

Available online on 15.06.2019 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

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Research Article

Method development, validation and comparative study of generic Vs. branded generic formulations of amoxicillin trihydrate in capsule dosage form

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ABSTRACT

The present work relates to development and validation of simple, precise and sensitive UV spectrometric and high-performance thin layer chromatographic (HPTLC) method for the analysis of Amoxicillin Trihydrate in Capsule dosage form. Method were developed and applied for comparative study of generic vs branded generic formulations of Amoxicillin Trihydrate. Amoxicillin Trihydrate in methanol shows maximum absorbance at 229 nm and the data of linear regression analysis indicated a good linear relationship over the concentration range of 5-30 µg/ml with a correlation coefficient (R^2) 0.998 by UV- Spectroscopy and the concentration range of 2000-12000 ng/band with a correlation coefficient (R^2) 0.997 by HPTLC. The main objective was to compare and evaluate the price and quality of branded generic and generic formulations of Amoxicillin Trihydrate 250 mg Capsule. The evaluation parameters like content of active ingredients, content uniformity test, mass variation, disintegration test, and in vitro release studies were performed as described in Indian Pharmacopeia 2010.

Keywords: Branded Generic, Generic, Quantitative and Qualitative Determination, Ultraviolet Spectroscopy, HPTLC, Comparative Studies.

Article Info: Received 23 April 2019; Review Completed 25 May 2019; Accepted 29 May 2019; Available online 15 June 2019



Cite this article as:

Gandhi SV, Mahajan MR, Method development, validation and comparative study of generic Vs. branded generic formulations of amoxicillin trihydrate in capsule dosage form, Journal of Drug Delivery and Therapeutics. 2019; 9(3-s):186-192 <http://dx.doi.org/10.22270/jddt.v9i3-s.2821>

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INTRODUCTION

Analytical chemistry spreads its application in many allied areas. Especially in the pharmaceutical industry; quality of the manufactured drugs in tablets, solutions, suspension or other dosage form must be carefully controlled. Slight variation in composition or in the purity of drug itself can affect therapeutic value. Therefore, there is constant need to develop newer and better methods for pharmaceutical analysis. The main objective of this study is to carry comparative study and evaluate the price and quality for certain drugs for their IPQC tests and dissolution studies in Branded Vs Generic solid dosage forms. As there is belief that due to its cheaper cost and non-popularity generic medicines are of poor quality. The marketing cost is also saved, government takes responsibility to decide the price of medicines and due to competition between manufacturers the cost of generic medicines is less compare to branded ones. In case of branded medicines, the time and its development cost need to be paid more for the original company that's why the branded medicines are costlier than the generic medicines [1,2]. The generic and branded generic formulation of the drug should have same strength, quality

and physicochemical properties [3]. We need to ensure that both formulations are pharmaceutically and therapeutically same.

Amoxicillin is [[2S [2α,5α,6β, (S*)]]-6-[[Amino(4-hydroxyphenyl) acetyl] amino]-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid]. 1) is an oral semi-synthetic penicillin structurally related to ampicillin. Amoxicillin is β-lactam antibiotic that belong to the group of penicillin. It is semi-synthetic, broad spectrum, acid stable, orally absorbed antibiotics that inhibit bacterial cell, it is normally used for the treatment of common bacterial infection [4].

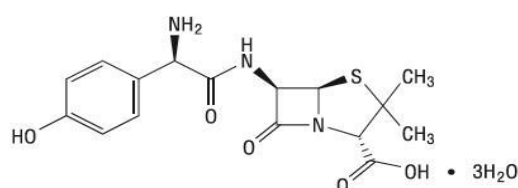


Fig 1: Chemical structure of Amoxicillin Trihydrate

Various methods were observed during literature survey for the quantitative determination of Amoxicillin Trihydrate, which includes UV [5-9], HPLC [10,11], HPTLC [12-14].

As per the literature survey the data represents that there is no reported comparative study for the estimation of Amoxicillin Trihydrate in pharmaceutical Capsule dosage, hence based on these observations we have developed a comparative study on generic and branded generic formulations for the determination of Amoxicillin Trihydrate in bulk and Capsule dosage form in accordance with International Conference on Harmonization Guidelines^[15].

MATERIALS AND METHODS

Reagents and chemicals

Amoxicillin Trihydrate Capsules I.P labeled to contain Amoxicillin Trihydrate 250 mg was purchased from local market. Methanol (AR grade), Methanol (HPTLC grade),

Acetic acid (AR grade), Ethyl acetate (AR grade), were used in study.

Preparation of standard stock solution:

Standard stock solution of drug Amoxicillin Trihydrate was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000 µg/ml. From this standard solution 1 ml was taken in 10 ml volumetric flask and volume was made up with methanol to get solution of concentration 100 µg/ml. Further 1 ml of this solution was diluted to 10 ml with methanol to get resulting solution of concentration 10 µg/ml.

Selection of detection wavelength:

From the standard stock solution (1000 µg/ml) further dilutions were made using methanol and scanned over the range of 200-400 nm and the spectra were obtained. It was observed that the drug showed linear, stable and considerable absorbance at 229 nm. Representative UV spectrum was obtained shown in Figure 1.

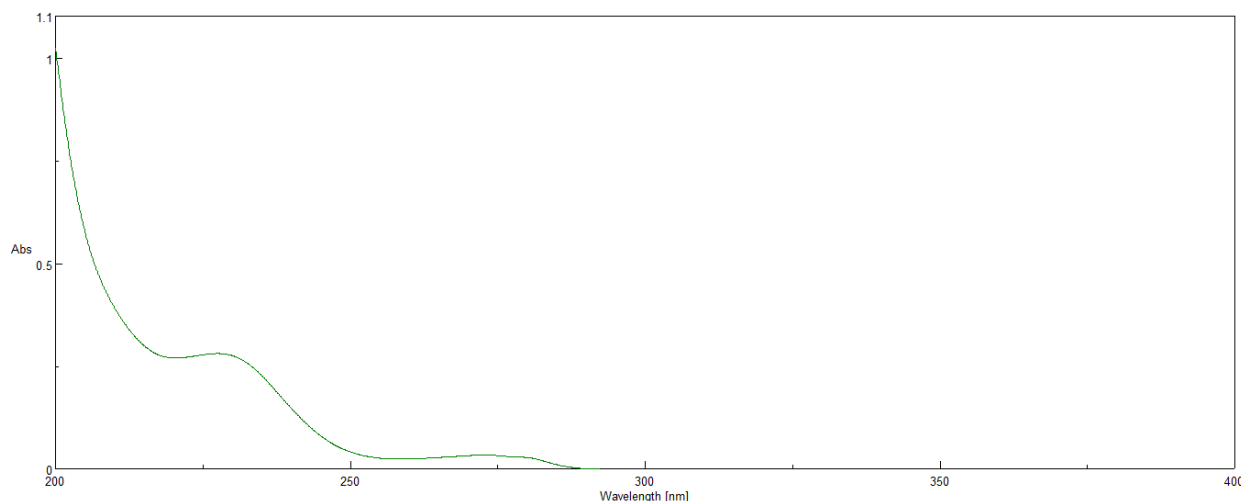


Figure 1: UV Spectrum of Amoxicillin Trihydrate (10 µg/ml)

Chromatographic conditions:

Chromatographic conditions were optimised by using CAMAG HPTLC system equipped with Camag Linomat V sample applicator, Hamilton syringe (100 µL), Camag TLC Scanner-3 with winCATS software version 1.4.2 and Camag twin- trough chamber (10×10 cm) were used for the present study. Chromatographic separation was established by using precoated Silica gel 60 F₂₅₄ TLC plates (10×10 cm, layer thickness 0.2 mm, E. Merck, Germany) as stationary phase and Ethyl Acetate: Acetic acid: Water (6: 2: 2, v/v/v) as mobile phase. Detection wavelength of 229 nm was employed during scanning and the band width 6 mm was fixed during spotting. Representative HPTLC chromatograph is given in Fig. 2.

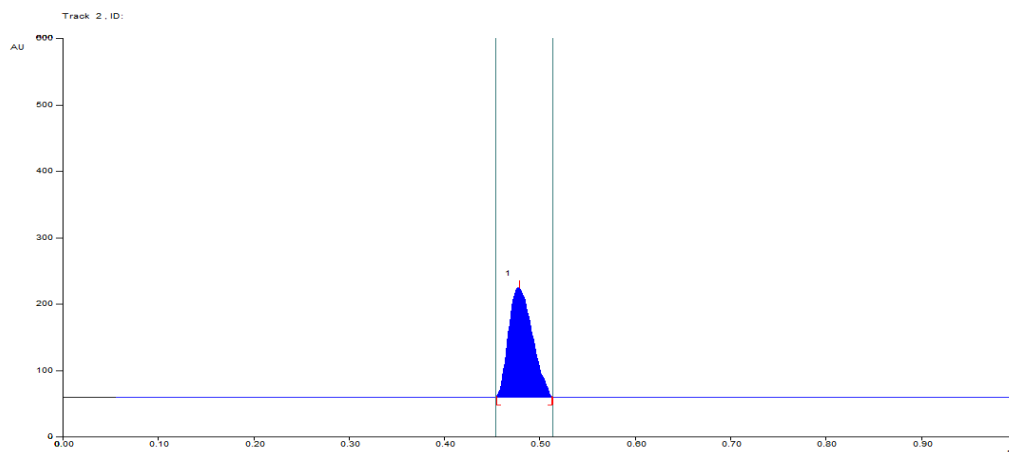


Figure 2: HPTLC Densitogram of Amoxicillin Trihydrate (2000 ng/band)

Preparation of sample solution:

Contents from the twenty capsules of Amoxicillin trihydrate were removed. The quantity of capsule powder equivalent to 10 mg of Amoxicillin Trihydrate (label claim: 250 mg Amoxicillin Trihydrate per Capsule) was transferred to a 10 ml volumetric flask containing 5 ml of methanol. The mixture was subjected for ultra-sonication for the period of 10 min and the resulting sample stock solution was filtered with Whatman filter paper 41 and the volume was made up with the methanol. 1.0 ml of this solution was diluted to 10 ml with the methanol to prepare a final sample stock solution of 100 µg/ml.

RESULTS AND DISCUSSION

Validation of analytical method [15]

Linearity and Range:

From standard stock solution (1000 µg/ml) of Amoxicillin Trihydrate, solution was prepared containing 100 µg/ml of Amoxicillin Trihydrate in methanol. Further 1ml was taken and diluted to 10ml with methanol in volumetric flask to get 100 µg/ml. This solution was further diluted with methanol to get range of solution containing different concentrations 5-30 µg/ml for the Ultraviolet spectroscopy. Absorbance was recorded. From standard stock solution (1000 µg/ml) of Amoxicillin Trihydrate spotting was done in the concentration range of 2000 – 12000 ng/band for the HPTLC. Area was recorded. The equation of calibration curve by UV- Spectroscopy and HPTLC was found to be $y = 0.0195x + 0.0318$ and $y = 0.9995x + 480.77$ respectively. The absorbance/area of drug was plotted against the corresponding concentrations to obtain the calibration curve as shown in Figure 3 and Figure 4 respectively.

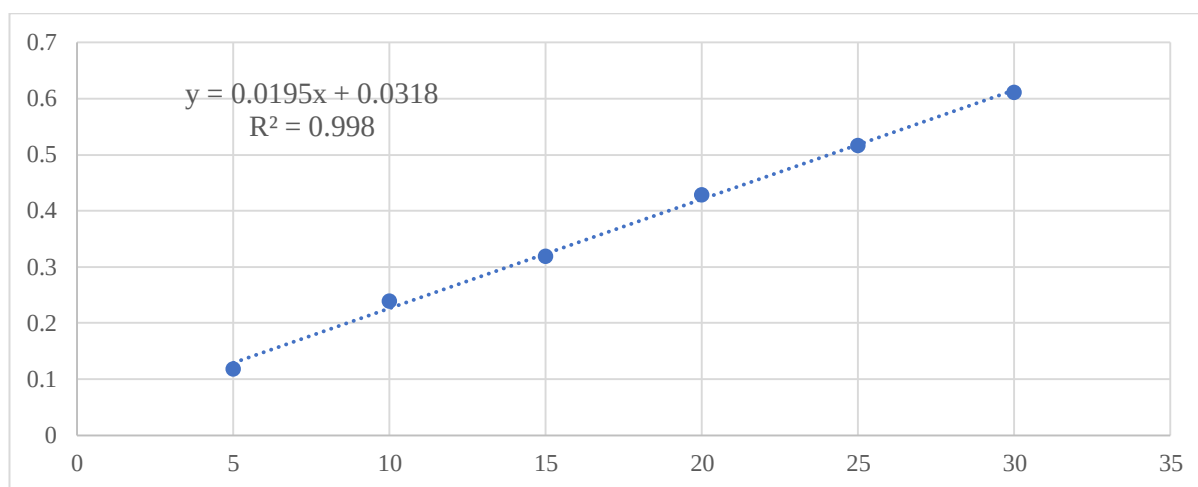


Figure 3: Linearity curve of Amoxicillin Trihydrate by UV- Spectroscopy (5-30 µg/ml).

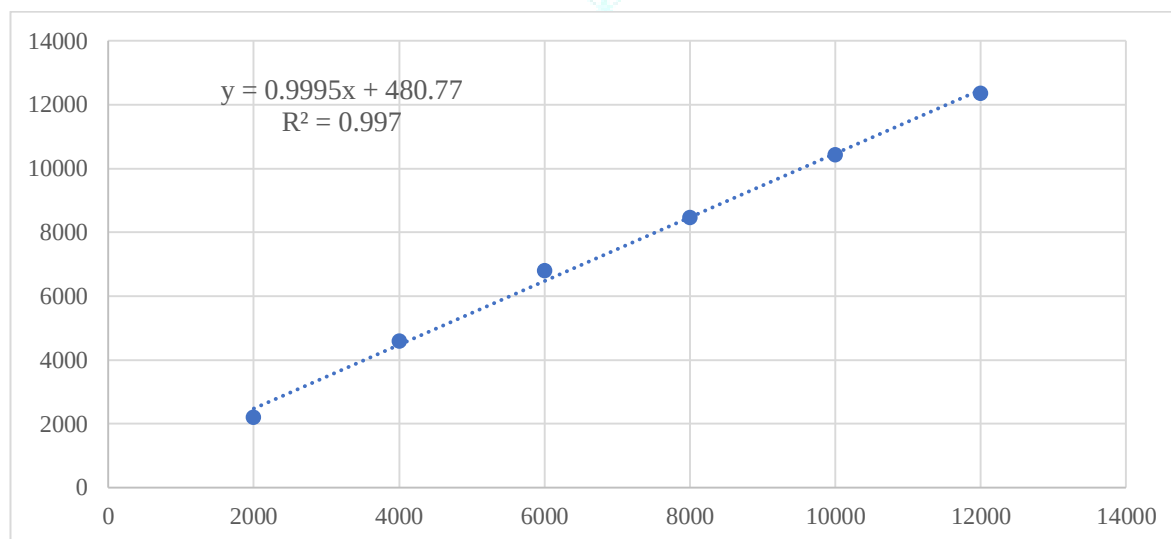


Figure 4: Linearity curve of Amoxicillin Trihydrate by HPTLC (200-1200 ng/band).

Precision:

The precision of the method was demonstrated by intra-day and inter-day variation studies. In the Intra-day studies, 3 replicates of 3 different concentrations were analyzed in a day and percentage RSD was calculated. For the inter-day

variation studies, 3 different concentrations were analyzed on 3 consecutive days and percentage RSD was calculated. The results obtained for intra-day and inter-day variations by UV-Spectroscopy and HPTLC are shown in Table 1 and Table 2 respectively.

Table 1: Intra-day and Inter-day variation studies data for Amoxicillin Trihydrate by UV-Spectroscopy.

Replicates	Intra-day Precision			Inter-day Precision		
	Conc. (µg/ml)			Conc. (µg/ml)		
	10	15	25	10	15	25
	Abs	Abs	Abs	Abs	Abs	Abs
1	0.2307	0.3294	0.5171	0.2297	0.3289	0.529
2	0.2258	0.3289	0.5263	0.2258	0.3287	0.5203
3	0.2294	0.3252	0.5201	0.2304	0.3302	0.5161
Mean	0.2286	0.3278	0.5212	0.2286	0.3293	0.5218
SD	1.302	0.784	0.962	1.271	0.278	1.350
%RSD	1.29	0.77	0.96	1.26	0.27	1.34

Table 2: Intra-day and Inter-day variation studies data for Amoxicillin Trihydrate by HPTLC.

Replicates	Intra-day Precision			Inter-day Precision		
	Conc. (µg/ml)			Conc. (µg/ml)		
	10	15	25	10	15	25
	Area	Area	Area	Area	Area	Area
1	4487.3	6407.6	10581.8	4424.4	6507.1	10381.9
2	4474.5	6481.7	10636.3	4527	6581.3	10436.4
3	4569.6	6504.3	10518.7	4425.3	6404.4	10418.5
Mean	4510.5	6464.5	10578.9	4458.9	6497.6	10412.3
SD	1.291	0.843	0.589	1.475	1.481	0.278
%RSD	1.28	0.85	0.58	1.48	1.48	0.28

Limit of detection (LOD) and limit of quantitation (LOQ):

From the linearity data the LOD and LOQ was calculated, using the formula $LOD = 3.3 \sigma/S$ and $LOQ = 10 \sigma/S$, where σ = standard deviation of the y intercept of linearity equations and S = slope of the calibration curve of the analyte. The LOD and LOQ by UV-Spectroscopy were found to be 0.455 µg/ml and 1.378 µg/ml, respectively and The LOD and LOQ by HPTLC were found to be 36.24 µg/ml and 109.82 µg/ml, respectively.

Accuracy:

To check accuracy of the method, recovery studies were carried by spiking the standard 250 mg Capsule sample solution, at three different levels around 50, 100 and 150%. Basic concentration of sample solution chosen was 10 µg/ml for UV and 4000 ng/band for HPTLC of Amoxicillin Trihydrate. % recovery was determined from linearity equation. The results obtained are shown in Table 3 and Table 4.

Table 3: Accuracy of Amoxicillin Trihydrate by UV-Spectroscopy.

Level	Conc. of Sample solution (µg/ml)	Conc. of Standard solution spiked (µg/ml)	Wymox			Aequimox		
			Absorbance	Amount recovered (µg/ml)	% recovery	Absorbance	Amount recovered (µg/ml)	% recovery
50%	10	5	0.3191	14.733	98.92	0.3209	14.826	100.536
			0.322	14.882		0.3331	15.451	
			0.3224	14.903		0.3236	14.964	
100%	10	10	0.4306	20.451	102.69	0.4331	20.579	102.803
			0.4317	20.508		0.4362	20.738	
			0.4346	20.656		0.4289	20.364	
150%	10	15	0.5293	25.513	100.47	0.5273	25.410	101.682
			0.5187	24.969		0.5287	25.482	
			0.5169	24.877		0.5265	25.369	

Table 4: Accuracy of Amoxicillin Trihydrate by HPTLC.

Level	Conc. of Sample solution (µg/ml)	Conc. of Standard solution spiked (µg/ml)	Wymox			Aequimox		
			Area	Amount recovered (µg/ml)	% recovery	Area	Amount recovered (µg/ml)	% recovery
50%	10	5	6507.1	6029.34	100.331	6407.6	5929.79	99.78
			6581.3	6103.58		6481.7	6003.93	
			6404.4	5926.59		6504.3	6026.54	
100%	10	10	8345.3	7868.46	99.005	8445.4	7968.61	99.43
			8398.3	7921.49		8498.8	8022.04	
			8448.1	7971.32		8348.5	7871.67	
150%	10	15	10581.9	10106.18	101.365	10781.8	10306.18	101.03
			10636.4	10160.71		10536.3	10060.56	
			10618.3	10142.60		10418.7	9942.90	

Assay:

Amoxicillin Trihydrate Capsules I.P labeled to contain Amoxicillin Trihydrate 250 mg formulation analysis was carried out as mentioned under section preparation of sample solution and absorbance/area was recorded. Basic

concentration of sample chosen was 10 µg/ml for UV and 4000 ng/band for HPTLC from Capsule solution. Procedure was repeated for six times. Concentration and % recovery was determined from linear equation. The results obtained by UV-Spectroscopy and HPTLC are shown in Table 5 and Table 6 respectively.

Table 5: Assay of marketed formulation by UV-Spectroscopy.

Sr. No.	Wymox			Aequimox		
	Absorbance	Amount Recovered (µg/ml)	% Recovery	Absorbance	Amount Recovered (µg/ml)	% Recovery
1	0.2256	9.94	99.38	0.2298	10.154	101.538
2	0.2299	10.16	101.59	0.2306	10.195	101.949
3	0.2238	9.85	98.46	0.2292	10.123	101.231
4	0.2284	10.08	100.82	0.2214	9.723	97.231
5	0.2323	10.28	102.82	0.2244	9.877	98.769
6	0.2306	10.19	101.95	0.2249	9.903	99.026
Mean	0.228	10.084	100.838	0.227	9.996	99.957
SD	0.003	0.164	1.643	0.004	0.189	1.887
%RSD	1.402	1.629	1.629	1.623	1.888	1.888

Table 6: Assay of marketed formulation by HPTLC

Sr. No.	Wymox			Aequimox		
	Area	Amount Recovered (µg/ml)	% Recovery	Area	Amount Recovered (µg/ml)	% Recovery
1	4564.5	4085.77	102.14	4464.4	3985.62	99.64
2	4497	4018.24	100.46	4597	4118.29	102.96
3	4525.2	4046.45	101.16	4425.3	3946.50	98.66
4	4487.3	4008.53	100.21	4587.8	4109.08	102.73
5	4474.5	3995.73	99.89	4576.6	4097.88	102.45
6	4569.6	4090.88	102.27	4469.7	3990.93	99.77
Mean	4519.683	4040.934	101.023	4520.133	4041.384	101.035
SD	40.337	40.357	1.009	75.258	75.296	1.882
%RSD	0.892	0.999	0.999	1.665	1.863	1.863

In-vitro dissolution studies:^[16]

The in-vitro dissolution study was carried out by using IP Type-2 dissolution apparatus at 100 rpm. For the dissolution study randomly 6 Capsules of both branded generic and generic formulations are selected. Media used was RO

purified water. Sufficient volume was removed, filtered by Whatman filter paper and diluted up to 10 ml in 10 ml volumetric flask and scanned over the UV-Spectroscopy. The % drug release was plotted against the corresponding time to obtain the curve as shown in Figure 5.

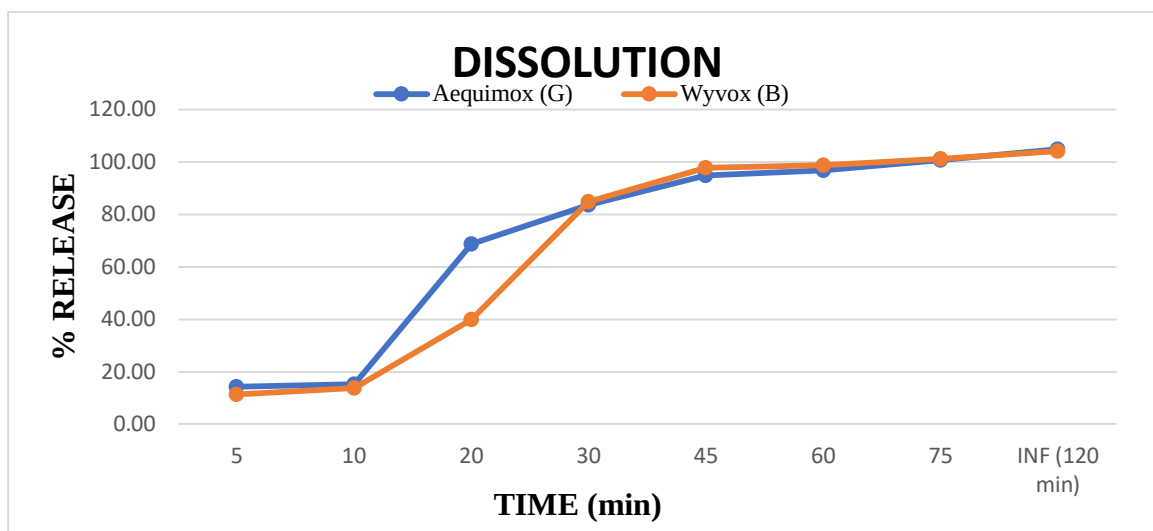


Figure 5: Comparison of Dissolution Profile of Generic and Branded generic Capsul

Appearance^[17]

Capsules produced on a small or a large scale should be uniform in appearance. Visual or electronic inspection should be undertaken to detect any flaws in the integrity and appearance of the capsule. Evidence of physical instability is demonstrated by gross changes in appearance, including hardening or softening, cracking, swelling, mottling, printing mistake or discoloration of the shell. Defective capsules should be rejected.

Size and Shape^[17]

Hard capsules are made in a range of sizes, the standard industrial ones in use today for human medicines range from size from 000 (the largest, 1.40 ml) to 5 (the smallest, 0.13 ml) are commercially available.

Unique Identification Markings^[17]

Capsule surfaces may bear symbols or other unique identification markings for better identification.

Content of Active Ingredients^[17]

For this test a sample of the contents is assayed as described in individual monographs and calculates the amount of active ingredient in each capsule. According to IP the range for the content of active ingredient stated in the monograph is based on the requirement that 20 capsules. In the circumstances where 20 capsules cannot be obtained, a

smaller number, which must not be less than 5, may be used, but to allow for sampling errors the tolerances are widened in accordance with the limits and proportionately smaller or larger allowances should be made.

Uniformity of Mass^[17]

For this test weigh an intact capsule. Open the capsule without losing any part of the shell and remove the contents as completely as possible. Weigh the shell. The weight of the contents is the difference between the weighing. Repeat the procedure with a further 19 capsules. Determine the average mass. According to IP capsules not more than 2 of the individual masses deviate from the average mass by more than the percentage deviation as per the limits and none deviates by more than twice that percentage.

Disintegration Test^[17]

The disintegration apparatus, described in I.P was used for the study. It contains 2 basket rack assembly. Each basket rack assembly consists of 6 glass tubes that are 3 inches long, open at the top and held against 10 mesh size at the bottom. Each Capsule was placed in each tube, and the basket rack was positioned in 1-L beaker of distilled water. The 37±2°C temperature was maintained throughout the study.

Table 7: Identification test: ^[17]

Sr No.	Drug name	Amoxicillin Trihydrate	
		Branded generic - Wymox	Generic - Aequimox
1	Manufacturer	Pfizer Ltd.	Zydus Healthcare Ltd.
2	Shape	Cylindrical	Cylindrical
3	Description	Each hard gelatin capsule contains: Amoxicillin Trihydrate I.P. equivalent to Amoxicillin 250 mg	Each hard gelatin capsule contains: Amoxicillin Trihydrate I.P. eq. to Amoxicillin 250 mg
4	Color	Pink and Black	Red and White
5	Dosage	As prescribed by the physician	As directed by the physician
6	Storage	Store protected from moisture at a temperature not exceeding 30° C.	Store protected from light and moisture, at a temperature not exceeding 30° C.
7	Mfg. Lic. no.	SP- 46/76	48/UA/SC/P-2013
8	Mfg. date	09/2017	04/2018
9	Exp. date	08/2019	03/2020
10	Price (PER 10 CAPSULES)	Rs.: 22.14	Rs.: 23.10

Table 8: Physiochemical evaluation: [17]

Sr No.	Drug name	Amoxicillin Trihydrate	
		Wymox	Aequimox
1	Content of active ingredients (Limits: between 90 and 110 %)	UV: 98.46-102.82 % HPLC: 99.89-102.27%	UV: 98.23-102.97 % HPLC: 98.66-102.96%
2	Uniformity of mass (not more than 2 of the individual masses deviate from the average mass by more than the percentage deviation)	Test complies with I.P.	Test complies with I.P.
3	Disintegration time (Not more than 30 min)	18 min	15 min
4	Dissolution test (Not less than 70 per cent of the stated amount)	% release Not less than 98.53 %	% release Not less than 94.89 %

CONCLUSION

Based on such studies; their result and possible outcomes, opinion and doubt about the generic medicines need to be alter in order to help society and availability of medicines in reasonable prices. During the study it was observed that both variants of capsules were found to be in permissible range for all the quantitative and qualitative parameters as described in Indian Pharmacopoeia. And there was no any significant difference between both the branded generic and generic variant.

ACKNOWLEDGEMENT

Authors are thankful to the Principal and Management, AISSMS College of Pharmacy, Pune for providing required facilities for research work.

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