Asian Journal of Scientific Research

ISSN 1992-1454

science alert
http://www.scialert.net

ANSInet
an open access publisher
http://ansinet.com

Asian Journal of Scientific Research 7 (3): 335-340, 2014
ISSN 1992-1454 / DOI: 10.3923/ajsr.2014.335.340

Spectrophotometric Determination of Amoxycillin in Different Formulations

[1]N. Anusha and [2]Uday B. Kamath
[1]School of Chemical and Biotechnology, SASTRA University, Thanjavur, 613401, Tamilnadu, India
[2]Karnataka Antibiotics and Pharmaceuticals Ltd., Bangalore, 560058, India

Corresponding Author: N. Anusha, School of Chemical and Biotechnology, SASTRA University, Thanjavur, 613401, Tamilnadu, India Tel: 919443627165

ABSTRACT

A simple, accurate, specific and reliable colorimetric method was developed for the quantification of amoxycillin in different salt forms in different formulations. On treating amoxycillin with acidic formaldehyde, intra molecular aminolysis occurs. As a result, α-amino benzoyl group present in the side chain of Amoxycillin attacks the carbonyl group present in β-lactam ring nucleophilically, resulting in the formation of pyrazine derivative. The λ_{max} was found to be 341 nm. This helps in the determination of amoxycillin without any interference from sulbactam. The method was found to be linear from 5.8 to 40.6 μg mL^{-1} with a correlation coefficient of 0.999.

Key words: Amoxycillin, aminolysis, β-lactam ring, correlation coefficient

INTRODUCTION

Amoxicllin is a well-known drug with anti-microbial activity (Zheng *et al*., 2013). Amoxicllin belongs to the class of aminopencillins, which are derived from pencillin (www.hipra.com). The presence of amino group in aminopencillins imparts additional activity against gram-negative bacterial species, compared to plain pencillin. Amoxycillin acts by perturbing the processes responsible for repair of mucopeptide wall of bacterial species (www.hipra.com). Owing to widespread use and acceptability of Amoxycillin, the same has been used in isolation or with other drugs in formulation. Reliable and fast analytical methods are required for estimation of Amoxycillin in combined formulations in various dosage forms such as tablets, parenteral etc. The requirements of a reliable analytical method are (i) Accuracy, (ii) Reproducibility, (iii) Ruggedness and (iv) Wider range of linearity. Some of the techniques widely used for estimation of Amoxycillin are HPLC (Anusha and Kamath, 2012), UV-Visible Spectroscopy, conductimetry etc. (Kilic *et al*., 1995). Methods are available to estimate the amount of Amoxycillin in specific salt forms. However, an analytical method capable of estimating the quantity of Amoxycillin accurately, irrespective of the salt form can be used as a universal method.

Amoxycillin Sodium, an aminopencillin with phenolic group, is sodium (6R)-6-(α-D-4-hydroxy-phenylglycylamino) penicillanate. Spectrum of activity of Amoxycillin sodium is broader than that of benzyl penicillin, especially when activity against gram-negative bacilli is considered. The present work is an attempt to increase the stability of the Amoxycillin solution and to accurately quantify the amount of Amoxycillin in any salt form in different formulations. We define the developed method as "UV-formaldehyde" method.

MATERIALS AND METHODS

Materials: Working standards of Amoxycillin trihydrate was obtained from Karnataka Antibiotics and Pharmaceuticals Limited, Bangalore, India. The working standard was equivalent to 89.56% of amoxycillin. The sample used for the method development was Amoxirum forte injection and 300 mg of Amoxirum forte injection was procured from Karnataka Antibiotics and Pharmaceuticals Ltd., India. The contents of the injection were Amoxycillin (200 mg) and Sulbactam (100 mg), as per the label claim. These drugs were present in their respective sodium salt forms. The average molecular weight was 340.4 mg, which was used in subsequent calculations. All the reagents such as hydrochloric acid, formaldehyde and sodium hydroxide were of AR grade and were used as such without any further purification.

METHODS

Preparation of standard solution

Standard stock solution-I: An accurately weighed quantity of Amoxycillin trihydrate equivalent to 50 mg of amoxycillin was transferred to a 100 mL standard flask. To this, 5 mL of 1 N HCL and 50 mL of distilled water were added. The 1 mL of formaldehyde solution was transferred to this mixture which was heated on a boiling water bath at 100°C for 90 min. The flask was subsequently cooled and the total volume made to 100 mL with water.

Standard stock solution-II: For the preparation of standard stock solution-II, 5 mL of 40% NaOH solution was added to 5 mL of standard stock solution-I and the total volume was made up to 100 mL using distilled water.

Preparation of sample solution

Sample stock solution-I: The contents of three Amoxirum forte injection were mixed to prepare a master sample. An accurately weighed mass of this blend measuring 85.2 mg equivalent to 50 mg of Amoxycilhn was taken in a 100 mL standard flask. The 50 mL of distilled water was then added to the contents of the flask, followed by addition of 5 mL of 1 N HCL. The 1 mL of formaldehyde solution was transferred to this mixture which was heated on a boiling water bath at 100°C for 90 min. The flask was subsequently cooled and the total volume made to 100 mL with water.

Sample stock solution-II: For the preparation of sample stock solution-II, 5 mL of 40% NaOH solution was added to 5 mL of sample stock solution-I and the total volume was made up to 100 mL using distilled water.

Preparation of blank solution

Blank stock solution-I: The 50 mL of water and 5 mL of 1 N HCl were taken in a 100 mL volumetric flask. The 1 mL of formaldehyde solution was transferred using a dry graduated pipette. The mixture was on a boiling water bath at 100°C for 90 min. The flask was subsequently cooled and the total volume made to 100 mL with water.

Blank stock solution-II: For the preparation of blank stock solution-II, 5 mL of 40% NaOH solution was added to 5 mL of blank stock solution-I and the total volume was made up to 100 mL using distilled water. Absorbance for different standards, samples and blank

solutions were measured using a double beam, UV-Visible Spectrophotometer (UV-1601, Shimadzu, Japan). All measurements were made at a wavelength of 341 nm.

RESULTS

Using "UV-formaldehyde" method, the percentage of Amoxycillin in the amoxirum vials was determined for six different trials, whose results are shown in Table 1.

To further fine-tune the method, the effect of heating temperature (60-100°C) and duration of heating (90-120 min) on the assay results was performed. The assay results obtained in these experiments are shown in Table 2.

When an analytical method is developed based on chemical reactions, it is imperative to ascertain whether the reaction product is stable for sufficient period of time to facilitate performance of off-hine analysis for the purpose of quality control and assurance. Hence, assays were performed at different time points, upon completion of the reaction. Table 3 shows the comparison of assay results, carried out after allowing the solutions to stand for different time periods. It may be observed from Table 3 that there is no significant difference in assay values obtained from samples that were left standing over different time periods. The influence of concentration of HCl on the assay was studied by performing the assay at a lower concentration (0.1 N). The experiment was repeated twice using 0.1 N HCl. The percentage of Amoxycillin, as determined from assay using 0.1 N HCl was 77.54 and 80.70% for the two experimental runs.

Table 1: Assay results, in terms of percentage of amoxycillin, obtained using "UV-formaldehyde" method

Trial No.	Percentage of Amoxycillin (%)
1	99.86
2	99.46
3	98.67
4	102.47
5	100.63
6	100.24
Average	100.22

Table 2: Influence of heating temperature and duration of heating on assay results

Heating temperature (°C)	Duration of heating (min)	Mass of amoxycillin trihydrate (g)	Mass of amoxirum forte blend (g)	Absorbance for standard	Absorbance for sample	Percentage of amoxycillin (%)
60	90	0.0571	0.0852	0.590	0.526	90
90	90	0.0574	0.0851	0.686	0.687	96
60	120	0.0572	0.0851	0.568	0.547	92
100	120	0.0579	0.0851	0.738	0.776	102
100	90	0.0583	0.0852	0.728	0.729	99.98

Table 3: Influence of heating temperature and duration of heating on assay results

Time of standing	Absorbance of standard	Absorbance of sample	Amoxycillin sodium found (%)
1 min	0.729	0.730	99.98
15 min	0.728	0.732	99.96
30 min	0.729	0.733	99.89
60 min	0.728	0.731	99.91
120 min	0.729	0.729	99.79
24 h	0.729	0.728	99.46

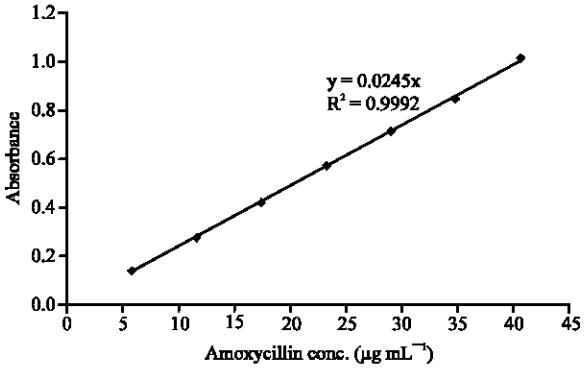

Fig. 1: Linearity of the developed method over a wide range of amoxycillin concentrations

Table 4: Results of calibration and linearity

Conc. of amoxycillin trihydrate (µg mL^{-1})	Absorbance at 341 nm
5.80	0.138
11.6	0.274
17.4	0.419
23.2	0.569
29.0	0.712
34.8	0.842
40.6	1.008

Table 5: Results of experiments carried out to ascertain precision of the developed method for estimation of amoxycillin

Run No.	Mass of amoxycillin trihydrate (g)	Mass of sample blend (g)	Absorbance of standard at 341 nm	Absorbance of sample at 341 nm	Amoxycillin found (%)
1	0.0581	0.0851	0.729	0.733	99.86
2	0.0583	0.0852	0.728	0.728	99.46
3	0.0579	0.0854	0.685	0.689	98.67
4	0.0582	0.0852	0.738	0.776	102.47
5	0.0581	0.0849	0.685	0.694	100.636
	0.0580	0.0851	0.750	0.757	100.24
Mean					100.22
RSD (%)					1.4233

The variation of absorbance with concentration of Amoxycillin using the developed method (1 N HCl, 5 mL 40% NaOH) are shown in Table 4 and Fig. 1. The correlation coefficient was found to be 0.9992 over a concentration range of 5.8 to 40.6 µg mL^{-1}. The results of six repetitions conducted over different days at different times are shown in Table 5.

DISCUSSION

When Amoxycillin is treated with acidic formaldehyde, intramolecularaminolysis occurs. This results in the attack of carbonyl group in beta lactam ring nucleophilically by α-amino benzoyl group in the side chain. The product of this reaction is a pyrazine derivative, which has a characteristic wavelength corresponding to maximum absorption (λ_{max}) at 341 nm. This forms the basis of the method developed here. As the method is dependent on reaction of formaldehyde with

Amoxycillin, this method can be applied for estimation of Amoxycillin in formulations, irrespective of salt form. We denote this method as "UV-formaldehyde" method. The average of assay results for Amoxycillin was found to be 100.22% with a relative standard deviation of 0.013%, as evident from Table 1.

The study on the effect of heating temperature (Table 2) indicates that the assay results were lower when the heating temperature was maintained lower than 100°C, irrespective of duration of heating. This indicates that temperature is the key parameter influencing assay results. At higher temperatures, the molecules taking part in reaction possess higher thermal energy, which helps to overcome the energy barrier for the chemical reaction. Hence irreversible reactions can proceed to completion at relatively higher temperatures. The fact that increase in duration of heating from 90 to 120 min, for heating temperature of 100°C does not influence assay, shows that reaction is complete at 90 min itself, providing satisfactory assay results. Hence, the heating temperature and duration of heating may be taken as 100°C and 90 min, respectively. The solutions were found to be stable even after 24 h, after their preparation (Table 3).

The method is sensitive to the concentration of HCl on two accounts: (i) The reproducibility is lost at lower concentration of hydrochloric acid (77.54 and 80.70% for two runs) and (ii) The percentage of Amoxycillin determined using assay carried out at lower concentration of hydrochloric acid are different and much lower than the actual values. The high value of correlation coefficient (0.9992) over a wide range of Amoxycillin concentration (5.8 to 40.6 μg mL^{-1}) testify the linearity of the developed method (Fig. 1). The UV assay for Amoxycilhn repeated six times on the same day and also on different days, show that the percentage relative standard deviation was found to be less than 2%, an indication of high degree of precision.

The formulation used for the study was available in the form of powders without any appreciably visible lumps. The uniform particle size of formulation enabled ease of preparation of standard and sample solutions for the purpose of method development. With uniform fine particles sizes, the specific surface area is increased. Hence, dissolution is promoted due to better interactions between high surface area particles and the fluid medium (Rajan *et al*., 2006, 2007a, b, 2008a, b, 2010). Hence, the condition of the sample must be examined to ensure that large aggregates and lumps do not interfere during preparation of standard and samples.

CONCLUSION

A method to estimate the percentage of Amoxycillin in different salt forms in combined formulation has been developed, based on the specific reaction between aminolysis reaction between acidic formaldehyde and Amoxycillin. The method shows linearity over a concentration range of (5.8 to 40.6 μg mL^{-1}) with correlation coefficient of 0.9992. The precision of the method was ascertained through calculation of percentage (%) Relative Standard Deviation of six different experimental runs at different time periods, which was found to be less than 2%. Hence, the developed method can be used for estimation of Amoxycillin present in any salt form in any dosage form.

ACKNOWLEDGMENT

The authors thank Karnataka Antibiotics and Pharmaceuticals Limited for providing instrument facilities, working standards of drugs and the batch samples of amoxirum forte injection vials to carry out this study.

REFERENCES

Anusha, N. and B. Uday Kamath, 2012. Simultaneous estimation of amoxicllin and sulbactam in a parenteral formulation by reverse phase HPLC method. Int. J. Pharm. Pharm. Sci., 4: 330-336.

Kilic, E., F. Koseoglu, A. Kenar and M.A. Akay, 1995. Conductimetric titrimetry for assay of selected antibiotics in non-aqueous media. J. Pharm. Biomed. Anal., 13: 1453-1458.

Rajan, K.S., S.N. Srivastava, B. Pitchumani and B. Mohanty, 2006. Simulation of gas-solid heat transfer during pneumatic conveying: Use of multiple gas inlets along the duct. Int. Commun. Heat Mass Trans., 33: 1234-1242.

Rajan, K.S., B. Pitchumani, S.N. Srivastava and B. Mohanty, 2007a. Two-dimensional simulation of gas-olid heat transfer in pneumatic conveying. Int. J. Heat Mass Trans., 50: 967-976.

Rajan, K.S., S.N. Srivastava, B. Pitchumani and B. Mohanty, 2007b. Simulation of countercurrent gas-solid heat exchanger: Effect of solid loading ratio and particle size. Appl. Therm. Eng., 27: 1345-1351.

Rajan, K.S., K. Dhasandhan, S.N. Srivastava and B. Pitchumani, 2008a. Studies on gas-solid heat transfer during pneumatic conveying. Int. J. Heat Mass Trans., 51: 2801-2813.

Rajan, K.S., S.N. Srivastava, B. Pitchumani and K. Dhasandhan, 2008b. Experimental study of thermal effectiveness in pneumatic conveying heat exchanger. Appl. Therm. Eng., 28: 1932-1941.

Rajan, K.S., S.N. Srivastava, B. Pitchumani and V. Surendiran, 2010. Thermal conductance of pneumatic conveying preheater for air-gypsum and air-sand heat transfer. Int. J. Thermal Sci., 49: 182-186.

Zheng, F., S. Wang, S. Wen, M. Shen, M. Zhu and X. Shi, 2013. Characterization and antibacterial activity of Amoxycillin-loaded electrospun nano-hydroxyapatite/poly(lactic-co-glycolic acid) composite nanofibers. Biomaterials, 34: 1402-1412.