drugs, and good resolution with both piperacillin sodium and sulbactam sodium, and also presented a symmetric peak. A representative chromatogram of piperacillin sodium, sulbactam sodium, and salicylic acid is shown in Fig. 2.

The stability of both standard and sample solutions was determined by monitoring the peak area responses of solutions of the standard mixture of piperacillin sodium and sulbactam sodium and a tablet sample over a period of one week. The results show that for both solutions, the retention times and peak areas of piperacillin sodium and sulbactam sodium remained almost unchanged (RSD% less than 2.0) and no significant degradation was observed within the given period, indicating that both solutions were stable for at least one week.

Method Validation

System Suitability

System performance parameters of the developed HPLC method were determined by analyzing standard working solutions. Chromatographic parameters, such as number of theoretical plates (N), resolution (Rs), capacity factor (k), and selectivity factor (α) were determined. The results are shown in Table 1, indicating the good performance of the system.

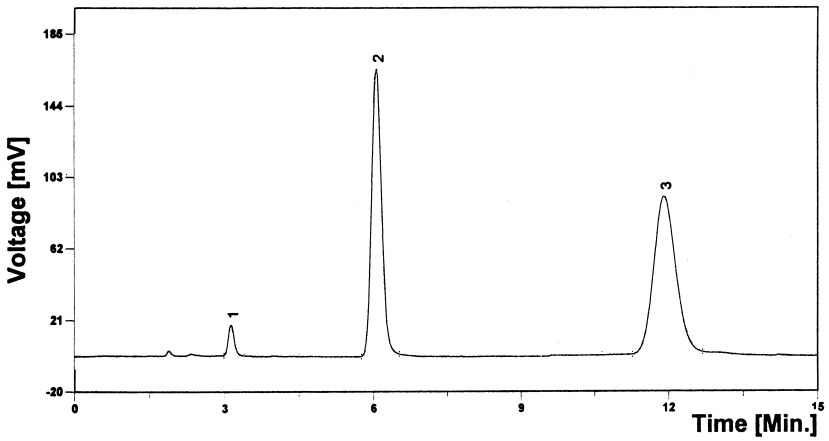

Figure 2. A typical chromatogram of a mixture of sulbactam sodium, salicylic acid and piperacillin sodium. Peak: 1. sulbactam sodium; 2. salicylic acid (internal standard); 3. piperacillin sodium. The chromatographic conditions used were: Diamonsil C_{18} column, mobile phase of methanol −0.01 M tetrabutylammonium hydroxide (TBAH) (50 : 50, v/v), flow rate of 1.0 mL/min, detection wavelength of 220 nm, room temperature.

System repeatability was determined by five replicate injections of a working standard solution, and the relative standard deviations (RSD) of peak areas of both drugs and internal standard were calculated to evaluate the repeatability. It was found that RSD for both drugs and internal standard was less than 2.0%.

Table 1. System performance parameters for sulbactam sodium, salicylic acid, and piperacillin sodium ($n = 5$).

Peak no.	Compounds	t_R	N	k	Rs	α
1	Sulbactam sodium	3.18	3,291	1.62		
					17.0	2.51
2	Salicylic acid	6.08	3,534	4.07		
					16.6	2.20
3	Piperacillin sodium	11.93	4,320	8.94		

Note: The chromatographic conditions used were: Diamonsil C_{18} column, mobile phase of methanol −0.01 M TBAH (50 : 50, v/v), flow rate of 1.0 mL/min, detection wavelength of 220 nm, room temperature.

The above results indicate that the described method showed adequate column efficiency, good resolution, and repeatability, and could be applied for simultaneous determination of the two drugs.

Linearity

Under the experimental conditions described above, linear calibration curves for both piperacillin sodium and sulbactam sodium were obtained throughout the concentration ranges studied. Regression analysis was done on the ratios of peak-areas of the two drugs to that of the internal standard (y) vs. concentration (x). The regression equations obtained for piperacillin sodium and sulbactam sodium were $y = 0.0057x + 0.0264$ ($r = 0.9998$, $n = 5$) and $y = 0.0014x - 0.0033$ ($r = 0.9998$, $n = 5$), respectively. The linear range was 103.0–1030.0 μg/mL for piperacillin sodium and 24.0–240.0 μg/mL for sulbactam sodium, respectively.

Limit of Quantitation

Limit of quantitation (LOQ) was established at a signal-to-noise ratio of 10. Limit of quantitation of piperacillin sodium and sulbactam sodium and were experimentally determined by six injections of each drug at the LOQ concentration. The LOQ of piperacillin sodium and sulbactam sodium were found to be 22.05 μg/mL and 5.48 μg/mL, respectively.

Accuracy

Accuracy was determined by applying the described method to synthetic mixtures containing known amounts of each drug corresponding to 80, 100, and 120% of label claim. The accuracy was then calculated as the percentage of analyte recovered by the assay. Mean recoveries for piperacillin sodium and sulbactam sodium from the formulations are shown in Table 2 indicating good accuracy of the method for simultaneous determination of the two drugs.

Precision

Precision of the method was determined with the product. An amount of the product powder equivalent to 80, 100, and 120% of label claim of piperacillin sodium was accurately weighed and assayed in five replicate determinations for each of the three weighing amounts. The results for precision are shown in Table 2, indicating that acceptable precision was

Table 2. Accuracy and precision of the HPLC method for simultaneous determination of sulbactam sodium and piperacillin sodium.

	Accuracy		Precision		
Drugs	Mean recovery ± SD	RSD (%, $n = 9$)	80%	100%	120% of label claim
Sulbactam sodium	99.9 ± 0.87	1.1	1.0	0.71	0.88
Piperacillin sodium	100.1 ± 0.75	0.95	0.91	0.79	0.61

Note: The chromatographic conditions used were: Diamonsil C_{18} column, mobile phase of methanol −0.01 M TBAH (50 : 50, v/v), flow rate of 1.0 mL/min, detection wavelength of 220 nm, room temperature.

achieved for piperacillin sodium and sulbactam sodium, as revealed by relative standard deviation data (RSD < 2.0% in all of the levels of the two drugs).

Method Application

The validated LC method was applied to simultaneous determination of piperacillin sodium and sulbactam sodium in sterile powder for injection. Three batches of the product were assayed. The assay results, expressed as a percentage of the label claim, are shown in Table 3, which indicates that the amount of each drug in the product meets the requirements.

Table 3. Assay results for simultaneous determination of sulbactam sodium and piperacillin sodium in sterile powder for injection (Mean ± SD, %).

Batch no.	Sulbactam sodium	Piperacillin sodium
1	102.3 ± 0.88	99.5 ± 0.72
2	101.4 ± 1.03	99.4 ± 0.67
3	101.9 ± 1.11	99.4 ± 0.81

Note: The chromatographic conditions used were: Diamonsil C_{18} column, mobile phase of methanol −0.01 M TBAH (50 : 50, v/v), flow rate of 1.0 mL/min, detection wavelength of 220 nm, room temperature.

CONCLUSIONS

The HPLC method developed for simultaneous determination of piperacillin sodium and sulbactam sodium in sterile powder for injection has sufficient accuracy, precision, and selectivity. In conclusion, the developed method can be used for the assay of the two drugs in this new product.

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