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Research Article

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Stability study of six brands of amoxicillin trihydrate and clavulanic acid oral suspension present in Yemen markets

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ABSTRACT

Amoxicillin/clavulanate is a combination of a ß-lactam with a ß-lactamase inhibitor which restores the potency of amoxicillin against strains producing ß-lactamases. The decomposition of active ingredients in pharmaceutical preparation includes, hydrolysis, oxidation-reduction, recemixation, decorboxylation, ring cleavage and photolysis.This is to evaluate the stability of six brands amoxicillin trihydrate in amoxicillin trihydrate with clavulanic acid oral suspension collected from Yemen markets, reconstituted with distilled water and store at different temperatures. Also to prove that the degradation follows a first order kinetic. Using HPLC method, the concentrations of amoxicillin trihydrate and clavulanic acid brands, A, B, C, D, E and F, at zero time were within the pharmacopial limit when reconstituted with distilled, decreases gradually with time and the reaction followed first order kinetics.

Keywords: Amoxicillin, clavulanic acid, suspension, stability, markets

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INTRODUCTION

Amoxicillin/clavulanate is a combination of a ß-lactam with a ß-lactamase inhibitor which restores the potency of amoxicillin against strains producing ß-lactamases [1,2]. Combination of amoxicillin and potassium salt of clavuanic acid are effective against B-lactamase producing strains of staph. Aureus, E. coli, K. pneumoniae, Enterobacter H. influences. Moraxella catarrhalis, and H. ducreyi, which are resistant to amoxicilling alone. Clavalanic acid is acid stable. It can not undergo penicillanic acid formation because it lacks an amide side chain [3,4,5].

Amoxicillin and potassium clavulanate oral suspension contain the equivalent of not less than 90% and not more than 120% of the labeled amount of amoxicillin ($C_{16}H_{19}N_3O_5S$) and the equivalent not less than 90% and not more than 125% of the labeled clavuanic acid ($C_8H_9NO_5$) [6].

Changes in the chemical, physical and microbiological properties of the medicament or preparation may affect the therapeutic value of a preparation or increase its toxicity [7]. Loss of potency usually results from a chemical changes [8,9].

Study show that 56% of the tested amoxicillin capsules formulations did not meet the United States Pharmacopoeia (USP) requirements for quality in some Arab countries [10].

The chemical stability of suspension may affect the potency of the products and inversely effect on its toxicity [8]. The decomposition of active ingredients in pharmaceutical dosage forms occurs through several pathway means, hydrolysis, oxidation-reduction, recemixation, decorboxylation, ring cleavage and photolysis [11].

Protection against the heat is only possible by storing products in cold storage room or refrigerators [12]. Clavulanice acid stability in aqueous solution is not good, but is optimal at pH 6.0 to 6.3 [13]. Stability of pharmaceutical products and of stability testing techniques is essential for many reasons [14].

The aim of this study is to evaluate the stability of six brands of Amoxicillin/clavulanate combination oral suspension reconstituted with distilled water and stored at different temperatures. Also to prove that the degradation follows first order kinetics.

EXPERIMENTAL SECTION

2.1.Materials

Oral suspension of amoxicillin trihydrate and potassium clavulanate includes, Augmex, Augmaclave and Augmen (Yemen), Neocalve and JuImentin (Emarate) and Augmentin (Britin). Amoxicillin trihydrate standard gift from Labopharm(Yemen), potassium clavulanate standard (Bio, Korea), Other materials used were of HPLC and analytical grade includes, methanol (BDH laboratory, England), sodium phosphate monobasic. HPLC, Auto sampler (PerkinElmer, USA), oven WT and incubator, (Binder, Germany), electronic balance, (Sartorius, Germany), Sonics, (Kerry, England), oven, (Gallenkamp (40C°) England).

2.2. Methods: Six bottles from each brands (Amoxicillin trihydrate with potassium clavulanate) were reconstituted with 50 ml distilled water stored at 25C° and at 40C° for seven days period is given in Table 1.

Table 1: Country of origin, Registration, batch number, manufacture day, expiry dates of six brands of amoxicillin trihydrate and clavulanic acid oral suspension present in the Yemen market

Brand Symbols	Country	Registration	Batch No	Manuf. Date	Expiry Date
A	Yemen	Yes	Not clear	2/2010	2/2012
B	Yemen	Yes	10306	8/2010	8/2012
C	Yemen	Yes	10464	11/2010	11/2012
D	Emarate	Yes	0003	12/2010	12/2012
E	Emarate	Yes	Not clear	6/2010	6/2012
F	Britin	Yes	518919	3/ 2011	3/2013

2.3. Assay and preparation of mobile phase: The mobile phase was prepared by mixing sodium phosphate buffer PH 4.4 [15] with methanol (95:5) and filter through 0.5um membrane filter. Stainless steel column 250x4.6mm contains C18, 5Mm packing material, flow rate 2ml/min., wave length 220nm, sample size 20M1 were prepared. Then 20ul of each standard preparation and test preparation were injected separately into the chromatograph, record the chromatograms and measuring the responses for the major peaks. Kinetic stability parameters were calculated include, zero, first and diffusion order with slope, intercept, k and t_{90}.

RESULTS AND DISCUSSION

The results of stability study show that the concentration of amoxicillin trihydrate in amoxicillin trihydrate with clavulanic acid for six brand A , B, C, D, E and F collected from the market at zero time were found within the pharmacopoeia limit [6] and when reconstituted with distilled and stored at different climatic conditions decreases gradually with time.

As illustrated in Table 1. The concentration of amoxicillin trihydrate in amoxicillin trihydrate with clavulanic acid, stored at 25C° for seven days in brand A, B. decrease gradually from 114% to 70.1% and 103% to 68.7% respectively and becomes out of pharmacopeial limit in the third day. Brand D decrease from 109% to 80.0% and become out of the limit in the fourth day. While brands C, E and F decreases from 117.8% to 95.91%, 114.2% to 94.33% and 113.8% to 89.13% respectively but remain within the pharmacopeial limits till the end of the study.

For those brands stored at 40C°, A, B and F the concentration decrease gradually from 102% to 58.2%, 103% to 52.6% and 110.1% to 59.2% respectively and become out of the pharmacopeial limit at second day, where brand C, D and E decrease from 105 % to 53.77%, 109 % to 60.2% and 106.4 %to 52.33% respectively and becomes out of the limit in the third day.

As shown in Table 2. The concentration of clavulanic acid in brands A, B, C, D, E and F stored at 25C° decrease gradually with the time and become out of the limit in the second day for A, B, D and E, where brand C and F in the

fourth and third day. While all brands stored at 40C° decrease gradually with the time and become out of the pharmacopial limit in the second day.

Table 2 : Effect of storage condition on the stability of Amoxicillin tihydrate in six brands of amoxicillin trihydrate and clavulanic acid oral suspension present in Yemen markets reconstituted with distilled water and stored at 25C° and 40C° for seven days

Storage	Brand names											
Time	A		B		C		D		E		F	
(Days)	25C°	40C°	25C°	40C°	25C°	40C°	25C°	40C°	25C°	40C°	25C°	40C°
0	114	102	103	103	117.8	105	109	109	114.2	110.1	113.8	106.4
1	99.5	91.4	95.3	91.5	117.3	99.58	98.3	98.2	112.9	87.76	111.6	96.68
2	69.1	77.2	90.8	84.6	114.6	90.17	96.1	90.8	110.6	89.9	108.2	83.95
3	85.2	73.7	85.7	78.5	111.2	78.55	94.8	85.3	106.4	78.88	105.3	70.41
4	80.0	68.9	80.9	72.7	105.4	69.41	87.5	82.1	103	73.11	100.3	64.13
5	77.2	66.0	76.0	70.1	102.2	63.45	83.6	79.2	99.79	67.37	95.73	59.92
6	73.3	61.5	71.3	60.9	98.48	56.91	81.3	72.6	96..04	61.88	91.27	54.34
7	70.1	58.2	68.7	52.6	95.91	53.77	80.0	60.2	94.33	59.2	89.13	52.33

As shown in Tables 3,4. The Kinetic reaction followed first order kinetics for all brands.

The t_{90} for amoxicillin trihydrate in amoxicillin trihydrate with clavulanic acid reconstituted with distilled water and stored at 25C° can be arranged in the following manner C > E >F > D > B > A. 4.93 > 4.69 > 3.72 > 3.21 > 2.39 > 2.13 days respectively .

For those stored at 40C° D > A > F > B > E > C. 1.91 > 1.83 > 1.67 > 1.6 > 1.53 > 1.35 days respectively.

Table 3 : Effect of storage condition on the stability of Clavulanic acid in six brands of amoxicillin tihydrate and clavulanic acid oral suspension present in Yemen markets reconstituted with distilled water and stored at 25°C and 40°C for seven days

Storage	Brand names											
Time	A		B		C		D		E		F	
(Days)	25C°	40C°	25C°	40C°	25C°	40C°	25C°	40C°	25C°	40C°	25C°	40C°
0	119	102	93.1	89.1	120	108	97.7	97.7	104.4	115.2	120.5	110.1
1	74.2	82.5	83.2	71.4	112.7	77.85	81.2	81.8	98.25	81.54	110.8	79.23
2	66.2	76.8	75.9	62.5	100.9	41.43	75.4	77.3	88.5	55.55	98.88	42.41
3	52.6	51.1	73.4	57.9	90.46	21.12	72.5	67.5	75.51	29.25	85.35	23.18
4	48.1	44.1	72.2	51.6	73.56	2.27	76..9	72.1	56.26	3.31	66.15	17.18
5	47.3	27.4	63.4	35.2	58.54	0	67.9	56.4	52.09	2.09	52.28	2.24
6	36.3	20.9	62.5	26.4	47.04	0	66.2	49.2	43.33	0	38.20	1.65
7	26.5	17.2	61.5	19.1	40.57	0	64.3	42.8	39.1	0	31.46	1.39

Table 4 : Kinetic study of Amoxicillin tihydrate in six brands of amoxicillin tihydrate and clavulanic acid oral suspension present in Yemen markets, reconstituted with distilled water and stored at 25°C and 40°C for seven days

Brand Names		CV%			Kinetic parameter			
	Temp	Zero	First	Diff.	Int.	Slope	K $(min)^{-1}$	T90(day)
A	**25°C**	4.91392	0.78892	264.385	2.02998	-0.028316	0.06521	2.1254
	40°C	6.28108	1.04261	137.663	1.98393	-0.03338	0.07687	1.803
B	**25°C**	1.5167	0.19226	165.37	2.00854	-0.025237	0.05812	2..3847
	40°C	3.01416	0.80153	244.975	2.00972	-0.038166	0.08789	1.5995
C	**25°C**	1.24224	0.28181	224.234	2.0804	-0.013907	0.03203	4.3279
	40°C	3.78896	0.6041	352.75	2.03143	-0.044751	0.10307	1.3447
D	**25°C**	2.8431	0.53993	123.444	2.02408	-0.01874	0.043135	3.2132
	40°C	3.7538	1.00539	259.442	2.03238	-0.031644	0.072876	1.91.1
E	**25°C**	0.83539	0.19188	179.128	2.06354	-0.012846	0.02957	4.687
	40°C	4.59206	0.61089	202.421	2.03082	-0.039407	0.09075	1.527
F	**25°C**	1.00779	0.25908	219.225	2.06327	-0.016199	0.037306	3.715
	40°C	17.4432	4.0308	795.12	2.0117	-0.038439	0.08853	1.5656

Usually the temperature has a pronounced effect on drug stability and a rise in temperature increase the frequency of collision of the reactants molecules and hence increase degradation, while a decrease in temperature reduce collision and reduce degradation [15] which confirm results obtain.

Only four brand out of six confirm with the pharmacopeia limit for those stored at 25C° while those store at 40C°fill, thus may be due to stabilizer additives or buffer system used in this suspension which may not be highly effective to stabilize the drug against the hydrolysis or maintains the pH. Metal ions especially Cu catalyses B

lactam of antibiotics by forming a complex with them and this complex is observed to be rapidly hydrolyzed into penicilloic acid and this cause the low stability of the suspensions [16].

A regression analysis for all brands in the study show that the correlation coefficient (r) between times and log concentration is not less than 0.9927 and the low CV% obtained confirms that, all brands of oral suspensions follows first order.

CONCLUSION

We can conclude that amoxicillin trihydrate with clavulanic acid suspension reconstituted with distilled water are affected by an increase in temperature. So it should be store below the room temperature.

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