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DETERMINATION OF DEGRADATION PRODUCT FOR COMBINATION CONTAINING AMOXICILLIN AND DICLOXACILLIN IN CAPSULE DOSAGE FORM BY LC-MASS SPECTROSCOPY

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ABSTRACT
A study was carried out using LC-MASS Spectroscopy to determine the degradation products of formulation (capsules) containing Amoxicillin and Dicloxacillin. Previously an RP-HPLC method was performed to determine the stability of five batches of four different brands of formulation (capsules) containing Amoxicillin and Dicloxacillin which were expiring in consecutive months (7th, 8th, 9th, 10th and 12th month of 2010). The Dicloxacillin content in all the five batches steadily decreased over the period of consecutive 5 month Assays (one month interval between each Assays), thereby determined the degradation product of Dicloxacillin by LC-MASS Spectroscopy.

Keywords: *LC-MASS Spectroscopy; RP-HPLC; Amoxicillin; Dicloxacillin; Degradation Product*

INTRODUCTION

Amoxicillin trihydrate [(6R)-6-(α-4-hydroxyphenyl-D-glycylamino)penicillanic acid trihydrate [1]] is a broad spectrum antibiotic and Dicloxacillin sodium is monosodium (2S,5R,6R)-6-[3-(2,6-dichlorophenyl)-5-methyl-4-isoxazolecarboxamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylate monohydrate[2]. The Dicloxacillin in capsule formulations containing Amoxicillin and Dicloxacillin was found to steadily decrease with time. It was therefore decided to determine the degradation products and the reason for degradation. A Capsule Formulation was chosen for the purpose and the degraded products were determined by LC-MASS Method. The Degradation was studied by LC-MASS rather than by GC-Mass, because in GC-Mass the degradation can be brought about by the high temperatures involved in the vaporization process, under such circumstances it would be difficult to justify that the specific fragments pertain to the original degradation of the formulation on storage or whether it is the degradation due to the high temperature in the vaporization process.

MATERIALS AND METHODS

Chemicals and Reagents

Standard Amoxicillin trihydrate and standard Dicloxacillin were obtained from Kaushik Therapeutics (Pvt) Ltd, Porur, Chennai. Three batches of 2 brands purchased from open market Chennai, while 2 batches of a particular brand were obtained as free samples. All the reagents and chemicals were of analytical purity, purchased from E-Merck.

LC-MS Conditions

LC-MS analysis was carried out on linear ion trap 3200 Quadrupole LC/MS/MS Mass spectrometer, with HPLC equipped with UV-VISIBLE detector and Agilent 1100 LC Quartnery Pump. The Turbo ion spray voltage was maintained at 10 e^{-5} torr. Analyst software was used for data acquisition and data processing. LC-MS spectra were acquired from m/z 50-1700 in 0.1amu steps with 3s dwell time. LC-MS analysis of the standard and sample was carried out using Thermo ODS Hypersil with dimension of 250 X 4.6 mm, 5µm particle size [3]. The mobile phase consisted of 5µm Ammonium acetate, 0.1% formic acid in water (A) and 0.1% formic acid in Acetonitrile (B). Flow rate and gradient program is given in Table 1.

Table-1 : *LC-MS Gradient Program*

Time (minute)	Flow rate (µl/min)	A %	B %
0	800	90	10
2	800	90	10
7	1000	25	75
10	1000	25	75
12	1000	80	20
15	800	80	20
20	800	80	20

Results and Discussion

a) The LC-Mass spectra of the Standard and Sample are shown in Fig 1 & 2 respectively. In the standard LC-Mass Spectrum, Amoxicillin and Dicloxacillin were found to have retention time at 3.5 & 9.07 min with molecular weights 363.9 & 469 respectively.

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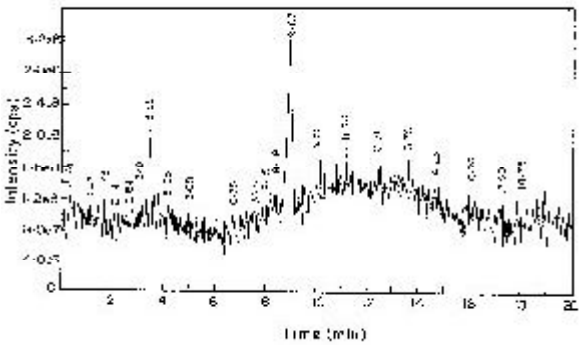


Fig. 1: *Standard LC-MASS Spectra*

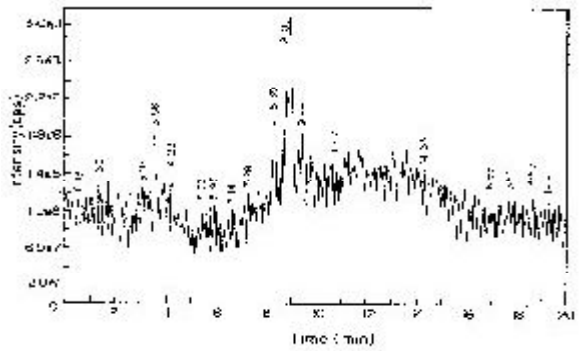


Fig. 2: *Sample LC-MASS Spectra*

b) In the LC-Mass Spectrum of the sample two additional peaks were found with high intensity at an RT of 8.35 & 9.46 adjacent to the Dicloxacillin peak. On scanning at these retention times, the m/z peaks were found at 443 and 487 respectively which have been shown in the Fig 3 & 4.

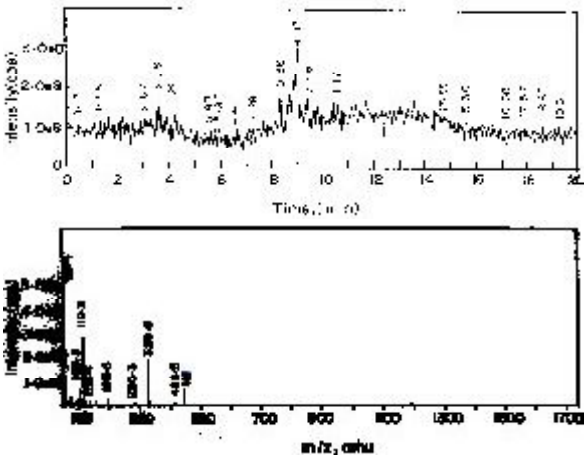


Fig. 3: *At 8.35 RT, m/z of 443 has been found which corresponds to Penilloic acid of dicloxacillin.*

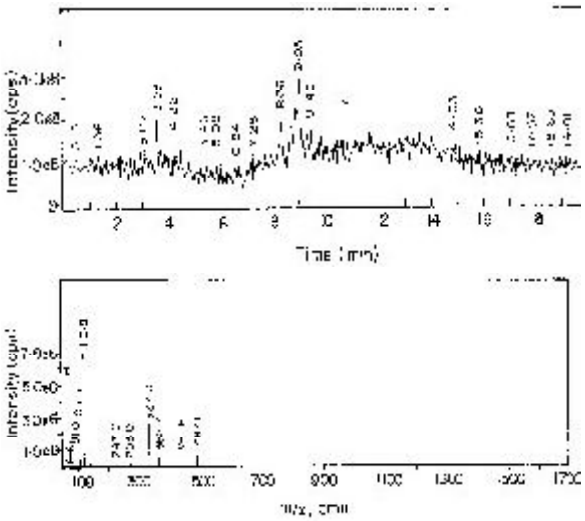


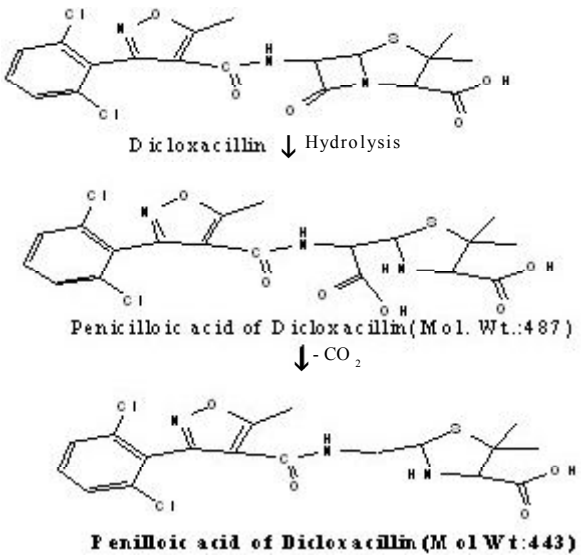
Fig. 4: *At 9.46 RT, m/z of 487 has been found which corresponds to Penicilloic acid of dicloxacillin.*

c) The LC-Mass spectral fragmentation information was juxtaposed with the known degradation products of the penicillin's and the following conclusions were drawn.

1. The Fragment with the m/z of 487 corresponds to the formation of Penicilloic acid of dicloxacillin.
2. The Fragment with the m/z of 443 corresponds to the formation of Penilloic acid of dicloxacillin. This occurs by the evolution of CO_2 (carbon dioxide) from the Penicilloic acid of dicloxacillin.

d) Schematic Representation of Degradation Pattern

The possible degradation pattern of dicloxacillin may be illustrated with the following chemical reactions.



CONCLUSION

The degradation product of Dicloxacillin was determined by using LC-MASS spectroscopy methods. The one part of the degraded product was found to be penicilloic acid of dicloxacillin with molecular weight of 487 and the other part of the degraded product was found to be Penilloic acid of dicloxacillin with molecular weight of 443. The degradation of Dicloxacillin is so rapid and it could be because of presence of small amount of water which could have been present in the alcohol used for granulation process, because these types of degradation occur normally as a result of hydrolysis.

Acknowledgement

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