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ORIENTAL JOURNAL OF CHEMISTRY
An International Open Free Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2011, Vol. 27, No. (4):
Pg. 1659-1664

Simultaneous Estimation of Ampicillin Sodium and Sulbactam Sodium in Injectable Dosage Form by High Performance Liquid Chromatography

MADHUKAR A. BADGUJAR and KIRAN V. MANGAONKAR*

Analytical Chemistry Research Laboratory SVKM's Mithibai College,
Vile-Parle (West), Mumbai - 400 056 (India).
*Corresponding author: E-mail: bmbadgujar@yahoo.com

(Received: September 10, 2011; Accepted: October 22, 2011)

ABSTRACT

A reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Ampicillin sodium and Sulbactam sodium in injectable formulation. The separation was achieved by Inertsil C_{18} column (250mm × 4.6mm i.d 5µm particle size) and Acetonitrile: Ammonium acetate buffer pH = 6.0 (17 : 83) as mobile phase at a flow rate of 1.0mL/min. The retention times of Ampicillin sodium and Sulbactam sodium were found to be 5.86mins & 4.33mins respectively. The proposed method was validated for linearity, accuracy and precision. The calibration curve was linear over the range of 50-150 µg/mL for Sulbactam sodium and 100 - 300 µg/mL for Ampicillin sodium. The proposed method can be applied successfully in routine analysis.

Key words: Ampicillin Sodium (AMP), Sulbactam Sodium (SB), RP-HPLC, Validation.

INTRODUCTION

Ampicillin(AMP) a derivative of 6-aminopenicillanic acid has a wide therapeutic use. But it is ineffective against organisms producing β-lactamase. Hence it needs to be administered with a β-lactamase inhibitor such as Sulbactam. Sulbactam(SB)[1] is a molecule that is given in combination with beta-lactam antibiotics to inhibit beta-lactamase, an enzyme produced by bacteria that destroys the antibiotics. Sulbactam is an irreversible inhibitor of beta-lactamase, it binds the enzyme and does not allow it to interact with the antibiotic Sulbactam is able to inhibit the most common forms of beta-lactamase but is not able to interact with the ampC cephalosporinase. Thus, it confers little protection against bacteria such as *Pseudomonas aeruginosa*, Citrobacter, Enterobacter, and Serratia, which often express this gene.

A detail Literature survey for AMP and SB revealed that few analytical methods are reported for the determination of AMP with SB by HPLC[1-9], but these methods are time consuming. Hence we focused on developing rapid, sensitive and cost effective method. The manuscript describes the development and subsequent validation of a screening method to simultaneously quantify AMP and SB by HPLC.

EXPERIMENTAL

Chemicals and reagents

AMP and SB working standard were obtained from Unichem (Mumbai, India), Injectable dosage form containing AMP (1000mg) and SB (500mg) were obtained from Unichem (Mumbai, India), HPLC grade methanol and acetonitrile were purchased from Baker (Mumbai, India). AR grade Ammonium acetate was purchased from Merck (Mumbai, India).

Preparation of stock solutions

Preparation of Buffer

Buffer solution was prepared by dissolving accurately weighed 0.081g of ammonium acetate (CH_3COONH_4) in 1 liter of distilled water (HPLC Grade). The concentration of the CH_3COONH_4 solution was 0.01M.

Preparation of Mobile phase

Mobile phase was prepared by using the above prepared buffer and acetonitrile, mixing them in the volume ratio of (83:17, v/v). The pH of this mixture was 6.0. The mobile phase was then sonicated for 10min to remove the dissolved gases which may cause interference to the HPLC system.

Preparation of Standard stock solution

50mg of Sulbactam (SB) sodium and 100 mg of Ampicillin sodium (AMP) were accurately weighed and transferred to a 100cm^3 volumetric flask. It was dissolved in a minimum quantity of double distilled water and then diluted up to the mark with water. The concentration of the solution obtained was 500 µg/mL for Sulbactam and 1000 µg/mL for Ampicillin sodium (Solution A).

5cm^3 of this solution A was taken in a 25 cm^3 volumetric flask and diluted upto the mark with distilled water. The concentration of the solution obtained was 100 µg/mL for SUB and 200 µg/mL for AMP(100% level).

Preparation of Sample solution

The sample solution of Sulbactam sodium and Ampicillin sodium was prepared from injectable dosage form. The content of the Injectable dosage form are 0.5gm of anhydrous Sulbactam sodium and 1.0 gm anhydrous Ampicillin sodium. 150mg of the sample was taken in 100 cm^3 volumetric flask. It was dissolved and diluted by sterile water upto the mark. Then 5cm^3 of this solution was taken in a 25 cm^3 volumetric flask and diluted up to the mark with sterile water.

Chromatographic Conditions

The chromatographic system consist of a Waters HPLC system having Waters 501 isocratic pump equipped with Waters ™ 717plus autosampler and a Waters 486 tunable absorbance UV-detector. The data was recorded using Millenium^{32} chromatographic software. Separation was performed on a 250 mm × 4.6 mm i.d., 5 µ particle size Inertsil ODS 3V C_{18} column. Mobile phase consisted of a mixture of buffer: acetonitrile (83 : 17) (pH = 6.0). Flow rate was kept at 1.0 mL/min. Wavelength was set at 230 nm.

Method Validation

The method was validated as per ICH guidelines for specificity, linearity, precision, accuracy, recovery and stability. Specificity was investigated by analyzing the blank diluents and samples of 100% level for any interference of the endogenous material at the retention times of AMP and SB. The accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drugs into a 100% level sample solution. The recovery was performed at three levels (110%, 120%, and 130%) of the label claim per injectable dosage form (0.5gm of SUB and 1.0 gm of AMP). Three samples were prepared for each recovery level. The sample solution contains 100mg of Ampicillin and 50mg of Sulbactam. The 0% level can be considered as 100% level. In this sample solution 10%, 20% and 30% of the standard drugs were added and injected into the system. The precision of the method was demonstrated by interday and intraday variation studies, six repeated injections of standard and sample were made and percentage RSD was calculated. In the intra-day variation studies six repeated injections of standard and sample solution was carried out by injecting on the same day at different intervals and percentage RSD was calculated. In the interday variation studies six repeated injections of standard and sample solution were made for three consecutive days and percentage RSD was calculated. The linearity of the

method was demonstrated at seven concentration levels of the mixed standards of AMP and SB.

The robustness of the method was checked by changing the chromatographic conditions. The organic phase of the mobile phase was varied by ±5% while pH of the buffer was varied by ±0.2 units. The three different sample solutions were injected in each varied condition and the assay was checked.

RESULTS AND DISCUSSIONS

Optimization of the chromatographic conditions

In order to develop an isocratic reverse phase stability indicating HPLC method for the simultaneous determination of AMP and SB in Injectable dosage form, the chromatographic conditions were optimized. For better separation and resolution the different buffers were tried. It has been found that ammonium acetate buffer (pH = 6.0) gave better peak shape than other buffers. The different compositions of mobile phase were changed for getting better separation of these analytes. Thus the mobile phase composed of the mixture of buffer (0.01M ammonium acetate pH= 6.0) and acetonitrile in the ratio of (83:17 v/v) was finalized. The better separation, peak symmetry and reproducibility were obtained with Inertsil C18, 250mm x 4.6mm, 5ìm column compared to Thermo BDS Hypersil C8, 150 mm x 4.6mm, 5ìm column. Both these analytes gave better response at 230 nm wavelength using UV detector. The flow rate kept was 1.0mL/min. There was no peak tailing observed under these optimized chromatographic conditions. The retention times of AMP and SB were found to be 5.8 mins and 4.3 mins respectively. It has been found that there was no any interference of the excipients and degraded products of the analytes at the retention times of the analytes. Thus the developed method was stability indicating method.

Validation

The proposed method was shows short elution time and good separation between AMP and SB. The system suitability test was performed as per the international conference of harmonization (ICH)[10] guidelines to confirm the suitability and the reproducibility of the method. Standard solution having assay concentration of individual analyte (100% level) was prepared as described earlier and injected to HPLC. The %RSD values were found to be satisfactory and meeting the requirements. %RSD values were found to be 0.280 and 0.792 for AMP and SB respectively. The tailing factor and theoretical plates were found to be within the limits.

The method was linear over the range 100–300 µg/mL and 50–150 µg/mL for Anpicillin sodium and Sulbactam sodium respectively. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was Y = 9453 X + (-86618) (r^2= 0.9991) for AMP and Y = 4440 X - 10522 (r^2= 0.9998) for SB. The results

Table 1:

S. No	% Added	Original amount	Added amount	Total amount	Mean (n = 5)	% Recovery	S.D	%RSD
% Recovery of Ampicillin sodium: Amount of Ampicillin sodium in mg								
1	10	100	10	110	111.77	101.36	0.630	0.621
2	20	100	20	120	120.34	100.17	0.106	0.105
3	30	100	30	130	130.68	100.37	0.160	0.159
% Recovery of Ampicillin sodium: Amount of Ampicillin sodium in mg								
1	10	50	5	55	54.82	99.43	0.456	0.459
2	20	50	10	60	59.34	98.12	0.098	0.100
3	30	50	15	65	64.32	98.30	0.155	0.157

show that an excellent correlation between response factor and concentration of drugs.

The limit of quantification (LOQ) and limit of detection (LOD) was established at a signal-to-noise ratio. The signal-to-noise ratio(S/N) method was adopted for the determination of lower limit of quantification. The limit of quantification is estimated as ten times the S/N ratio. Quantification limit was achieved by injecting a series of possible dilute solutions of AMP and SB and the precision was established at quantification level. The LOQ and LOD of Ampicillin and Sulbactam were experimentally determined. The LOD of AMP and SB was found to be 0.8 µg/mL and 0.4 µg/mL respectively. The LOQ of AMP and SB was found to be 2.0 µg/mL and 1.0 µg/mL respectively.

The developed method was validated for system precision (repeatability) and method precision. Six injections of mixed standards of 200 µg/mL of AMP and 100 µg/mL of SB were injected and %RSD calculated for injection repeatability. Six samples were prepared at 100% levels and assayed according to the procedure. The average assay of three replicate analysis was found to be 98.02% for AMP and 98.39% for SB with a relative standard deviation of 0242% and 0.69% respectively.

The accuracy of the method was determined by the standard addition method at three different levels. The sample solution of 100% level was considered as a zero level and 10%, 20% and 30% of the standard drug of analytes were added respectively. Each determination was performed in

Table 2: Solution stability of Ampicillin sodium sample solution

Conditions	Level in %	Peak area	% Assay in Injectable sample	% Label claim
Initial - 0 hrs	100	1760546	99.99	99.99
Initial -6hrs	100	1765849	100.30	100.30
Initial -12hrs	100	1763624	100.17	100.17
Initial -24hrs	100	1755129	99.69	99.69
	Mean	1761287	100.04	100.04
	S.D.	4023	0.23	0.2
	%RSD	0.23	0.23	0.23
Solution stability of Sulbactam sodium sample solution				
Initial - 0 hrs	100	427102	50.09	100.18
Initial -6hrs	100	427628	50.15	100.30
Initial -12hrs	100	427893	50.18	100.36
Initial -24hrs	100	419927	49.25	98.49
Mean	425637	49.92	99.83	
S.D.	3309	0.39	0.8	
%RSD	0.78	0.78	0.78	

Table 3: Analysis of Injectable formulation: Sulbacin Vial (Label Claim: Ampicillin 1gm + Sulbactam 0.5gm)

Drug	Std wt (mg)	Avg. wt mg	Label Claim (mg)	Mean Std area	Mean Sample area	Amount Present	% Assay
Ampicillin Sodium	1000mg	1500	1000mg	1718400	1761287	100.04 mg	100.04%
Sulbactam Sodium	500mg		500mg	426344	425637	49.92mg	99.83%

triplicates. The accuracy was then calculated as the percentage of the standard drug recovered by the recovery study. Mean recoveries for AMP and SB from the combination formulation are shown in Table 1. The results are well within the acceptance limit and hence the method is accurate.

The stability of both the standard and the sample was determined by monitoring the peak area responses of the standard solution and the sample solution of AMP and SB. These solutions were analyzed at 0, 6, 12, and 24 hrs against a freshly prepared standard at each time interval. The relative standard deviations for the assay values, determined up to 24 hrs for Ampicillin and Sulbactam in assay preparation and standard preparation, were 0.21% and 0.09%, respectively. The assay values were within ±2% after 24 hrs. The results show that the solutions were stable for 24 hrs at room temperature. The results are shown in Table2.

The robustness of the method was performed by deliberately changing the chromatographic conditions. The organic strength of the mobile phase was varied by ±5% while pH of the buffer was varied by ±0.2 units. The standard solution and three different sample preparations were injected in each varied condition and the assay was checked. Under all varied conditions, it has been found that the %RSD for the assay values for AMP and SB were found to be well within the acceptance limit of 2%.

The specificity of the method was checked by injecting a sample solution. The chromatogram of the sample solution shows that there is not any interference of the exciepients at the retention time of the analytes.

Application

The validated stability indicating HPLC method was applied to the simultaneous

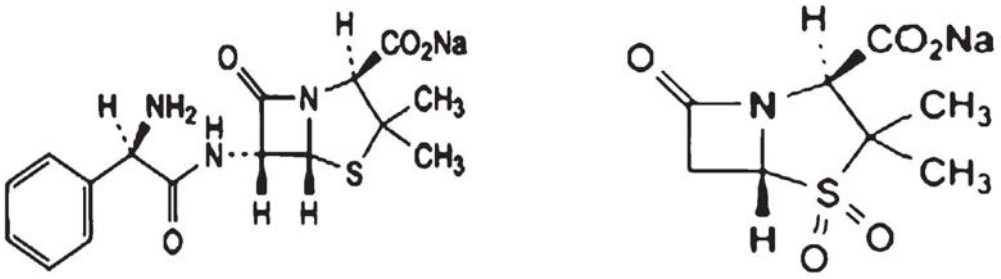

Fig. 1: Structural formula of (a) Ampicillin Sodium (b) Sulbactam Sodium

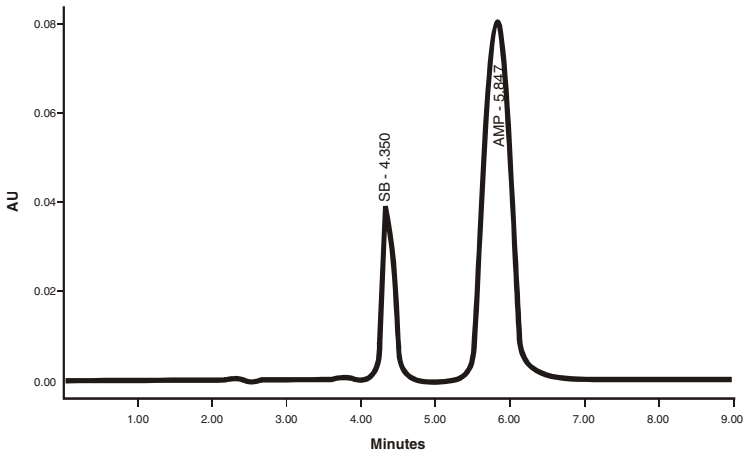

Fig. 2: Chromatogram of Ampicillin and Sulbactam in 100% sample solution

determination of AMP and SB in Injectable dosage form. The samples were analyzed and the assay results are as per the label claim shown in Table3.

CONCLUSION

The isocratic RP- HPLC method has proved to be simple, specific, precise and accurate and is suitable for simultaneous quantification of AMP and SB. The proposed method gives a good resolution among these analytes. High percentage of recovery shows that the method is accurate. The forced degradation study shows that there is no any interference of the exciepients and the degraded products at the retention times of AMP and SB.

ACKNOWLEDGEMENTS

The authors are thankful to Department of Chemistry, Mithibai College, Mumbai, India for their support.

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