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QUALITY EVALUATION AND COMPARISON OF AMOXICILLIN ANTIBIOTIC UNDER GOVERNMENT SUPPLY OF DELHI AND BRANDED FORMULATION OF INDIA

[1]Suraj Kumar Prajapati, [2]Amrita Parle

[1]M.Pharm Research Scholar, [2]Associate Professor

[1]Quality Assurance

[1]Delhi Institute of Pharmaceutical Sciences and Research, New Delhi, India

***Abstract*:** In the present study, an attempt was made to assess the quality and pharmaceutical equivalence of Six samples of Amoxicillin available in Delhi, India. Three samples were collected from market and three from government supply. Amoxicillin was selected for the present study because it is widely prescribed Antibiotic. The study was performed using *in-vitro* methods as per Indian Pharmacopoeia 2018. All six samples were assessed through different official and non-official tests like Hardness, Friability, Weight Variation, Disintegration Time, Dissolution Profile and Assay. All six samples met the prescribed limit and found to be of good quality, safe and effective. All samples were pharmaceutically equivalent.

***IndexTerms* – Amoxicillin, Weight Variation, Disintegration, Dissolution, Assay.**

I. INTRODUCTION

Major population of India depends upon government hospitals for the treatment and medicines. This study was designed to investigate whether patients are getting good quality antibiotics or not. Amoxicillin was chosen because it is most prescribed antibiotic in government hospitals.

This study also compared the outcome of following classes of Amoxicillin capsules.

a) Generic Vs Generic
b) Branded Vs Branded
c) Generic Vs Branded

Amoxicillin is a semi-synthetic β-lactam antibiotic belonging to penicillin group [1,2]. It inhibits the bacterial cell wall synthesis by binding with Penicillin Binding Proteins (PBPs) and block the process of transpeptidation (cross-linking process in cell wall synthesis) [3,4]. It results in the activation of bacterial autolytic process and leads to bacteria death. It is used to treat tonsillitis, bronchitis and Pneumonia [5-7]. It is also used to treat infections of ear, nose, throat, skin and urinary tract [8]. The bioavailability is approximately 60% with 27.7 L of volume of distribution. The half life of amoxycillin is 61.3 minutes [9-11].

II. MATERIALS AND METHODS

The first step of the study was collection of six samples. All samples were assessed according to Indian Pharmacopoeia 2018 for Identification, Weight Variation, Disintegration, Dissolution Profiling and Assay.

Sample collection

Six Amoxicillin capsules with label claim of 250 mg were collected. Three Generic samples were collected from the government hospitals of Delhi and other three Branded samples were purchased from the local medical stores of Delhi. The six different samples were labelled for quality assessment as shown in Table 1.

Table: 1 Details of samples

Sample Code	Company	Sample Type	Sample Collection Site	Manufacturing Date	Expiry Date	Prices
A1	Vivek Pharmachem India Ltd.	Generic	Mohalla Clinic Malviya Nagar New Delhi	July 2019	June 2021	-
A2	Daffodils Pharmaceutical Ltd.	Generic	Khanpur Dispensary New Delhi	May 2019	April 2021	-
A3	Unicure India Ltd.	Generic	ESIC Dispensary New Delhi	April 2019	March 2022	-
A4	Sun Pharmaceutical India Ltd.	Branded	Local Pharmacy Store	September 2018	October 2021	Rs. 34.75/strip
A5	Uni Medicilab.	Branded	Local Pharmacy Store	April 2019	April 2021	Rs. 36.24/strip
A6	Micro Labs Ltd.	Branded	Local Pharmacy Store	March 2019	February 2021	Rs. 46.50/strip

III. Chemicals used in the study

Monobasic potassium phosphate, sodium hydroxide, ethanol, acetonitrile, and distilled water or HPLC water. All chemicals used were of analytical grade.

IV. Equipments used in the study

UV Visible Spectrophotometer (Lamba 35 Perkin Elmer), Digital Balance (AL500 Mettler Toledo), Disintegration Test apparatus (ED2SAPO Electrolab), Dissolution Test Apparatus (DS 8000 Lab India), pH meter (seven compact pH/Ion S220 Mettler Toledo), Sonicator (Branson Sonicator Mettler Toledo), Water purification system (integral3Q-POD Millipore, Bedford, USA), HPLC System (Thermo Scientific Dionex Ultimate 3000U) were used.

1. METHODS EMPLOYED

1.1. Weight Variation

Weight variation test was performed to check that each capsule contains the labeled amount of Amoxicillin. The test was conducted by weighing twenty capsules using a digital balance. The average weight was calculated in milligrams. Percentage deviation from the average weight was calculated and is given in Table 8. Percentage deviation was calculated using the formula [12].

$$\% \text{ Deviation} = \frac{\text{Average weight of capsule - Individual weight of capsule}}{\text{Average weight of capsule}} \times 100$$

1.2. Identification

The identification of Amoxicillin was performed to verify the presence of Amoxicillin molecule in the capsule content. The identification was done from the Retention time (RT) obtained in the assay chromatogram. The permissible RT limit for samples is ± 10% of standard RT shown in Fig: 1.

Fig 2 to 7 depict the retention time of the six samples in the chromatograms obtained while performing the assay of these samples.

1.3. Disintegration Test

This test was performed to determine whether Amoxicillin capsules disintegrate within the prescribed time when placed in the liquid medium under the experimental conditions. Disintegration is the first step before dissolution. Faster the capsule disintegrates, faster it will dissolve in the gastric or enteric fluid. 06 capsules of each sample were placed in the disintegration apparatus. The volume of the disintegration medium was 900 ml of water and the temperature was maintained at 37 ± 0.5℃. The time taken by each capsule to break into small parts that can cross the mesh was observed and recorded. The average time was calculated in minutes.

1.4. Capsule Dissolution

For capsule, Dissolution is an important step to achieve the bioavailability and therapeutic effect. Dissolution test is important because it measures the rate and extent of solution formation from the dosage form. Dissolution plays a major role in drug absorption. If the dissolution of the drug is good then its absorption will be better and it will be more bioavailable for therapeutic effect.

This test was performed using USP type II apparatus and the samples were analysed by UV.

1.4.1. **Dissolution medium** - The dissolution medium was 900 ml of water. It was transferred to dissolution basket. Instrument was allowed to rotate at 100 RPM for 60 minutes and temperature was maintained at 37 ± 0.5 ^{0}C.

1.4.2. **Standard solution** - The standard solution was prepared by dissolving 32.6 mg of reference standard of (85%w/w) Amoxicillin in 100ml of dissolution medium, 8 ml aliquot was diluted to 25 ml by dissolution medium to get a solution of concentration of approximately 8.88 PPM (Parts Per Million).

1.4.3. **Sample solution** – 01 capsule was placed in 900 ml of dissolution medium using sinker. After 60 minutes the desirable quantity of sample was withdrawn and filtered. 8 ml of aliquot was diluted to 25 ml with dissolution medium to get a solution of concentration of approximately 8.88 PPM.

Absorbance of samples was recorded by UV spectrophotometer at the maximum of 272 nm. Percentage of content dissolved was calculated using the formula.

$$\% \text{ Content dissolved} = \frac{\text{Test area} \times \text{Std wt} \times \text{Test dilution} \times \text{Potency} \times 100}{\text{Std area} \times \text{Std dilution} \times \text{Test wt} \times 100 \times \text{Claim}}$$

1.5. Assay

This test was performed to determine the specified label claim or potency of drug in individual sample and to observe differences among the samples. The assay was performed using HPLC as recommended in IP 2018.

1.5.1. **Reference solution** – It is prepared by dissolving 30 mg of Amoxicillin Trihydrate Reference Standard (85% w/w) in 25ml of 0.68 % w/v solution of monobasic potassium phosphate to obtain concentration of 1.2 mg per ml.

1.5.2. **Test solution –** It is prepared by dissolving 30 mg of Amoxicillin powder from capsules in 25 ml of 0.68 % w/v solution of monobasic potassium phosphate to obtain concentration of about 1.2 mg per ml.

1.5.3. **Chromatographic condition**

Column – C18, 25 cm x 4.0 mm, 5 µm
Mobile phase – It is prepared by mixing 4 ml of acetonitrile with 96 ml of 0.68 % w/v solution of monobasic potassium phosphate whose pH was adjusted to 5, using potassium hydroxide.
Flow rate - 1.5 ml/min
Spectrophotometer - set at 230 nm
Injection volume – 10 µl

V. RESULTS

1. Weight variation

As per IP, the limit for weight variation for capsules of weight less than 300 mg is, not more than two individual weights of capsules should deviate from the average weight by ± 10%. All samples were within the specified limit and passed the test. The average weight and and % deviation of all samples of Amoxicillin is shown in Table 2.

Table: 2 Weight variation of Amoxicillin samples

Sample Code	Average weight (mg)	Range of % weight variation (n=20)	Limit in mg	Result
A1	**296.9**	**-1.7 to 1.8**	**267.21 - 326.59**	**Passed**
A2	**298.8**	**-2.3 to 2.8**	**268.92 - 328.68**	**Passed**
A3	**297.6**	**-2.2to 2.6**	**267.84 – 327.36**	**Passed**
A4	**313.1**	**-6.8 to 2.4**	**281.79 – 344.41**	**Passed**
A5	**291.4**	**-6.7 to 4.9**	**264.26 – 320.54**	**Passed**
A6	**299.8**	**-5.8 to 4.8**	**269.82 – 329.78**	**Passed**

2. Identification

Identification was done using the Retention time of Amoxicillin Reference Standard, which was found to be 6.772 minutes in the Assay as per IP 2018, the limit for retention time is ±10% of Standard RT. It is observed that all the samples correspond with that of standard. All samples were identified as Amoxicillin. The retention time of all the samples taken from Chromatograms, is given in Table 3.

Table: 3 Retention time of Amoxicillin samples

Sample Code	Retention time (in min)	Limit (±10% min)	Result
A1	6.767	6.092 to 7.444	Passed
A2	6.773		Passed
A3	6.773		Passed
A4	6.767		Passed
A5	6.767		Passed
A6	6.767		Passed

3. Disintegration time

As per IP, the disintegration time for hard gelatin capsules should not be more than 30 minutes. The disintegration time of all six samples of Amoxicillin are given in Table 4. Disintegration time of all the samples was found to be within the prescribed limit.

Table: 4 Disintegration time of Amoxicllin samples

Brand Code	Disintegration time (min-sec)	Limit	Result
A1	6.12	30min	Passed
A2	6.30		Passed
A3	6.09		Passed
A4	8.35		Passed
A5	8.53		Passed
A6	12.32		Passed

4. Dissolution test

The % content dissolved for all samples is given in Table 5. Sample A4 shows maximum dissolution while A5 shows the least among all the six samples but all the samples show dissolution above the prescribed percentage.

Table: 5 Dissolution profile of Amoxicillin samples

Sample Code	% label claim dissolved	Limit	Results
A1	97.55	Not less than 85%	Passed
A2	96.16		Passed
A3	92.97		Passed
A4	105.48		Passed
A5	96.06		Passed
A6	92.18		Passed

5. Assay

Chromatograms depicting the retention time and the principal peak are used for calculating percentage purity.

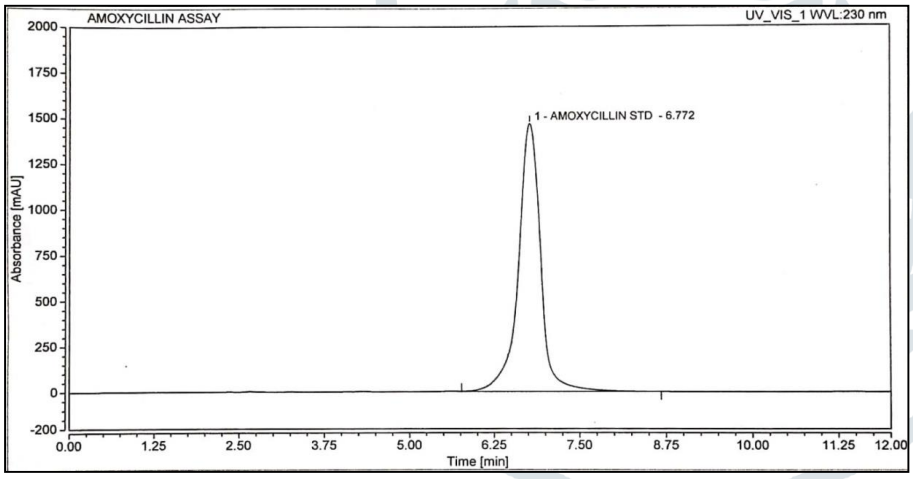

Fig: 1 Chromatogram of Standard Amoxicillin

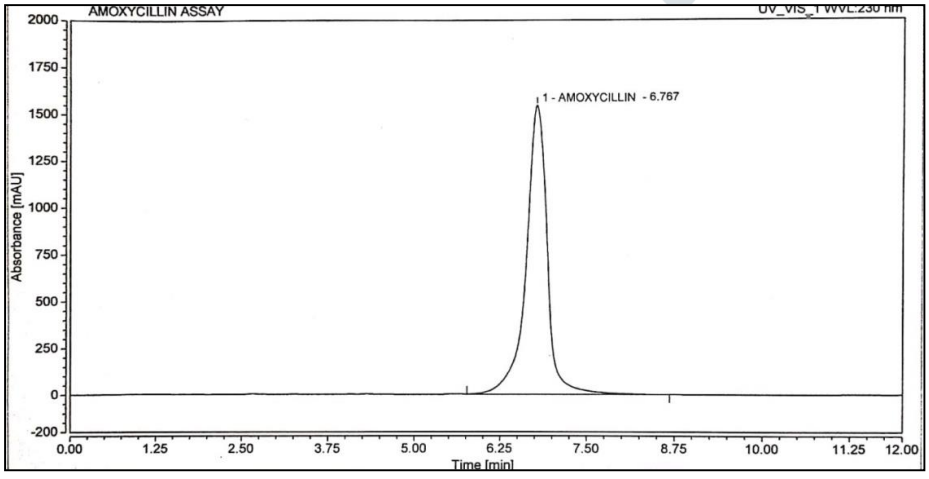

Fig: 2 Chromatogram of sample A1

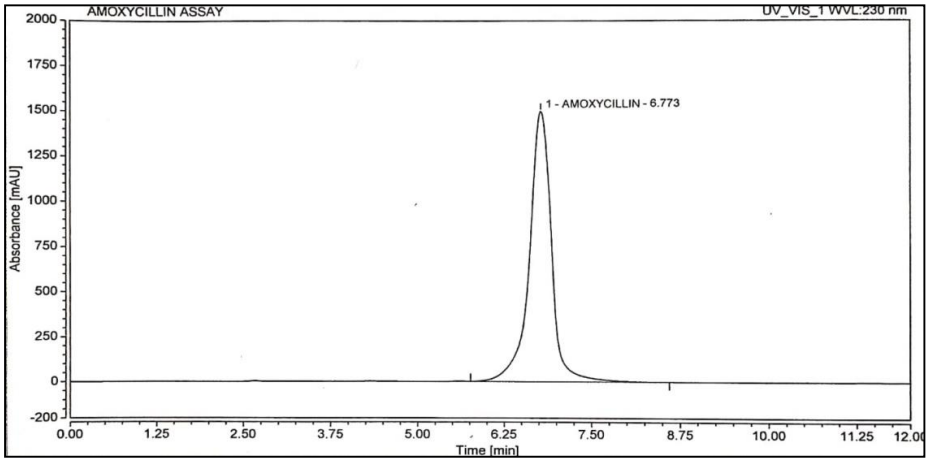

Fig: 3 Chromatogram of sample A2

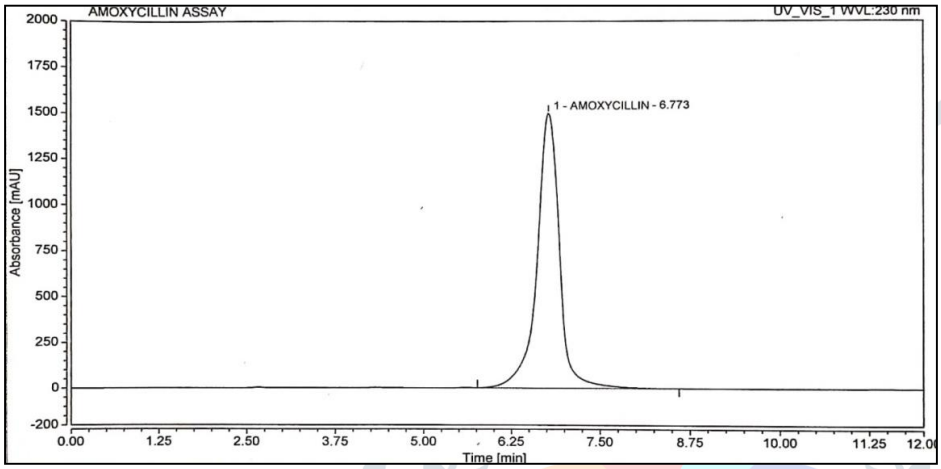

Fig: 4 Chromatogram of sample A3

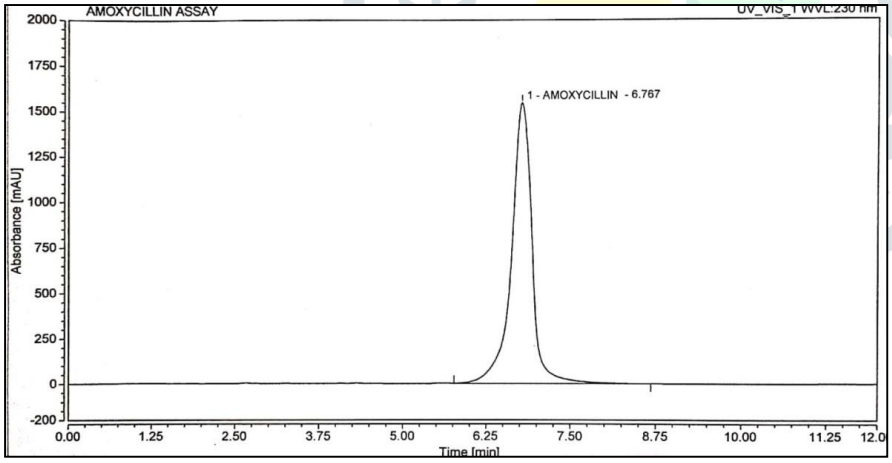

Fig: 5 Chromatogram of sample A4

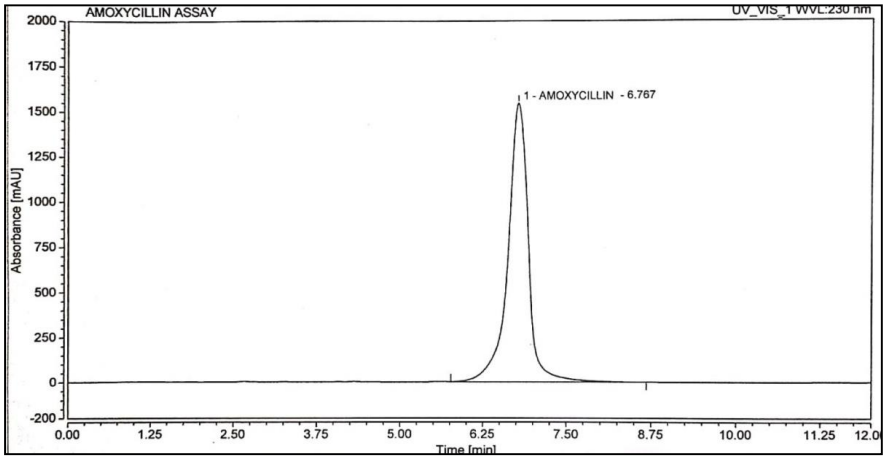

Fig: 6 Chromatogram of sample A5

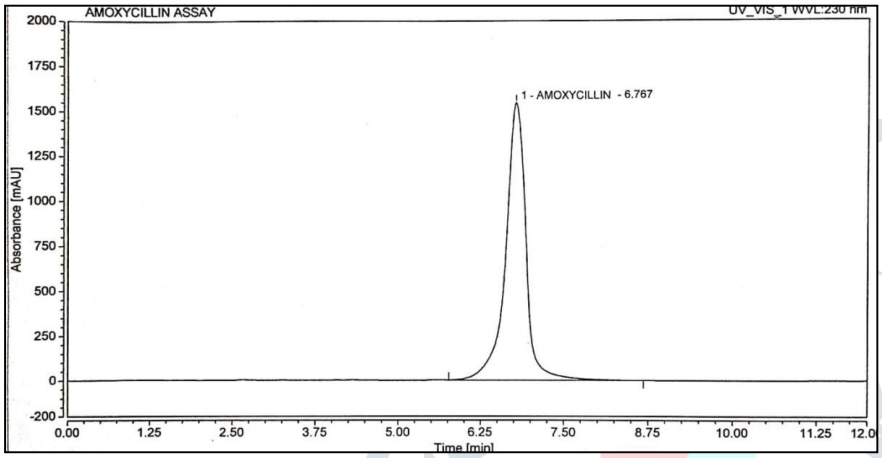

Fig: 7 Chromatogram of sample A6

Percentage purity was calculated using the formula

$$\% \text{ Assay} = \frac{\text{Test Response} \times \text{Std wt} \times \text{Test dilution} \times \text{Potency} \times \text{Avg wt} \times 100}{\text{Std Response} \times \text{Std dilution} \times \text{Test wt} \times 100 \times \text{Claim}}$$

The % purity for all samples is given in the Table 6. Sample A4 gives maximum content which is in correlation with the maximum dissolution shown by A4.

Table: 6 Assay results of Amoxicillin samples

Brand code	Amount (mg/tab)		Content %	Limit	Result
	Label claim	Assay (mg)			
A1	250	255.41	102.16	Not less than 90 % and not more than 110%	Passed
A2	250	256.50	102.42		Passed
A3	250	252.63	101.05		Passed
A4	250	273.64	109.45		Passed
A5	250	259.84	103.93		Passed
A6	250	253.30	101.32		Passed

VI. DISCUSSION

Our study compared the generic Amoxicillin capsules prescribed in government supply with branded Amoxicillin capsules sold in open market.The literature survey showed that previously no study is undertaken to compare the government supply Amoxicillin capsules with the branded Amoxicillin capsules. This makes our study unique.

Following studies have been undertaken by different researchers to study the Amoxicillin formulation for its content and quality control. **Kassaye et al.** from Ethiopia in 2013 conducted a comparative evaluation of nine commercially available brands of Amoxicillin capsules with the innovator brand (Amoxil) and found that only two brands passed quality test as per USP. **Huda et al.** from Bangladesh in 2009 performed a comparative evaluation of twenty national and four multinational brands of Amoxicillin capsules.The study concluded that 02 national brands failed the different quality test as per USP. **Obarisiagbon et al.** from Nigeria in 2019 conducted in-vitro pharmaceutical quality evaluation of five commercially available brands of Amoxicillin capsules and found that all the sample passed the quality test as per USP.
In our study, all the samples passed quality tests given in IP 2018.Thus, our study is in coungruence with results of Obarisiagbon et al. while it is not in a congruence with the results of Kassaye et al. and Huda et al. It is found that A4 sample has released maximum content in dissolution test which is in coherence with the maximum content found in assay as per IP 2018.

VII. CONCLUSION

In our study, all samples of 250 mg Amoxicillin capsules, complied with the limits specified for each quality assessment test prescribed in IP 2018. Negligible difference was found among generic vs generic, branded vs branded and branded vs generic samples. All samples are suitable for interchanging with each other. As all samples are found to be pharmaceutically equivalent, it is concluded that all samples are safe, effective, potent and suitable to use in clinical practice. Medical practitioners can replace the higher cost samples of Amoxicillin capsules with the lower cost samples, for the economical benefit of patients.

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