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DETERMINATION OF *IN VITRO* DRUG RELEASE OF AMOXYCILLIN AND POTASSIUM CLAVULANATE TABLETS IP AS PER INDIAN PHARMACOPOEIA

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ABSTRACT

Infections still represent a common cause of morbidity and mortality worldwide accounting among the most important reasons for hospital admissions and increased economic burdens for national health system in India. Amoxycillin and Potassium clavulanate is an oral antibacterial combination consisting of the semisynthetic antibiotic Amoxycillin and the β-lactamase inhibitor, clavulanate potassium. In-vitro drug release testing, a measure of release of the active pharmaceutical ingredient (API) from the drug product matrix in controlled laboratory environment, is a key evaluation in drug development and quality control. It involves subjecting the dosage form to a set of conditions that will induce drug release and quantitating the amount of drug released under those conditions. In vitro dissolution testing of oral dosage forms measures the dissolution rate of an amount of drug substance going from the solid state into solution per unit time under standardized conditions. The goals of a dissolution test include prediction of bioavailability, indication of drug product safety of dosage form and implication of variations in the manufacturing process. The main objective of this research work is to evaluate the quality of two brands of Amoycillin and Potassium Clavulanate Tablets IP mg marketed in North East region of India, in order to verify whether these products complies with the standard monograph or not.

KEY WORDS

Amoxycillin and Potassium clavulanate, Indian Pharmacopoeia, In vitro, Quality control

INTRODUCTION

Infections still represent a common cause of morbidity and mortality worldwide, with acute respiratory tract infections (RTIs) accounting among the most important reasons for hospital admissions and increased economic burdens for national health system in India. [1]. Despite the considerable number of newer antibacterial made available over the past decades, β -lactam antibiotics are still the most used antibacterial all over the world. The first β -lactams were licensed in the 1950s (penicillin G and V) and presented substantial inconveniences, most notably a limited range of activity, a short half-life, and the administration route had to be parenteral. The development of a semi synthetic pathway for their production in the 1960s led to the creation of newer Penicillins, with significant improvements in their range of activity.

Amoxycillin is the most striking innovations, effective not only in the treatment of upper and lower RTIs but also for urinary tract, soft-tissue and skin infections. Amoxycillin in particular, is a moderate-spectrum, semisynthetic β -lactam active against a wide range of Gram-positive and a limited range of Gram-negative organisms [2]. It was marketed in 1972, and still remains the most commonly utilized drug in this class because its oral absorption is better when compared with other β -lactam antibiotics [3]. Unfortunately, during the past decades, an increasing number of bacteria have become

resistant to antibiotics, making bacterial resistance one of the world's most pressing public health problems. β -Lactamase production is one of the most common mechanisms of bacterial resistance; these enzymes, that cleave the β -lactam ring, can be produced by several Gram-positive organisms, Gram-negative organisms and anaerobic organisms [4,5]. To overcome this problem, in the 1970s, a new area of research was focused on identifying compounds able to inhibit β -lactamase, and in 1972 Clavulanic acid was identified. Clavulanic acid is structurally related to Penicillins; it prevents inactivation of antibiotics, thus increasing their effectiveness when combined [6]. The association of Amoxycillin /Clavulanate was first marketed in 1981, and it is the only penicillin combined with a β -lactamase inhibitor available in oral formulation [7]. Amoxycillin /clavulanate potassium is an oral antibacterial combination consisting of the semisynthetic antibiotic Amoxycillin and the β-lactamase inhibitor, clavulanate potassium (the potassium salt of clavulanic acid). Amoxicyllin is an analog of ampicillin, derived from the basic penicillin nucleus, 6-aminopenicillanic acid. Clavulanic acid is produced by the fermentation of *Streptomyces clavuligerus*. It is a β-lactam structurally related to the penicillins and possesses the ability to inactivate a wide variety of β-lactamases by blocking the active sites of these enzymes. Clavulanic acid is particularly active against the clinically important plasmid-mediated β-lactamases frequently responsible for transferred drug resistance to penicillins and cephalosporins. Amoxycillin and clavulanate potassium are well absorbed from the gastrointestinal tract after oral administration of Amoxycillin /clavulanate potassium. Amoxycillin serum concentrations achieved with Amoxycillin /clavulanate potassium are similar to those produced by the oral administration of equivalent doses of Amoxycillin alone. The half-life of Amoxycillin after the oral administration of Amoxycillin /clavulanate potassium is 1.3 hours and that of clavulanic acid is 1.0 hour.

In-vitro drug release testing, a measure of release of the active pharmaceutical ingredient (API) from the drug product matrix in controlled laboratory environment, is a key evaluation in drug development and quality control. It involves subjecting the dosage form to a set of conditions that will induce drug release and quantitating the amount of drug released under those conditions. In development, it is an essential test in assessing differences between prototypes, predicting the timeframe of API release, and modeling in vivo behavior. During this phase, in vitro conditions are generally selected to simulate in vivo conditions. In quality control it is used to assess conformance of a batch to pre-determined criteria at time of manufacture and to assess the long-term API release stability. In this use, in-vitro test conditions are chosen to be discriminatory, meaning that they are capable of reflecting a change in API release profile that is related to a change in the drug product. It is an important tool in evaluating drug product performance for most dosage forms and is known as dissolution testing, in vitro release testing, and elution testing.

In vitro dissolution testing of oral dosage forms measures the dissolution rate of an amount of drug substance going from the solid state into solution per unit time under standardized conditions. The goals of a dissolution test include prediction of bioavailability (a surrogate parameter of the therapeutic efficacy), indication of the robustness of the dosage form (drug product safety) and implication of variations in the manufacturing process (which may have a critical influence on performance). USP <711> describes the apparatus types and procedural recommendations for testing immediate-, extended- and delayed-release dosage forms. Nomenclature for compendial apparatus for dissolution testing includes USP 1 baskets, USP 2 paddles, USP 3 reciprocating cylinders, USP 4 flow through cell, USP 5 paddle over disk, USP 6 cylinders and USP 7 reciprocating holders. In an attempt to mimic in vivo conditions, the choice of apparatus and method parameters such as medium composition, pH and sampling frequency vary depending on the dosage form and overall purpose of the test. Dissolution is commonly applied to tablets, capsules, suspensions, ointments, creams, suppositories, transdermals, implants, drug eluting stents, medicated gums, and has potential applicability with alternative formulations such as oral and injectable nanosuspensions.

The importance of good manufacturing practices (cGMP) for establishing the quality of pharmaceutical products has emerged as a very significant issue. In the manufacturing process of a pharmaceutical product quality control test plays a very significant role, it includes various parameters for eliminating or preventing every possible error for maintaining the quality of finished product. Quality as per ISO 8402-1986

is best defined as "the totality of features and characteristics of a product or service that bears its ability to satisfy as stated or implied needs" [8]. In process and Finished product quality control tests are done to measure the efficiency of the product before they get released commercially. On completion of the manufacturing process of finished product, quality control tests are done with reference to qualitative and quantitative characteristics. The compliance of the approval limits of the finished product during its entire shelf life is studied. Pharmacopoeias are standard monograph for all drugs. There are various official pharmacopoeias in which includes the Indian Pharmacopoeia (IP), United States Pharmacopoeia (USP), British Pharmacopoeia(BP), European Pharmacopoeia (EP), wherein they have laid down specified limits within which the product should fall to fulfill the requirements in order to be compliant as per the standards [9].

The main objective of this research work is to evaluate the quality of two brands of Amoycillin and Potassium Clavulanate Tablets IP mg marketed in North East region of India, in order to verify whether these products complies with the standard or not. The Indian Pharmacopoeia is an official document meant for overall Quality Control and Assurance of Pharmaceutical products marketed in India published by the Indian Pharmacopoeia Commission (IPC) on behalf of the Ministry of Health & Family Welfare, Government of India. The Indian Pharmacopoeia provides standards for drugs manufactured/marketed in India to control as well as assure the quality of medicines.

Table 1: List of Commercial Brands of Amoycillin and Potassium Clavulanate Tablets IP

Product code	Batch No.	Manufacturer	Mfd date	Exp date
A	BT170013	Theon Pharmaceuticals Limited	01/2017	12/2018
B	TEHET7001	Finecure Pharmaceuticals LTD	02/2017	07/2018

MATERIALS AND METHODS

In this study the active pharmaceutical ingredient (API), Amoycillin W/S and Potassium Clavulanate W/S was obtained from M/S Regent Biotech. We have procured two commercial brands of Amoycillin and Potassium Clavulanate Tablets IP from retail pharmacies located in Guwahati and they are listed in Table 1.

Quality control parameters

Identification Test

Reversed phase High Performance Liquid Chromatography (HPLC) was used for carrying out the identification test on (Waters HPLC) auto sampler integrated with UV detector. The software employed was Empower 2 chromatography data system [10].

Assay

The assay was also carried out by HPLC. This test was done to determine the actual amount of active ingredient present in the tablet and its compliance with the labeled amount. The chromatographic conditions maintained throughout the procedure were a stainless-steel column (30cm × 4mm) packed with octadecylsilane chemically bonded to porous silica (3-10µm). The mobile phase is a mixture of 95 volumes of 0.78 percent w/v solution of sodium phosphate, monobasic adjusted to PH 4.4 with orthophosphoric acid 5 volume Methanol. The mobile phase was pumped into the system at a flow rate of 2ml per minute with Spectrophotometer wavelength set at 220nm and injection volume of 20µl.The mobile phase prior to use was degassed under vacuum by filtration through 0.2µ nylon membrane [10].

Preparation of Reference solution

A solution containing 0.05 per cent w/v of Amoycillin trihydrate RS and 0.02 percent w/v of Potassium Clavulanate RS was prepared in water. The prepared solution was sonicated for 10 minutes making final concentration equivalent to 500mcg and filtered through 0.45µm filter [10].

Preparation of Test solution

Of all the two batches 20 tablets of each batch were weighed separately and powdered. An accurately weighed powder containing 50 mg of of Amoycillin trihydrate was transferred to 100ml volumetric flask with addition of water as diluent. The prepared solution was sonicated until complete mixing and filtered through 0.45µm filter [10].

DISSOLUTION

Dissolution test was carried out on all two different brands in Apparatus 1 of I.P. (TDT-08L, Electrolab) with six individual tablets of each brand. The dissolution medium used was 900ml of water with the speed and

time of apparatus set at 75rpm and 30 minutes respectively. During the entire analysis the temperature was maintained at 37±0.5°C. A suitable volume of the dissolution medium was withdrawn at the end of analysis and filtered through Whatman filter No. 40. The quantity of Amoycillin and Potassium Clavulanate released into the dissolution medium was calculated as percentage in relation to the value declared on product label. The dissolution was carried out by HPLC. This test was done to determine the actual amount of active ingredient released by the tablet and its compliance with IP. The chromatographic conditions maintained throughout the procedure were a stainless-steel column (30cm × 4mm) packed with octadecylsilane chemically bonded to porous silica (3-10µm). The mobile phase is a mixture of 95 volumes of 0.78 percent w/v solution of sodium phosphate, monobasic adjusted to PH 4.4 with orthophosphoric acid 5 volume methanol The mobile phase was pumped into the system at a flow rate of 2ml per minute with Spectrophotometer wavelength set at 220nm and injection volume of 20µl.The mobile phase prior to use was degassed under vacuum by filtration through 0.2µ nylon membrane [10].

Preparation of Reference solution

Working standard (WS) was prepared in dissolution medium to obtain a final concentration of 500 mcg for Amoxicyllin trihydrate and 226.8mcg for Potassium clavulanate [10].

Preparation of Test solution

Test solutions were prepared at a concentration equivalent to 555 mcg for Amoxicyllin and 138.88mcg for Potassium clavulanate [10].

Water content Analysis

Pharmaceutical products are often characterized by complex formulations. In pharmaceutical guidelines the Karl Fischer titration is described as common method for water determination. The IP specify that not more than 7.5 percent where the labeled amount of Amoxycillin in each tablet is 250 mg or less; not more than 10 percent where the labeled amount of Amoxycillin in each tablet is more than 250 mg but less than or equal to 500mg; not more than 11 percent where the labeled amount of Amoxycillin in each tablet is more than 500mg [10].

RESULTS AND DISCUSSION

Identification Test

This test was found to be in compliance with the criteria mentioned in I.P. which states that the principal peak in the chromatogram obtained with test solution in assay corresponds with the peak in the chromatogram obtained with reference solution.

Assay

In this test the determination of actual amount of active ingredient present in the formulation was found to be within the acceptance limit of (90-120) % in all the two different brands of Amoycillin and Potassium Clavulanate tablets IP under study and is listed in Table 2. Figures 1, 2 show the chromatograms of standard and tested Amoycillin and Potassium Clavulanate tablets IP obtained from HPLC.

Table 2: Results of Assay of the two brands of Amoxycillin and Potassium Clavulanate Tablet IP

Product code	Batch No.	Identification (HPLC)	Assay (HPLC) Amoycillin	Potassium Clavulanate
A	TEHET7001	Complies	107.74 % (90-120%)	95.93% (90-120%)
B	BT170013	Complies	110.84% (90-120%)	96.03% (90-120%)

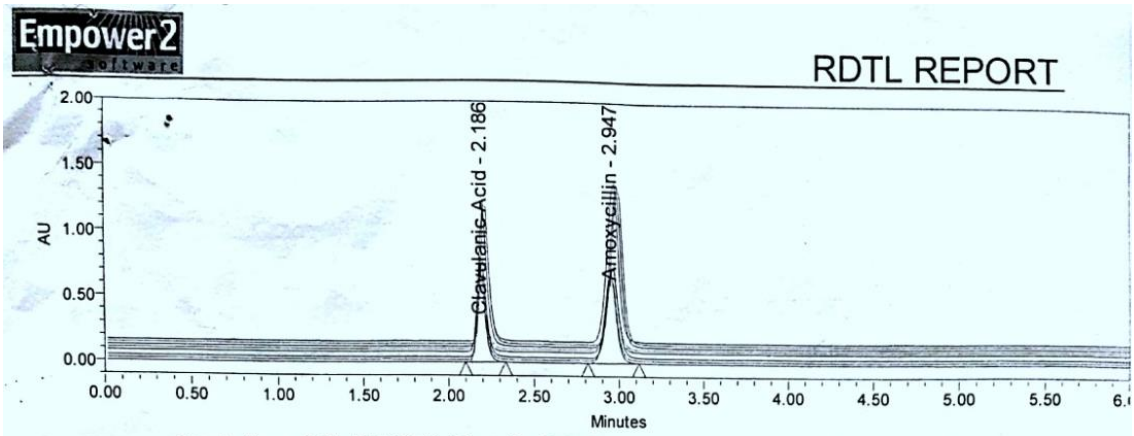
Table 3: Results of dissolution studies of the Amoxycillin in two brands of tablet

Sl No.	TEHET7001	Sl No	BT170013
Tablet 1	94.36	Tablet 1	97.8
Tablet 2	94.26	Tablet 2	91.9
Tablet 3	94.69	Tablet 3	98.7
Tablet 4	94.57	Tablet 4	99.3
Tablet 5	93.81	Tablet 5	94.6
Tablet 6	91.14	Tablet 6	98.8
Limit(NLT) 85%		Limit(NLT) 85%	

Table 4: Results of dissolution studies of the Potassium Clavulanate in two brands of tablet

Sl No	TEHET7001	Sl No	BT170013
Tablet 1	103.63	Tablet 1	101.5
Tablet 2	102.96	Tablet 2	96.7
Tablet 3	102.36	Tablet 3	103.9
Tablet 4	99.83	Tablet 4	102.2
Tablet 5	102.73	Tablet 5	102.2
Tablet 6	103.01	Tablet 6	103.9
Limit (NLT 80%)		Limit (NLT 80%)	

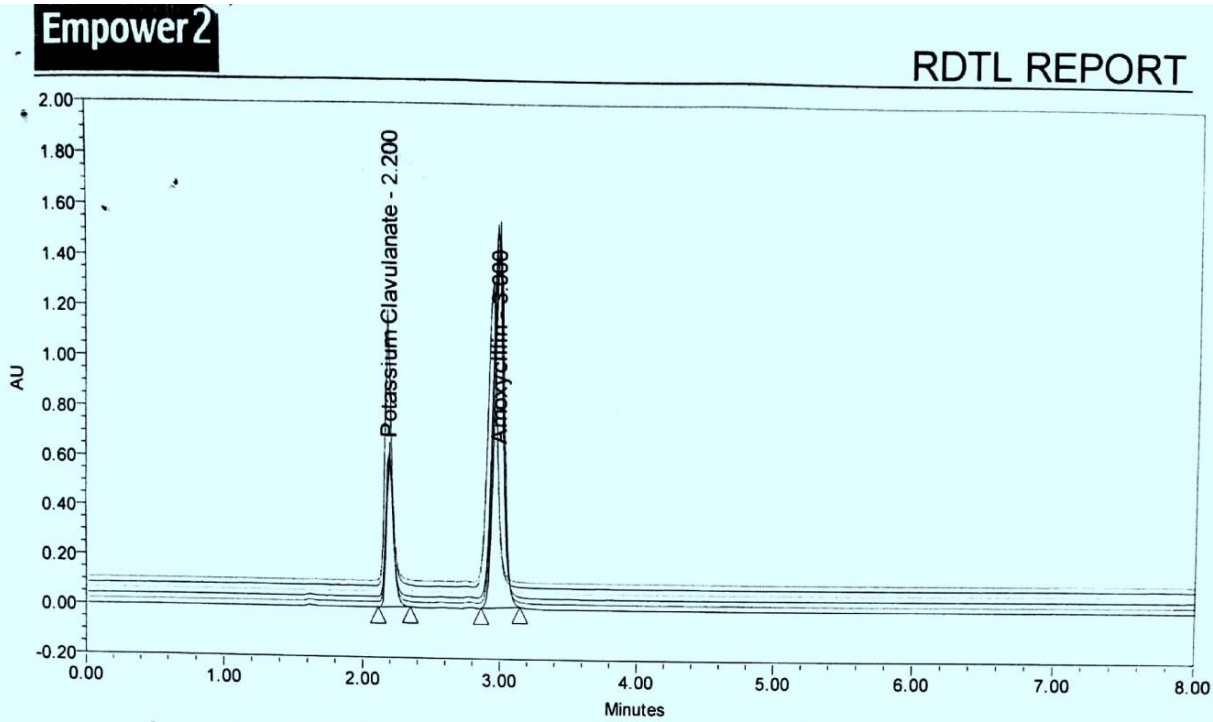
Figure 1



Peak Summary with Statistics
Name: Amoxycillin

	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	% Area	Height
1	Standard	1	1	Amoxycillin	2.962	5914966	61.93	1241337
2	Standard	1	1	Amoxycillin	2.987	5901465	62.00	1223942
3	Standard	1	2	Amoxycillin	2.977	5880434	61.89	1217506
4	B.No. BT170013 (Disso1)	7	1	Amoxycillin	2.947	3341934	57.97	693748
5	B.No. BT170013 (Disso2)	8	1	Amoxycillin	2.952	3141845	57.64	652840
6	B.No. BT170013 (Disso3)	9	1	Amoxycillin	2.961	3374380	57.61	695890
7	B.No. BT170013 (Disso4)	10	1	Amoxycillin	2.963	3393412	58.18	702504
8	B.No. BT170013 (Disso5)	11	1	Amoxycillin	2.956	3234234	57.48	663011
9	B.No. BT170013 (Disso6)	12	1	Amoxycillin	2.960	3376652	57.64	693527
Mean					3.0			
Std. Dev.					0.0			
% RSD					0.4			

Figure 2



Peak Summary with Statistics
Name: Amoxycillin

	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	% Area	Height
1	STANDARD	1	1	Amoxy cillin	2.947	5774540	62.11	1206844
2	STANDARD	1	2	Amoxy cillin	2.948	5782830	62.12	1208500
3	STANDARD	1	3	Amoxy cillin	2.943	5786394	62.12	1204514
4	B.No. TEHET7001 (Assay)	13	1	Amoxy cillin	2.986	7318377	78.23	1485940
5	B.No. TEHET7001 (Assay)	13	2	Amoxy cillin	2.993	7343761	78.28	1486950
6	B.No. TEHET7001 (Assay)	13	3	Amoxy cillin	3.000	7342130	78.30	1490396
Mean					3.0			
Std. Dev.					0.0			
% RSD					0.9			

Figure 3

Results report

Determination

Method	water in sample
Method saving date	2017-08-16 00:33:11 UTC+5:30
Determination start	2017-11-22 11:50:23 UTC+5:30
Run number	4
User (full name)	
User (short name)	Admin

Sample data

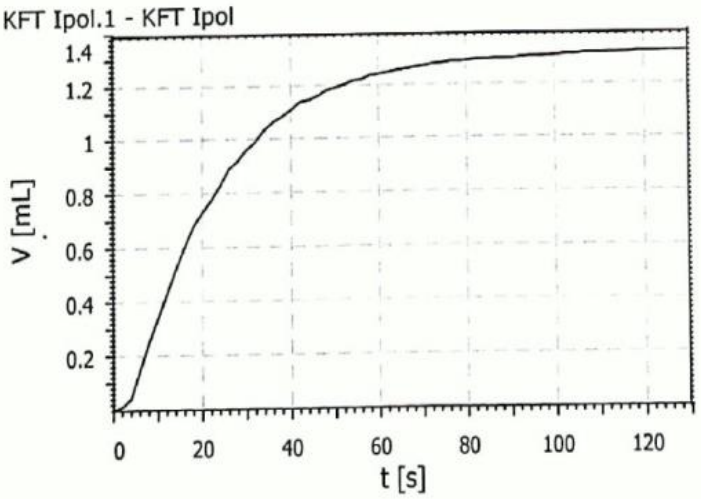
ID1	B.No.TEHET7001
Sample size	* 0.103 g

End points

KFT Ipol	KFT Ipol.1		
EP1	1.3215 mL	129.20 s	

Results

Result	**8.07 %**



Calculations

Result	8.07 %
Formula	= 'KFT Ipol.EP.VOL' * 'CV.KF factor' / 'MV.Sample size' / 10
KFT Ipol.EP.VOL	1.3215000629425049
CV.KF factor	6.2863
MV.Sample size	0.103

Figure 4

Results report

Determination

Method . . . . . water in sample
Method saving date . . . . . 2017-08-16 00:33:11 UTC+5:30
Determination start . . . . . 2017-11-10 15:27:57 UTC+5:30
Run number 10
User (full name) . . . . . .
User (short name) . . . . . Admin

Sample data

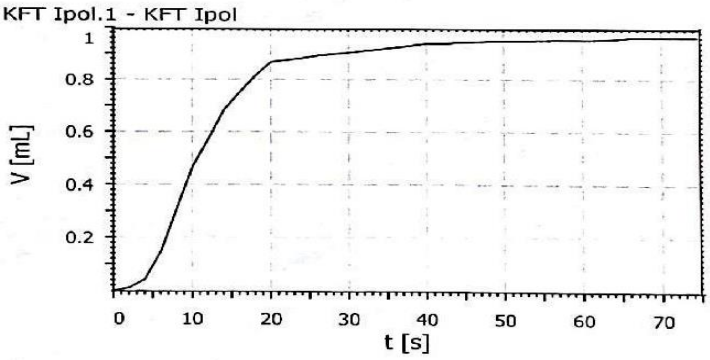
ID1 . . . . . B.No.BT170013
Sample size . . . . . * 0.064 g

End points

KFT Ipol KFT Ipol.1
EP1 . . . . . 0.9625 mL . . . . . 74.50 s

Results

Result . . . . . **7.30 %**



Calculations

Result . . . . . 7.30 %
Formula . . . . . = 'KFT Ipol.EP.VOL' * 'CV.KF factor' / 'MV.Sample size' / 10
KFT Ipol.EP.VOL 0.9625000953674316
CV.KF factor 4.8535
MV.Sample size 0.064

Water content Analysis report

The water content of Amoxycillin found in batches TEHET7001and BT170013 is 8.07%. and 7.30% respectively. Hence the sample complies Indian Pharmacopoeia.

CONCLUSION:

Amoxicillin and potassium clavulanate is an oral antibacterial combination drug. Therapeutic response of any formulation depends on its quality parameters. From the study it was found that Assay, Identification, Dissolution and Water content test of both Amoxycillin and Potassium Clavulanate Tablet IP brands complies the specification. Variation was obtained in assay and dissolution profile during the test procedure. It should be strictly considered that an ideal tablet must complies the tests mentioned in the standard monograph to maintain its mechanical stability or dissolution profile. Finally, as quality control parameters are related to one another from initial step to pharmacological action of the drug, a high-quality tablet should meet all the standard quality parameter for getting its desired therapeutic response.

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