Pharmacophore

ISSN-2229-5402

Journal home page: http://www.pharmacophorejournal.com

IMPACT OF SHELVES' STORAGE CONDITIONS ON DEGRADATION OF CO-AMOXICLAV

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ARTICLE INFO

Received:
22 March 2020
Received in revised form:
10 August 2020
Accepted:
11 Aug 2020
Available online:
28 Aug 2020

Keywords: Stability of Co-amoxiclav, pharmacies, medical stores, clavulanic acid, amoxicillin

ABSTRACT

Stability is the ability of a drug substance or drug product to persist within the established specifications, to sustain its physicochemical and pharmacological properties throughout its shelf life. Drugs can be degraded when exposed to conditions that are not suitable or labeled for it. Co-amoxiclav (amoxicillin-clavulanate potassium) samples of 7 brands (Amoxicillin: 125mg/5ml, Clavulanic Acid: 31.25mg/5ml) were evaluated to determine the parameters such as physical description (color, odor, and taste), pH, water content, and drug content analysis at days 0, 15, 30 and 45. The results showed that most of the variations were observed at day 45 of the storage of the drug product. It was concluded that the storage conditions at pharmacies/ medical stores are not fulfilling the regulatory guidelines which contributed to degrading the drug content of co-amoxiclav dry powder for suspension for oral utilization.

To Cite This Article: Saba Shaikh, Mirza Tasawer Baig, Shumaila Shaikh, Uzma Shahid, Ambreen Huma, Saira Shahnaz and *et al.*, (2020), "Impact of Shelves' Storage Conditions on Degradation of Co-Amoxiclav", ***Pharmacophore, 11(4),*** *65-70.*

Introduction

Stability [1-3] is the ability of a drug substance or drug product to persist within the established specifications [4], to sustain its physicochemical and pharmacological properties throughout its shelf life. Stability testing provides the information about quality of the drug and how it changes with the time associated with environmental factors (temperature, humidity, and light), also the type of packaging are the distinguishing parameters of the stability. Furthermore, stability studies support understanding the long-term effect(s) of the environment on drugs. Stability testing also provides information about the possible degradation of the drugs, their mechanisms of degradation, degradation pathways as well as the interaction between the drug and the excipients in the drug product [5]. Drugs can be degraded when exposed to conditions that are not suitable

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or labeled for it. According to U.S. Pharmacopeia's definition, the room temperature is taken in the range of 20 degrees Celsius to 25 degrees Celsius. Higher temperature and humidity quicken deterioration, not only during transportation from overseas but also in warehouses/pharmacies and at home [6]. A proper storage provision for a reconstituted antibiotic is defined as storing the medicines under refrigeration (2- 8°C). In Developing national homes even in rural areas, refrigerators may not be present or lack power supply, and even where there are refrigerator and power supply there may be inconsistent supply [7]. Antibiotic suspensions are taken for this study because reconstituted antibiotics usually require refrigeration; it may be difficult to meet all the resources in a limited environment. Studies in Iraq and Sudan had also revealed that antibiotics were the most frequently encountered drugs deposited and consumed by patients in their homes, especially the beta-lactam antibiotics of penicillin.

Drugs are chemicals that react to external stimuli such as microbial agents, humidity, heat, light, and dust. In many cases, such reactions can lead to physical modifications including discoloration of the drug product. In many other cases, the reaction may influence the drug more fatally resulting in the decrease or elimination of its efficiency and/or potency. There are cases of drugs that, when influenced, not only cause the failure of the drug to exert a beneficial therapeutic effect, but also lead to adverse impacts on the patient's health [8].

Amoxicillin (AMX) (Fig. 1) is β-lactam semisynthetic penicillin belonging to the aminopenicillin class with an extensive antibacterial spectrum, utilized to treat a large number of infections having susceptibility against Gram-negative and Gram-positive bacteria. It is one of the most frequently recommended penicillin derivatives within the class as, following oral administration, it is absorbed better than other β-lactam antibiotics. AMX is vulnerable to degradation by β-lactamase generating bacteria, which are resistant to a narrow spectrum of β-lactam antibiotics, such as natural penicillins. Consequently, it is regularly combined with clavulanic acid, a β-lactamase inhibitor [9].

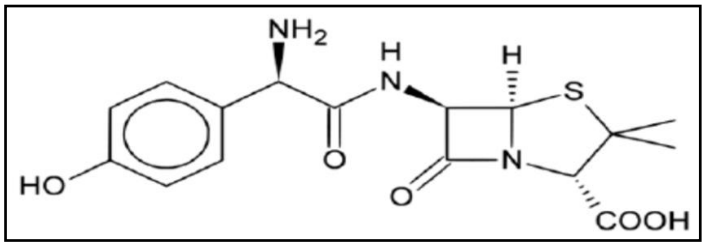

Figure 1: Structure of Amoxicillin [9].

Clavulanic acid (CLA) (Fig. 2) is an oxapenam derivative lacking the 6-acylamino side-chain feature for penicillin derivatives, which displays very weak antibacterial activity, consequently, is not suitable as an antibiotic. It is utilized combined with penicillin group antibiotics to overcome resistance to bacteria that produce β-lactamase. CLA can be defined as a "suicide inhibitor", covalently bonding to a serine residue in the active site of the β-lactamase. Combining these two drugs enhances efficacy by decreasing susceptibility to β-lactamase resistance. Amoxicillin–clavulanic acid (co-amoxiclav) [10] is a combination product containing the semisynthetic antibiotic amoxicillin (also spelled amoxycillin) with the beta-lactamase inhibitor clavulanic acid as a potassium salt. Clavulanic acid is a naturally existing beta-lactamase inhibitor acquired from *Streptomyces clavuligerus*. It contains a beta-lactam ring and the sulfur of the penicillin thiazolidine ring is substituted with oxygen to produce an oxazolidine ring. Clavulanic acid has weak intrinsic beta-lactam activity, but its clinical effectiveness is associated with its potent inhibition of many beta-lactamases and its capability to protect substrate drugs from hydrolysis [11].

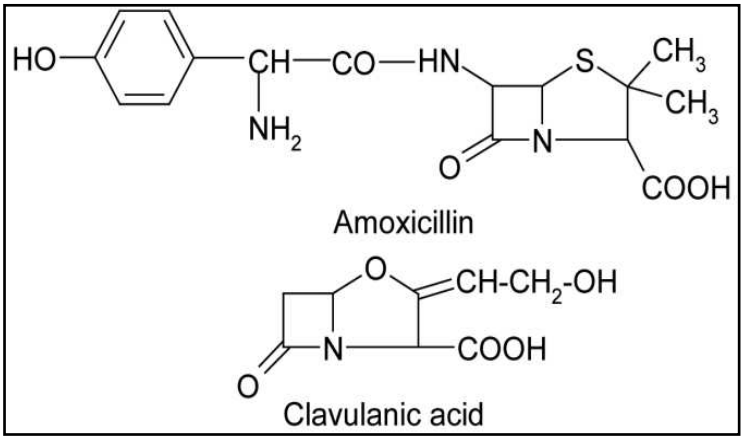

Figure 2: Chemical Structure of Amoxicillin and Clavulanic Acid [11].

A notable feature of the ß-lactam ring is its easiness of hydrolysis by aqueous acid, aqueous base, and enzymatic conditions. The central cause of antibiotic resistance to ß-lactam antibiotics rises from the irreversible hydrolysis of the amide bond in

the ß-lactam ring by ß-lactamase enzymes [12]. Additionally, amoxicillin oral formulations should be deposited in well-sealed bottles since high temperature and moisture in amoxicillin solid-state formulations (tablets, capsules, and powders) can result in the hydrolytic breakdown of the beta-lactam ring [13]. This study aims to assess the influence of unsuitable storage conditions on the drug content of Co-amoxiclav.

Materials and Methods

Co-amoxiclav (amoxicillin-clavulanate potassium) samples of Five National Pharmaceutical Manufacturers (NPM) and 2 Multinational Pharmaceutical Manufacturers (MPM) brands were bought from the Pharmacies/Medical Stores. Hence, 7 brands of co-amoxiclav (Amoxicillin: 125mg/5ml, Clavulanic Acid: 31.25mg/5ml) were evaluated (Table 1). All samples were in suspension form of pack size 60ml. Samples of seven brands of Co-amoxiclav (CLA) (156.25 mg/5ml) containing 125mg of amoxicillin (as amoxicillin trihydrate) and 31.25mg of clavulanic acid (as potassium clavulanate per five ml) for reconstitution in water for oral utilization were applied in this investigation. Five Pharmacies/Medical Stores from five divisions of Karachi were selected by convenient sampling, where room temperature wasn't maintained but were registered with the Health Department, Govt. of Sindh Pakistan. Twelve sample bottles of Co-amoxiclav dry suspension powder of each of the selected brands (within the labeled expiry date) were purchased. Only those products were purchased which were distributed to the pharmacy/medical store within 15 days of its manufacturing. The brands used were labeled as mentioned in table 1. All other chemicals used were of analytical grade. Three sample bottles of each brand were subjected to the determination of parameters such as physical description, pH, water content, and drug content analysis at day 0, while the remaining nine suspension samples (stored in their original container) of all the seven selected brands (NPM-A, NPM-B, NPM-C, NPM-D, NPM-E, MPM-A, and MPM-B) were requested to be stored in shelves of selected Pharmacies/Medical Stores, keeping a daily record of temperature and relative humidity of storage. It was made sure that ventilation and lighting were appropriate. Three sample bottles of suspension from all the seven selected brands were removed from the shelf after every 15 days and sent for the determination of parameters as were analyzed at day 0. In this study, it was made sure that the difference of the manufacturing date of selected brands of co-amoxiclav and sample purchasing date was within the limit of 15 days. Potassium Clavulanate and Amoxicillin Trihydrate United States Pharmacopeia (USP) reference standards were given as complimentary by Birds Chemotech. All other reagents used were of analytical grade (Merck, Germany).
Three samples (Co-amoxiclav dry suspension bottles of strength 156.25 mg/5ml) of one batch of each of the seven selected brands were taken and reconstituted with ultrapure water following the manufacturer's label instructions and the deliverable volume was measured according to the official method. Each sample bottle was physically examined concerning the taste, color, and smell of non-reconstituted and reconstituted samples. In both cases, the presence of clusters was evaluated. The determination of water content (≤11.0%) was carried out by Karl Fischer titration (USP29 method I). A pH meter (Mettler Toledo, China) was calibrated and utilized to measure in triplicate the pH values of each of the samples, made for the drug content determination at room temperature (25°C), confirming that they were within the official range established from 3.8-6.6 in suspension constitute [14].

Assay:

The separation of drugs was carried out utilizing HPLC (1525 HPLC, Waters, USA) with a UV detector and a symmetry C18 (250 x 6.4mm, 5µ m packing material) analytical column (Waters, USA). The flow rate utilized was two ml/min, the wavelength was 220nm, and the injection volume was twenty µl [15].

Standard preparation:

A precisely weighed quantity of amoxicillin trihydrate reference standard equivalent to about one hundred mg of amoxicillin anhydrous was utilized. A quantity of clavulanate Potassium reference standard equivalent to about twenty-five mg of clavulanic acid was transferred to two hundred ml volumetric flask. Standards were dissolved in distilled water and the sample volume was accustomed to two hundred ml [14].

Sample preparation:

A bottle of product for oral suspension was reconstituted as directed in the labeling. Five milliliters of product suspension was transferred into a 500 ml volumetric flask. The sample was dissolved with distilled water (sonication was used if necessary to complete dissolution), then the volume was adjusted to 500 ml. The solution was filtered through a suitable filter of 1µ m, and the filtrate was used. Calculations for the content of amoxicillin and clavulanic acid was carried out. A mixture of pH 4.4 sodium phosphate buffer and methanol (95:5) was prepared and filtered through a membrane filter of 0.5µ m pore size [14].

Determination of content of amoxicillin and clavulanic acid by HPLC

Amoxicillin content was determined for each sample of suspension reconstituted with ultrapure water by a validated high-performance liquid chromatography (HPLC) method verifying that it was within the official range established from 90% to 120% of the label claim (USP-41, 2018). Similarly, HPLC was used to determine the content of clavulanic acid with the help of HPLC.

Results and Discussion:

The effects of storage conditions of Pharmacies/Medical Stores oral suspension of Co-amoxiclav dry suspension (156.25 mg/5ml) have been depicted in tables 1 to 5. The results exhibited compliance of label at day zero which explains that the manufacturers of co-amoxiclav dry powder suspension for oral use are following the regulatory guidelines and USP monographs. However, the variations in the drug product after long placement at shelves in a pharmacy/medical store is due to improper storage conditions available for drug at pharmacies/medical stores. These factors along with improper use of antibiotics by self-medication [16] and overuse of antibiotics are the challenging tasks to overcome in developing countries like Pakistan [17]. The physical indicators showed variation in color at day 30 and day 45. It was surprising to observe in Tables 3 and 4, the variation in color in both of the multination brands (MPM-A and MPM-B). Out of 5 national brands, the color change was observed among two brands (Tables 3 and 5). Water content was found to be decreased by the passage of time among all the selected brands, which may be due to high temperature at the storage site but remained within the prescribed limit. The pH of the selected brands was found to be increased, maybe due to loss of water content and malfunctioning of the added buffer. The impact of improper storage of Co-amoxiclav on the drug content of Amoxicillin and Clavulanic Acid was greatly observed at day 45. Table 5 exhibited the loss of drug content beyond the lower limit of USP at day 45 among 3 national and both the multinational selected brands. This demonstrates that the climatic conditions can expose medications to risky temperatures that can possibly degrade the drug [18]. The key cause of degradation of amoxicillin is the reactivity of the strained lactam ring, for the most part to hydrolysis [19]. The course of the hydrolysis and the nature of the degradation products are affected by the pH of the solution. The temperature at pharmacies/medical stores influences the rate of deterioration: although the dry salts are stable at room temperature and do not need refrigeration, extended heating inactivates the penicillins [20].

Table 1: Determination of Physical Description, pH, and Content of Co-amoxiclav at Day Zero.

Sample Brand Code	Physical Description			pH	Water Content
	Color	Taste	Odor		
NPM-A	Yellowish White Powder	Sweet	Fruity	4.9 ± 2.2	7.8 ± 1.3
NPM-B	Yellowish White Powder	Sweet	Fruity	4.4 ± 1.7	8.2 ± 1.6
NPM-C	Yellowish White Powder	Sweet	Fruity	5.1 ± 3.7	7.2 ± 4.7
NPM-D	Yellowish White Powder	Sweet	Fruity	5.2 ± 1.5	6.9 ± 0.8
NPM-E	Yellowish White Powder	Sweet	Fruity	5.2 ± 2.8	5.9 ± 3.2
MPM-A	Yellowish White Powder	Sweet	Fruity	5.4 ± 1.6	5.2 ± 2.9
MPM-B	Yellowish White Powder	Sweet	Fruity	5.2 ± 6.3	6.9 ± 2.4

Table 2: Determination of Physical Description, pH, and Content of Co-amoxiclav at Day 15

Sample Brand Code	Physical Description			pH	Water Content
	Color	Taste	Odor		
NPM-A	Yellowish White Powder	Sweet	Fruity	5.2 ± 1.9	7.3 ± 2.2
NPM-B	Yellowish White Powder	Sweet	Fruity	4.7 ± 3.7	7.7 ± 5.7
NPM-C	Yellowish White Powder	Sweet	Fruity	5.5 ± 2.5	6.7 ± 3.1
NPM-D	Yellowish White Powder	Sweet	Fruity	5.5 ± 2.6	6.4 ± 1.5
NPM-E	Yellowish White Powder	Sweet	Fruity	5.6 ±4.2	5.4 ± 3.9
MPM-A	Yellowish White Powder	Sweet	Fruity	5.7 ± 1.3	4.8 ± 2.3
MPM-B	Yellowish White Powder	Sweet	Fruity	5.5 ± 2.9	6.4 ± 1.1

Table 3: Determination of Physical Description, pH, and Content of Co-amoxiclav at Day 30

Sample Brand Code	Physical Description			pH	Water Content
	Color	Taste	Odor		
NPM-A	Yellowish White	Less Sweet	Fruity	5.7 ± 1.8	6.6 ± 3.4
NPM-B	Yellowish White	Less Sweet	Fruity	5.1 ± 2.1	7.0 ± 0.4
NPM-C	Pale Yellow	Less Sweet	Fruity	6.0 ± 1.4	6.1 ± 2.2
NPM-D	Pale Yellow	Less Sweet	Fruity	6.0 ± 5.3	5.8 ± 6.1
NPM-E	Yellowish White	Less Sweet	Fruity	6.1 ± 1.6	5.0 ± 1.4
MPM-A	Pale Yellow	Less Sweet	Fruity	6.2 ± 0.9	4.4 ± 2.3
MPM-B	Pale Yellow	Less Sweet	Fruity	6.0 ± 1.3	5.8 ± 2.6

Table 4: Determination of Physical Description, pH, and Content of Co-amoxiclav at Day 45

Sample Brand	Physical Description	pH	Water

Code	Color	Taste	Odor		Content
NPM-A	Yellowish White	Less Sweet	Fruity	6.4 ± 6.3	5.8 ± 1.3
NPM-B	Yellowish White	Less Sweet	Fruity	5.8 ± 1.1	6.1 ± 0.5
NPM-C	Yellowish Brown	Slight Bitter	Less Fruity	6.8 ± 1.3	5.3 ± 5.7
NPM-D	Yellowish Brown	Slight Bitter	Less Fruity	6.8 ± 2.7	5.1 ± 1.1
NPM-E	Pale Yellow	Less Sweet	Fruity	6.9 ± 1.6	4.3 ± 2.4
MPM-A	Yellowish Brown	Slight Bitter	Less Fruity	7.1 ± 4.5	3.8 ± 0.7
MPM-B	Yellowish Brown	Slight Bitter	Less Fruity	6.8 ± 1.3	5.1 ± 2,2

Table 5: Assay of different co-amoxiclav products, n=3 (accepted limit: amoxicillin 90-120%; clavulanic acid 90-125%)

Product	Day 0		Day 15		Day 30		Day 45	
	Amoxicillin (%±SEM)	Clavulanic Acid (%)	Amoxicillin (%)	Clavulanic Acid (%)	Amoxicillin (%)	Clavulanic Acid (%)	Amoxicillin (%)	Clavulanic Acid (%)
NPM-A	120 ± 3.1	122.5 ± 3.5	115.2 ± 2.1	113.9 ± 5.1	109.4 ± 3.1	103.7 ± 4.7	101.8 ± 3.8	90.2 ± 2.3
NPM-B	105.4 ± 2.4	123.2 ± 3.2	101.2 ± 5.3	114.6 ± 1.8	96.1 ± 1.7	104.3 ± 2.2	89.4 ± 1.7	90.7 ± 1.6
NPM-C	107.2 ± 1.8	119. 3± 4.7	102.9 ± 1.3	110.9 ± 2.7	97.8 ± 1.1	101.0 ± 3.6	90.9 ± 2.9	87.8 ± 5.8
NPM-D	105.1 ± 6.3	118.5 ± 4.5	100.9 ± 1.7	110.2 ± 1.1	95.9 ± 1.9	100.3 ± 1.9	89.1 ± 6.2	87.2 ± 3.9
NPM-E	118.6 ± 1.4	123.7 ± 1.9	113.9 ± 2.4	115.0 ± 3.2	108.2 ± 2.8	104.7 ± 4,1	100.6 ± 1.7	91.1 ± 1.1
MPM-A	108.2 ± 1.5	119.8 ± 3.7	103.9 ± 1.6	111.4 ± 2.7	98.7 ± 5.1	101.4 ± 1.5	91.8 ± 4.2	88.2 ± 2.6
MPM-B	110.2 ± 2.9	118.8 ± 1.8	105.8 ± 2.8	110.5 ± 6.1	100.5 ± 3.6	100.5 ± 1.1	93.5 ± 1.5	87.5 ± 1.7

Conclusion:

It was concluded that the storage conditions at pharmacies/medical stores are not fulfilling the regulatory guidelines which contributed to degrading the drug content of co-amoxiclav dry powder for suspension for oral use.

Acknowledgments:

Birds Chemtech.

Author's contribution:
All authors contributed equally.

Conflict of interest:
The authors have no conflict of interest.

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