Journal of Pharmaceutical Research Vol. 11, No. 1, January 2012 : 1-5.

DETERMINATION OF STABILITY AND DEGRADATION RATE FOR COMBINATION CONTAINING AMOXICILLIN AND DICLOXACILLIN IN CAPSULE DOSAGE FORM

Jerad Suresh A*, Mohan Kumar A, Suyaraj T, Vinod D
Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai - 600003, India.

Received on : 25.01.2011 Revised : 05.11.11 Accepted : 12.11.11

ABSTRACT
A study was carried out using Reverse Phase High Performance Liquid Chromatography to determine the stability of four different brands of formulation (capsules) containing Amoxicillin and Dicloxacillin. Five batches of four different brands expiring in consecutive months (7^{th}, 8^{th}, 9^{th}, 10^{th} and 12^{th} month of 2010) were obtained and studied. During 5 months study, all the five batches were stored at room temperature **(NMT 25 °C)**. The 5 batches were assayed periodically at one month interval by Reverse Phase High Performance Liquid Chromatography method. The Dicloxacillin content in all the 5 batches steadily decreased over the months indicating that formulations of Amoxicillin and Dicloxacillin, which are available in the market are generally unstable. The Dicloxacillin content also reached 0% in some of these batches.

Keywords: *HPLC; Amoxicillin; Dicloxacillin; Stability; Degradation Rate.*

INTRODUCTION

Amoxicillin trihydrate [(6R)-6-(á-4-hydroxyphenyl-D-glycylamino)penicillanic acid trihydrate[1]] is a broad spectrum antibiotic [2] and Amoxicillin Capsules contains not less than 90% and not more than 110% of the stated amount of Amoxicillin ($C_{16}H_{19}N_3O_5S$).[3]

Dicloxacillin sodium is sodium (6R)-6-[3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carboxamido]penicillinate monohydrate[4] . Dicloxacillin capsules contain not less than 90% and not more than 120% of the stated amount of dicloxacillin, $C_{19}H_{17}Cl_2N_3O_5S$ [5]. The aim of the work was to determine whether the formulations are stable if not then determine its degradation rate. Five batches of four different brands were obtained. Four brands were chosen based on their expiry i.e. July 2010, August 2010, September 2010, October 2010 and December 2010 of brands A_1, A_2, B, C, D respectively, and all these brands were containing equal Strength of Amoxicillin and Dicloxacillin (i.e.250mg:250mg) ensuring that every batch expires every subsequent months, this ensures that a pattern will emerge in the event of detecting the stability of marketed formulations.

MATERIALS AND METHODS

Chemicals and reagents

Standard Amoxicillin trihydrate and standard Dicloxacillin were obtained from Kaushik Therapeutics (pvt) Ltd, Porur, Chennai.

All the five brands were purchased from open market Chennai. All the reagents and chemicals were of Analytical purity and Acetonitrile was purchased from E-Merck.

Preparation of Buffer

1.36 gm of Potassium dihydrogen orthophosphate was dissolved in 500ml of water and pH was adjusted to 5 using 0.1M sodium hydroxide.

Preparation of mobile phase

Mobile phase was prepared with 60% of buffer and 40% of acetonitrile (i.e.) 60:40 volumes of buffer and acetonitrile respectively.

Preparation of Standard solution

50 mg of standard Amoxicillin trihydrate and 50 mg of standard Dicloxacillin sodium were dissolved in 50 ml of mobile phase and sonicated for 10 minutes. From the resultant solution, 2 ml was pippetted out and made up the volume to 10 ml with the mobile phase.

Preparation of Sample solution

20 capsules are weighed and average weight was calculated by removing the empty capsules shell weight.100mg equivalent of sample was dissolved in 50 ml of mobile phase, sonicated for 10 minutes and filtered. From the resultant filtrate 2 ml was pippetted out and made up the volume to 10ml with the mobile phase.

Chromatographic conditions

Reverse Phase High Performance Liquid Chromatography was carried out using MERCK HITACHI of L7100 pump model and UV- Detector of

***Correspondence :** ajsuresh2001@yahoo.co.uk

L7400 model and stainless steel column filled with octadecyl silicagel.

Assay

50µl of standard solution and sample solution were injected separately with the flow rate of 1 ml/ minute of the mobile phase containing buffer and Acetonitrile 60:40 proportion at the detection wavelength of 246nm and calculated the amount of Amoxycillin and Dicloxacillin in samples, from the chromatogram obtained with reference to both the Standards (i.e.) Amoxicillin and Dicloxacillin.[6]

RESULTS

The Percentage content of Amoxicillin and Dicloxacillin (Table 1-5) was calculated using the following formula

Percentage content of Amoxicillin

$$\text{Amount present} = \frac{\text{Peak Area sample} \times \text{Dilution factor} \times \text{Avg. Wt} \times \text{conversion factor}}{\text{Peak Area standard} \times \text{weight of sample taken} \times \text{label claim}}$$

The conversion factor is 0.8711 [Molecular weight of Amoxicillin (365.404) / molecular weight of Amoxicillin trihydrate (419.45)]

In order to calculate the amount equivalent to Amoxicillin as mentioned in label claim conversion factor is employed.

Percentage content of Dicloxacillin

$$\text{Amount present} = \frac{\text{Peak Area sample} \times \text{Dilution factor} \times \text{Avg. Wt} \times \text{conversion factor}}{\text{Peak Area standard} \times \text{weight of sample taken} \times \text{label claim}}$$

The conversion factor is 0.9210. [Molecular weight of Dicloxacillin sodium (470) / molecular weight of Dicloxacillin Sodium monohydrate (510.3)]

In order to calculate the amount equivalent to Dicloxacillin sodium as mentioned in label claim conversional factor is employed.

The figures 1 and 2 correspond to HPLC graphs of first and fifth month respectively. The Retention time for standard Amoxicillin and Dicloxacillin is represented in each graph.

Percentage Content of Amoxicillin and Dicloxacillin in each brands:

Table 1: *Percentage of Batch 1*

Months	Brand A_1 (Batch 1)	
	% of Amoxicillin	% of Dicloxacillin
1 st month	104.94	37.55
II nd month	117.02	26.98
III th month	109.93	20.88
IV th month	111.35	12.58
V th month	105.76	---

Table 2: *Percentage of Batch 2*

Months	Brand A_2 (Batch 2)	
	% of Amoxicillin	% of Didoxacillin
1 st month	98.84	23.66
II nd month	117.13	20.95
III th month	101.31	----
IV th month	108.43	----
V th month	106.00	----

Table 3: *Percentage of Batch 3*

Months	Brand B (Batch 3)	
	% of Amoxicillin	% of Dicloxacillin
1 st month	110.39	84.14
II nd month	106.41	82.01
III th month	111.41	81.96
IV th month	100.67	53.68
V th month	110.94	53.25

Table 4: *Percentage content of Batch 4*

Months	Brand C (Batch 4)	
	% of Amoxicillin	% of Dicloxacillin
1 st month	105.79	78.61
II nd month	102.59	69.21
III th month	118.19	48.80
IV th month	105.95	47.41
V th month	103.29	40.29

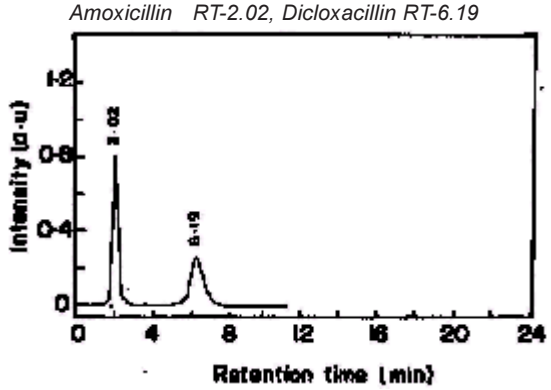
Table 5: *Percentage content of Batch 5*

Months	Brand D (Batch 5)	
	% of Amoxicillin	% of Dicloxacillin
1 st month	102.83	37.31
II nd month	109.75	26.97
III th month	110.40	12.52
IV th month	93.12	----
V th month	115.01	----

Fig 1: *HPLC graph of First month for five batches*

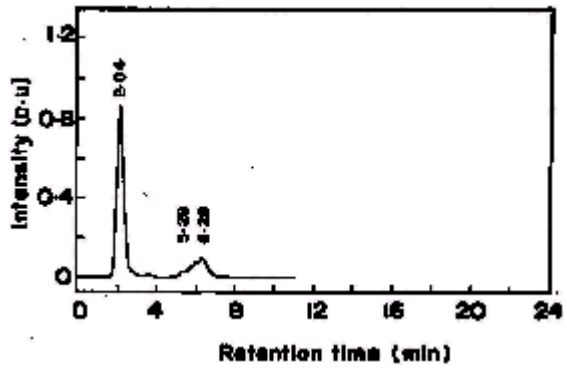
STANDARD

Amoxicillin RT-2.02, Dicloxacillin RT-6.19



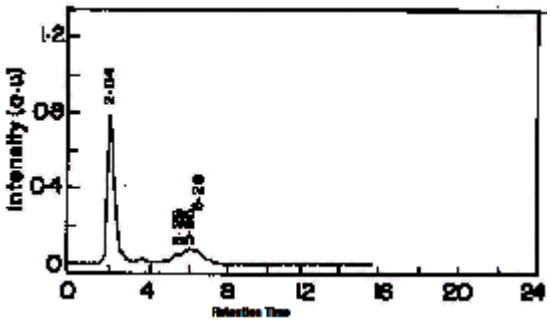
BATCH 1

Amoxicillin RT-2.04, Dicloxacillin RT-6.28



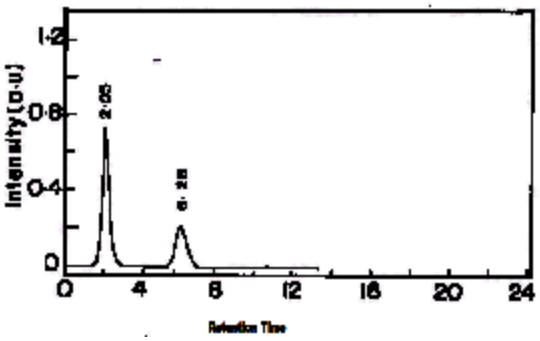
BATCH 2

Amoxicillin RT-2.04, Dicloxacillin RT-6.26



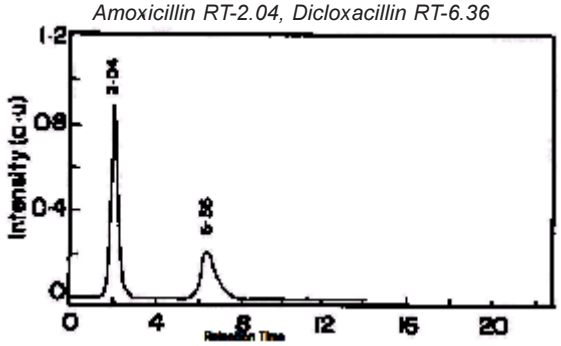
BATCH 3

Amoxicillin RT-2.05, Dicloxacillin RT-6.28



BATCH 4

Amoxicillin RT-2.04, Dicloxacillin RT-6.36



BATCH 5

Amoxicillin RT-2.07, Dicloxacillin RT-6.53

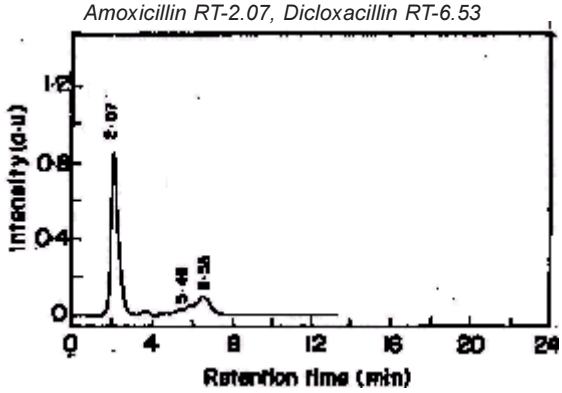
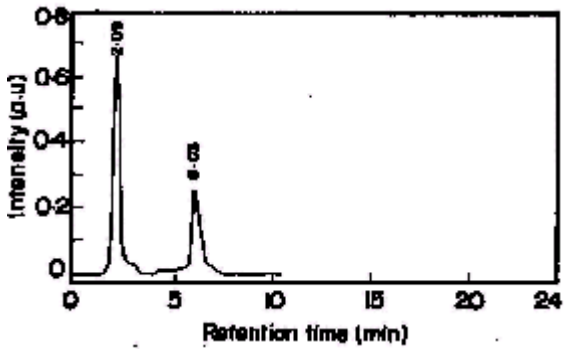


Fig. 2: *HPLC graph of Fifth month for five batches*

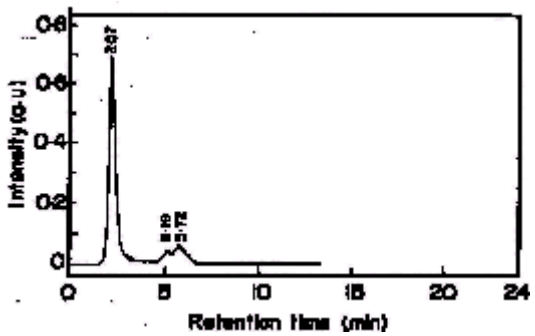
STANDARD

Amoxicillin RT-2.09, Dicloxacillin RT-6.03



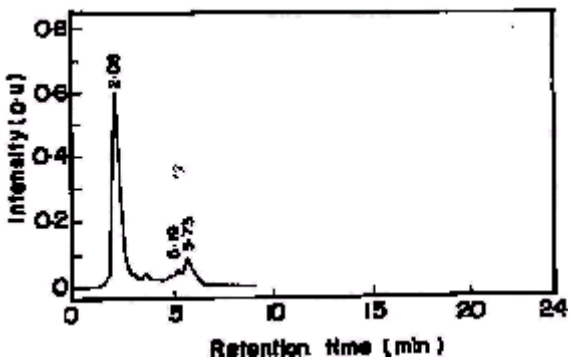
BATCH 1

Amoxicillin RT-2.07, Degraded Fragment RT-5.72, 5.19



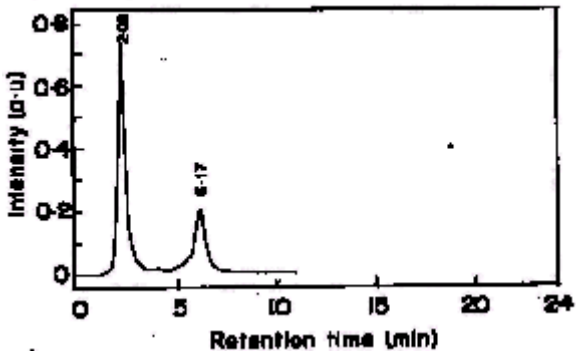
BATCH 2

Amoxicillin RT-2.08, Degraded Fragment RT-5.73, 5.19



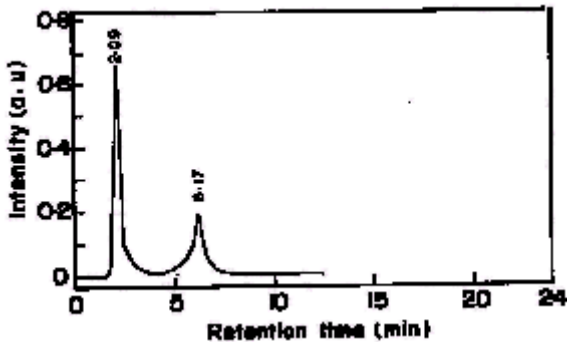
BATCH 3

Amoxicillin RT-2.09, Dicloxacillin RT-6.17



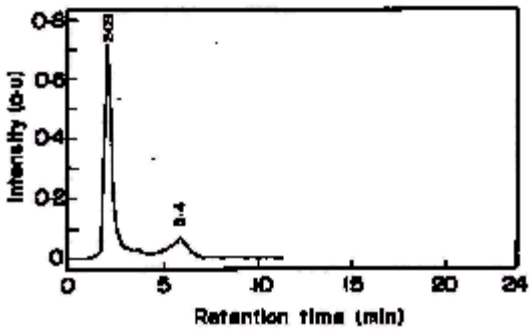
BATCH 4

Amoxicillin RT-2.09, Dicloxacillin RT-6.17



BATCH 5

Amoxicillin RT-2.09, Degraded Fragment RT-5.4



First order Degradation rate constant value of Dicloxacillin for Five Batches of Four brands:

The Formula to calculate the First order degradation rate constant (k) is

$$k = \frac{2.303}{t} \log \frac{a}{a-x}$$

Where t=time, a=initial concentration,x=concentration at time t.

The Specific Reaction rate listed in Table 6 were calculated by using the above equation and The equation express the fact that in a First order reaction the concentration is decreasing exponentially with time[7] (Figure 3).

Table 6: *First order degradation rate constant value for five batches*

MONTHS	BRAND-A_1 (batch 1)	BRAND-A_2 (batch 2)	BRAND-B (batch 3)	BRAND-C (batch 4)	BRAND-D (batch 5)
Ist month	7.1×10^{-2}	4.3×10^{-2}	4.3×10^{-2}	2.9×10^{-2}	1.4×10^{-2}
IInd month	4.5×10^{-2}	3.8×10^{-2}	3.8×10^{-2}	1.96×10^{-2}	0.89×10^{-2}
IIIrd month	3.23×10^{-2}	———	3.3×10^{-2}	1.06×10^{-2}	0.37×10^{-2}
IVth month	1.7×10^{-2}	———	1.5×10^{-2}	0.97×10^{-2}	———
Vth month	———	———	1.4×10^{-2}	0.74×10^{-2}	———

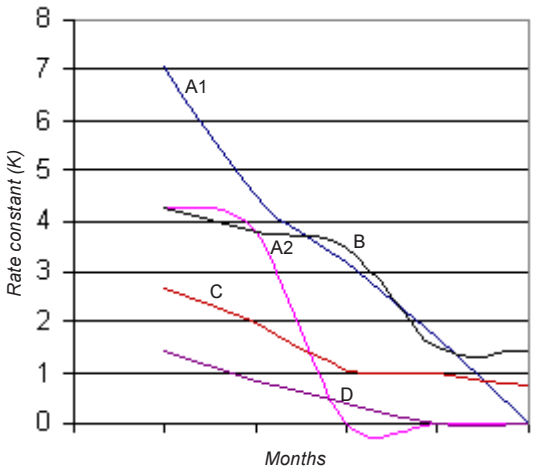


Fig. 3: *Graphical representation of First order degradation rate constant*

CONCLUSION

The assay of the five batches (of the four brands) Dicloxacillin was found to be least stable. The degradation was found to follow First order kinetics. The curve representing Amoxicillin tends to develop a shoulder, thereby increasing in AUC and hence the percentage of Amoxicillin increased. Brands A_1, A_2, C, were assigned shelf life of 24 months, and Brands B, D were assigned shelf life of 18 months. But all the five tested batches (of the four brands) failed to retain the label claim in respect of Dicloxacillin, though they were stored at the correct storage conditions.

Acknowledgement

We would like to thank Kowshik Therapeutics (Pvt) Ltd Porur, India for helping us to carry out the HPLC assay work and their constant support during the test period.

REFERENCES

1. *Indian Pharmacopoeia*. Government of India, The Indian Pharmacopoeia Commission, Ghaziabad, 2010, volume II, p 816.

2. Tripathi KD. *Essentials of Medical Pharmacology*, 3rd edition, published by Jaypee brother's medical publishers, New Delhi, p 660.

3. *Indian Pharmacopoeia*. Government of India, The Indian Pharmacopoeia Commission, Ghaziabad, 2010, volume II, p 813.

4. *Indian Pharmacopoeia*. Government of India, The Indian Pharmacopoeia Commission, Ghaziabad, 2010, volume II, p 1201.

5. *Indian Pharmacopoeia*. Government of India, The Indian Pharmacopoeia Commission, Ghaziabad, 2010, volume II, p 1202.

6. Sethi PD, *HPLC Quantitative Analysis of Pharmaceutical Formulations*. CBS Publishers and Distributors, New Delhi, 2008, p 569.

7. Alfred N Martin, James Swarbrick, Arthur Cammarata, Chun AHC. *Physical Chemical Principles in the Pharmaceutical Sciences.* 2nd edition, published by Lea & Febiger, Philadelphia, 1973, p 361.