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Research Article

Stability Study Of Aspirin Tablet In Crush Condition Stored In Glass And Plastic Container By Using The Reverse Phase High Performance Liquid Chromatography.

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1. Abstract:
The objective of the present to research work is to provide the detailed analytical stability study of anti-inflammatory drug product i.e. Aspirin on its different physical condition as well as different storage container. Different condition i.e. crush condition in which the surface area of drug product increased and it can lead to increase the impurity levels of drug product. And by using the different storage container, compatibility of packaging materials can be evaluated. Aspirin is available in the form of tablet with aluminium wrapped blister pack. In this study, tablets were crushed and stored in wide mouth plastic container which kept in different stability stations (30°C/65%RH and 40°C/75%RH). The forced degradation study performed in acidic, alkali, oxidative, thermal and photolytic conditions to check its degradation impurity profile. Alkali degradation shows more than 50% degradation of Aspirin drug substance. The samples kept in plastic container at different stability time points were analysed by using the Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) as per Indian Pharmacopeia (IP). Based on the analytical data, the Assay of tablet found more than 95.0% while related impurities were found less than 1.0% as per specification of Indian Pharmacopeia. The data shows the molecule stability in crush condition and null impact of packaging material on crushed tablets.

Keywords: Aspirin, Stability study, Forced degradation, Anit-inflammatory, Liquid Chromatography

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2. Introduction:

Aspirin is from the category of salicylate drug known as Acetyl salicylic acid shown in figure-1. In 1897, Aspirin was invented by scientists of Bayer company. Aspirin is non prescribed over the counter drug available in different forms like pills, powder, oral gel and soluble tablets [13]. Hence, easily available at medial store. It is nonsteroidal anti-inflammatory drugs (NSAIDs) which is used during the fever, pain and inflammation [2]. Specific inflammatory conditions in which aspirin is used to treat the Kawasaki diseases, pericarditis and rheumatic fever [3]. The World Health Organization predicts that by 2030, the number of people who die each year from cardiovascular diseases—which include stroke and myocardial infarction will be close to 25 million [1,9]. The cyclooxygenase (COX; technically known as prostaglandin-endoperoxide synthase, PTGS) enzyme necessary for prostaglandin and thromboxane synthesis is irreversibly inactivated by aspirin, which prevents prostaglandin and thromboxane synthesis from

occurring. When an acetyl group is covalently linked to a serine residue in the COX enzyme's active region, aspirin functions as an acetylating agent (suicide inhibition). This distinguishes aspirin from other NSAIDs, which are reversible inhibitors, such ibuprofen and diclofenac.

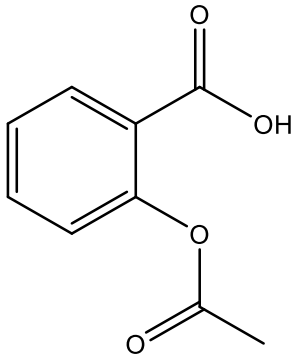

Figure 1. Acetyl salicylic acid

Using low-dose aspirin inhibits platelet aggregation for the duration of the afflicted platelet's existence (8–9 days) by permanently blocking the production of thromboxane A2 in platelets [2,3]. Aspirin's antithrombotic quality helps those who have experienced a heart attack, unstable angina, ischemic stroke, or transient ischemic attack to experience fewer heart attacks in the future [5,6]. A significant amount of the maximum thromboxane A2 release induced initially can be inhibited by 40 mg of aspirin per day, with minimal impact on prostaglandin I2 synthesis. For aspirin to further impede, higher dosages are required [6,7]. The body produces prostaglandins, which are local hormones that have a variety of functions, such as influencing inflammation, adjusting the hypothalamus thermostat, and sending pain signals to the brain. Blood clots are caused by platelets aggregating because of thromboxanes. Blood clots are the main cause of heart attacks, and low dosages of aspirin are thought to be a useful medicinal strategy to stop a recurrent acute myocardial infarction [2,3].

Aspirin acts on COX-1 and COX-2, two different kinds of cyclooxygenases. Aspirin alters the enzymatic activity of COX-2 and irreversibly suppresses COX-1 [2,3]. Prostanoids, the majority of which are proinflammatory, are generally produced by COX-2 [2,3]. The goal of the development of more recent NSAIDs, known as COX-2 inhibitors (coxibs), is to decrease the frequency of gastrointestinal adverse effects by selectively inhibiting COX-2. A few types of COX-2 inhibitors, like rofecoxib (Vioxx), have been withdrawn from the market after research showing that they raise the risk of stroke and heart attack [8,9]. It is suggested that COX-2 is expressed by endothelial cells that line the body's microvasculature. By directly suppressing COX-2, prostaglandin production (more precisely, PGI2; prostacyclin) is downregulated in relation to thromboxane levels, whereas COX-1 in platelets remains unaffected [9,10]. As a result, PGI2 loses its preventive anticoagulant function, which raises the risk of thrombus, heart attacks and other circulatory issues [2]. Once aspirin has irreversibly blocked the enzyme, platelets cannot synthesis new COX because they do not have DNA. This is a significant distinction from reversible inhibitors.

In addition, aspirin changes the activity of COX-2 from a prostaglandin-forming cyclooxygenase to an enzyme more akin to lipoxygenase [11]. This means that aspirin-treated COX-2 breaks down a range of polyunsaturated fatty acids into hydroperoxy products, which are subsequently broken down into specific proresolving mediators like aspirin-triggered lipoxins, aspirin-triggered resolvins, and aspirin-triggered maresins [10,11,12]. The anti-inflammatory properties of these mediators are strong. Aspirin is thought to have anti-inflammatory properties in part because it causes COX-2 to change from cyclooxygenase to lipoxygenase activity, which in turn leads to the creation of specific proresolving mediators [11,12]. Aspirin may lower the total chance of developing cancer as well as the risk of dying from it [14]. There is strong evidence that aspirin reduces the risk of colorectal cancer (CRC), however this effect requires aspirin use for at least 10–20 years [15]. Additionally, it might somewhat lower the risk of prostate and endometrial cancer [14,15]. Aspirin has shown anti-tumoral effects through inhibiting the PTTG1 gene, which is frequently overexpressed in malignancies [16]. An increasing body of research is also supporting the function of aspirin on bacterial and fungal biofilms [17].

3. Materials and Methods:

3.1 Reagents and Instruments:

Aspirin tablet (Label claim 325mg) purchased from medical store from X company. API of Aspirin and salicylic acid was provided by ACE Lab, Ankleshwar, Gujarat, India. Milli-Q-Water, Sodium dihydrogen ortho phosphate (AR Grade, make: S.D. Fine Chemicals), Orthophosphoric acid (HPLC grade, make: Merck), Acetonitrile (HPLC grade, make: Merck) and formic acid (LCMS grade, make: Sigma-Aldrich) were used for the analysis. The liquid chromatographic system was of waters (Alliance e2695) equipped with autosampler, UV and PDA detectors controlled by empower software.

3.2 Chromatographic conditions:

Buffer solution of 0.05M sodium dihydrogen orthophosphate prepared by adjusting the pH 2.0 with

orthophosphoric acid. Mobile phase was prepared by mixing the 25 volumes of acetonitrile and 75 volumes of buffer pH 2.0. USP (L1) column (250*4.6) mm, 5µ was used for analysis. Column flow set to 1.0mL per minute, 20µL injection volume and 280nm wavelength used to record the chromatograms.

Average Weight of 10 tablets: 450mg
Label Claim: 325mg of Aspirin

Test Solution: Transferred accurately weighed quantity of powdered tablets containing 0.3gm of Aspirin in 60mL of acetonitrile and 1mL of formic acid. Dispersed it about 15min and diluted to 100mL with acetonitrile. Shake well and filter the solution with 0.45µ syringe filter.

Standard Preparation: 0.0075% w/v solution of aspirin standard in 99 volumes of acetonitrile and 1 volume of formic acid.
Reference Solution (a): A 0.009% w/v solution of salicylic acid in a mixture of 99 volumes of acetonitrile and 1 volume of formic acid.
Reference Solution (b): A solution containing 0.3%w/v of aspirin standard and 0.009% w/v solution of salicylic acid in a mixture of 99 volumes of acetonitrile and 1 volume of formic acid.
The diluent chromatogram as figure 2 shows no interreferences at retention time of aspirin and salicylic acid peaks. The chromatogram of figure 3 shows the positive response of aspirin and chromatogram of figure 4 represents the well separation of aspirin with salicylic acid. Figure 5, chromatogram of test solution shows two unknown impurities at RT 8.55min (0.03%) and 9.64min (0.03%).

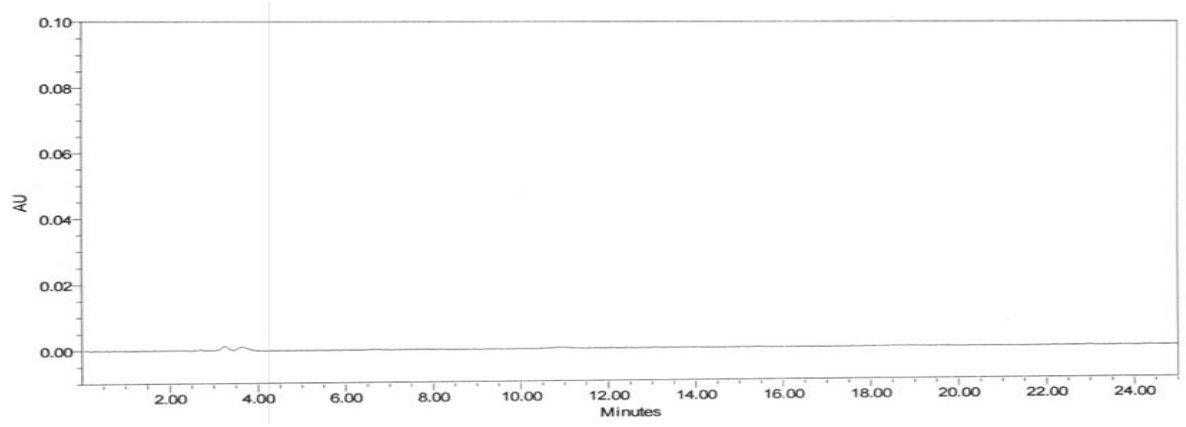

Figure 2. Typical Chromatogram of diluent

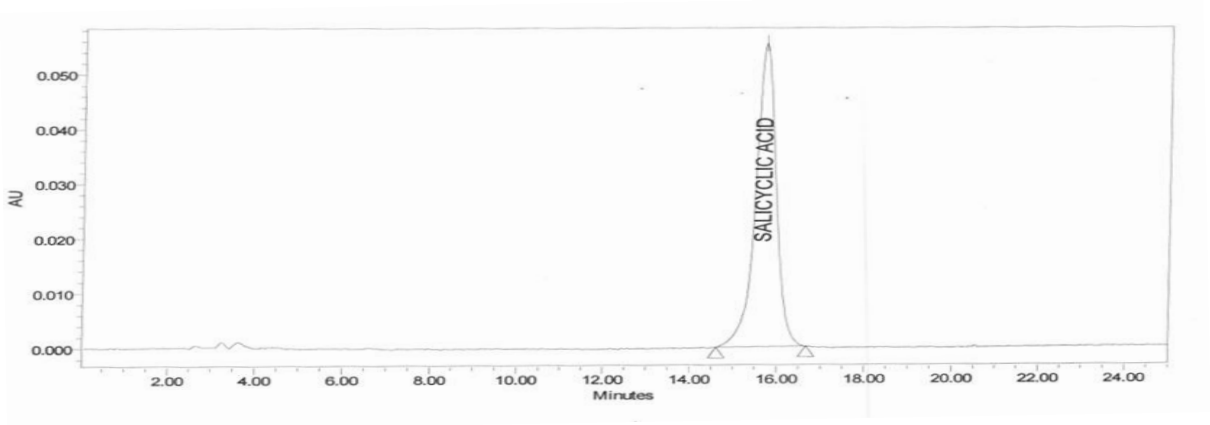

Figure 3. Typical Chromatogram of Reference Solution (a)

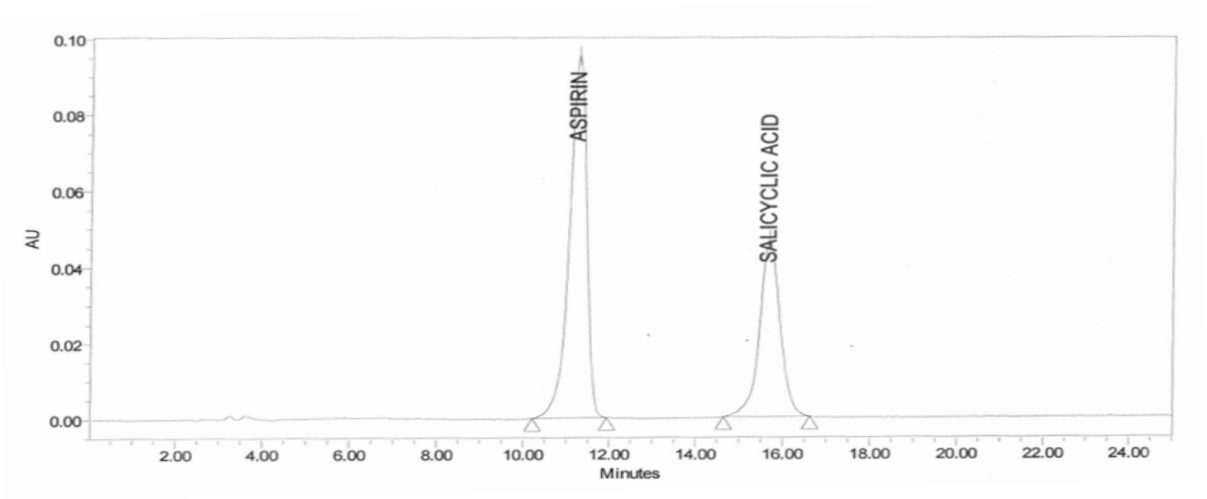

Figure 4. Typical Chromatogram of Reference Solution (b)

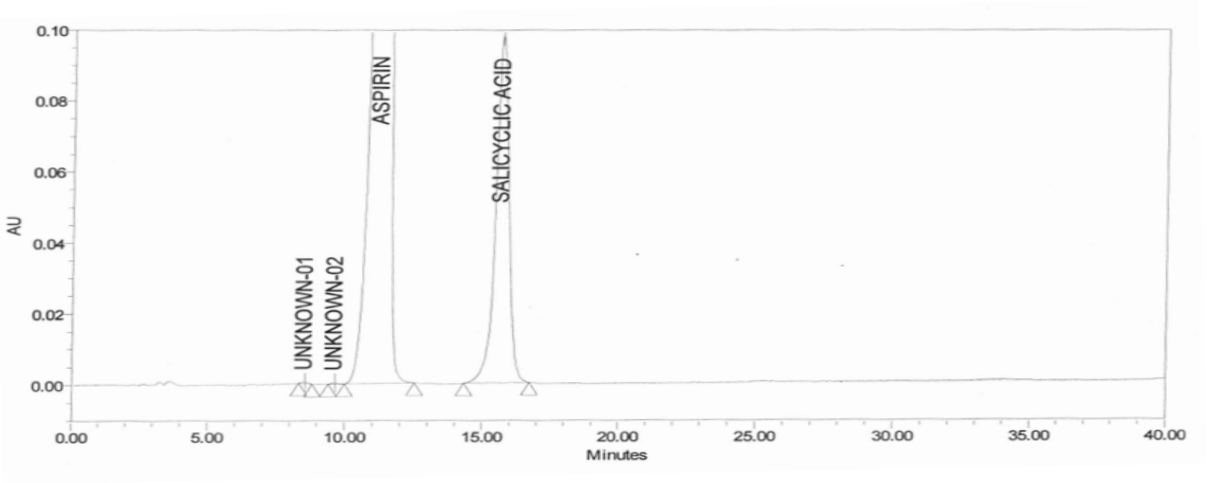

Figure 5. Typical Chromatogram of Test Solution

3.3 Forced Degradation:

In order to show the specificity of the stability indicating techniques and to reveal potential degrading impurities in the drug substance and drug products, forced degradation, or FD, is necessary. Studies on stress aid in the rapid generation of contaminants. Thus, formulation scientists are able to produce stable formulations more quickly. These days, FD studies are needed to complete the dossier and comprehend the drug development process in order to be accepted into globally controlled marketplaces. The inclusion of stress experiments involving acid and base hydrolysis, thermal degradation, photostability, and oxidation conditions is mandated by the ICH recommendations. Regarding the pH range, acid, base, and temperature concentrations, no particular regulation was given; instead, it can be evaluated depending on the chemical characteristics of the drug substance being used. According to ICH Q1(B) photostability study performed. A minimum of 1–10% drug substance the degradation should occur under stress. Not every stressful situation has to produce 10% of impurities. For a stressed sample to be utilized in the creation of stability signalling techniques, it must experience a minimum of 5% degradation under any given set of stress circumstances. Summary of degradation mentioned in table 1.

For the forced degradation 3.75mg/mL solution of drug substance was used.

3.3.1 Acidic degradation:

The drug was exposed with 5.0mL of 1M HCl at 60°C for 30minute. The drug degradation started with respective time and achieved degradation (72%) as per figure 6.

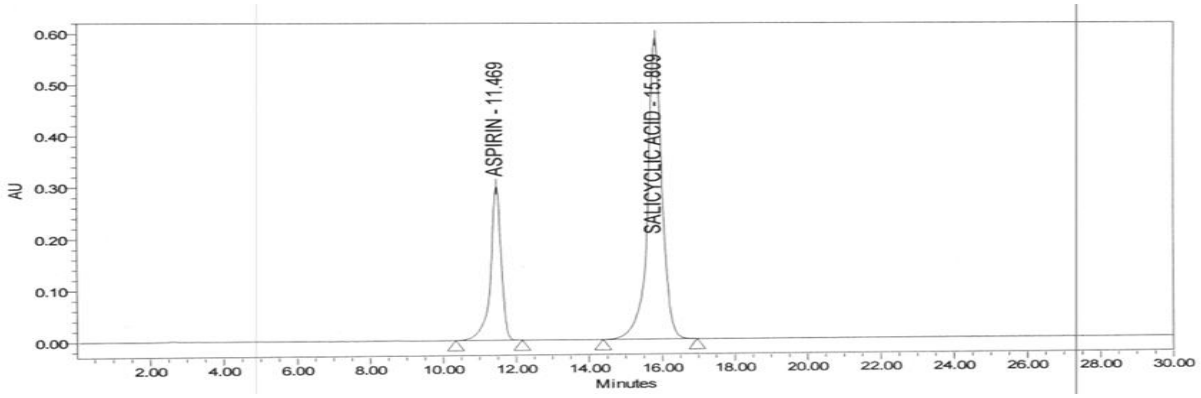

Figure 6. Typical Chromatogram of Acid Degradation

3.3.2 Alkali degradation:
The drug was exposed with 5.0mL of 1M NaOH at 60°C for 30minute. The drug degradation started with respective time and achieved degradation (95%) as per figure 7.

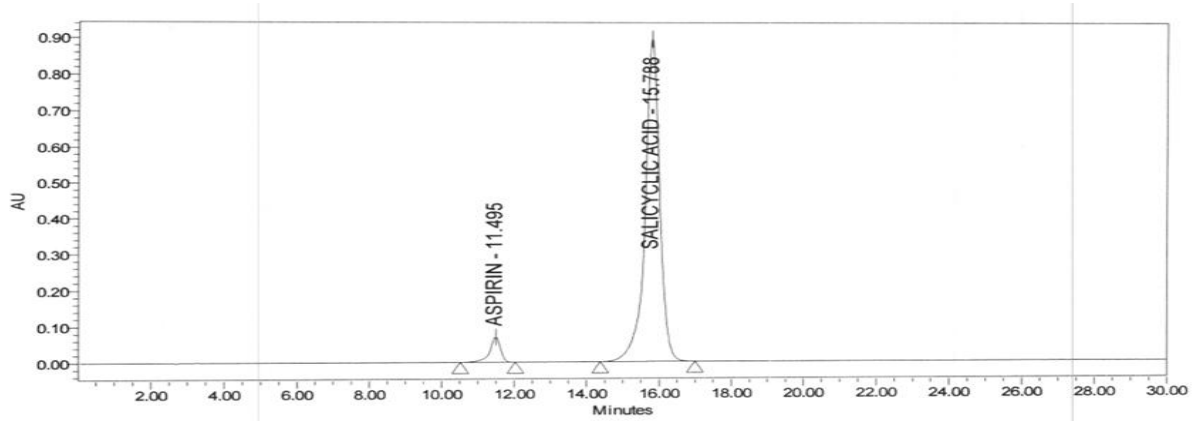

Figure 7. Typical Chromatogram of Alkali Degradation

3.3.3 Peroxide degradation: The drug was exposed with 5.0mL of 3%H2O2 at 60°C for 30min. The degradation started with respective time achieved degradation (61%) as per figure 8.

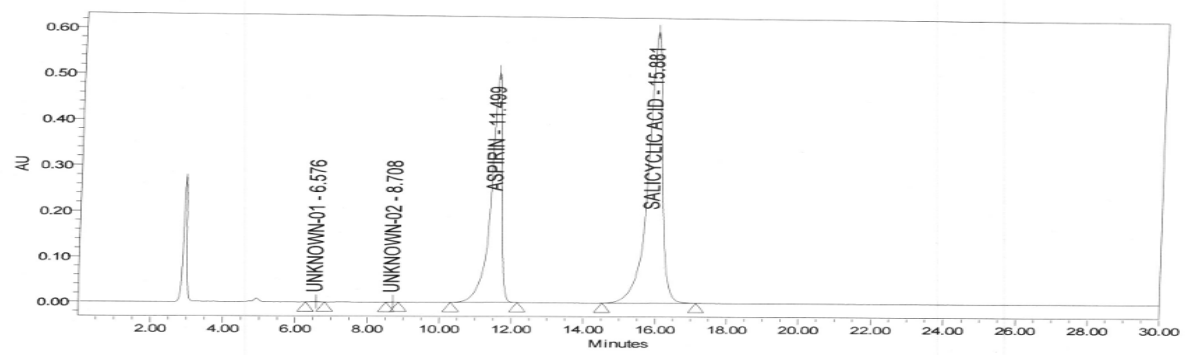

Figure 8. Typical Chromatogram of Peroxide Degradation

3.3.4 Thermal degradation:
The drug was exposed in chamber at 60°C ± 2°C, 75%RH ± 5%RH for 30min. The degradation achieved (11%) as per figure 9.

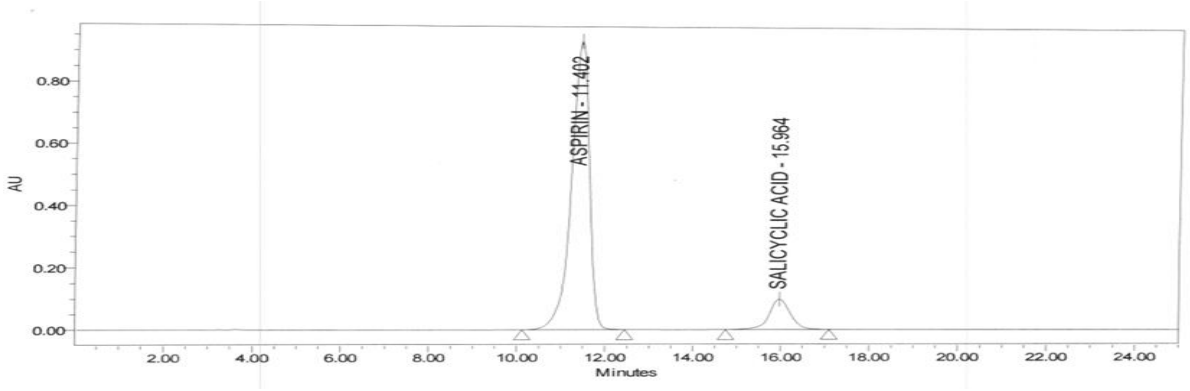

Figure 9. Typical Chromatogram of Thermal Degradation

3.3.5 Photolytic degradation:
The drug was exposed in chamber for 1.2 million lux hours. The degradation achieved (0.2%) as per figure 10.

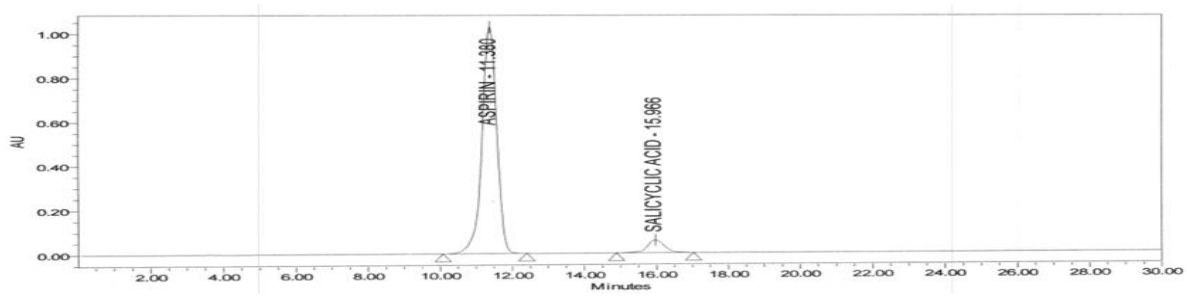

Figure 10. Typical Chromatogram of Photolytic Degradation

3.3.6 Neutral pH water degradation:
The drug was exposed with 5.0mL of neutral pH water at 60°C for 30min. The drug degradation started with respective time and achieved degradation (10%) as per figure 11.

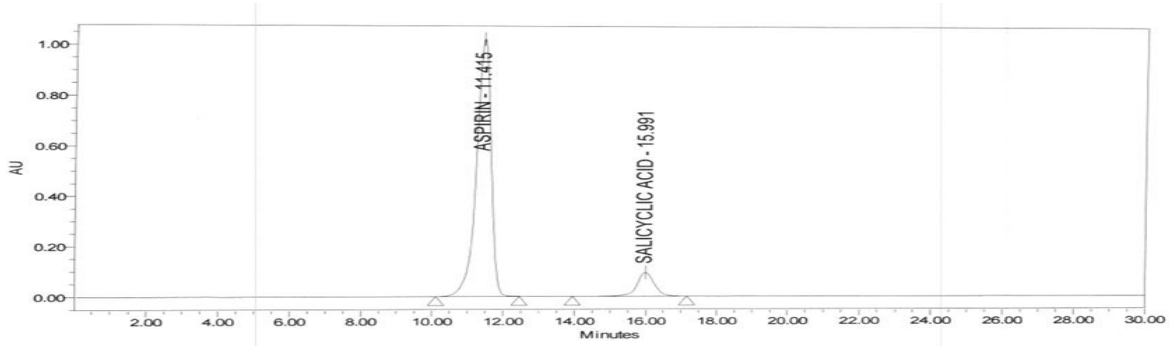

Figure 11. Typical Chromatogram of Neutral pH water Degradation

Table 1: Summary of degradation study

Stress Conditions	% Degradation	Purity angle	Purity threshold	Purity alert
Exposed with 1M HCl solution	72%	7.149	8.820	No
Exposed with 1M NaOH solution	95%	0.048	0.253	No
Exposed with 3% H2O2 solution	61%	3.108	7.763	No
Exposed at 60°C for 30min	11%	6.461	27.620	No
Exposed in chamber for 1.2 million lux hours	0.2%	6.321	25.150	No
Exposed with neutral pH water	10%	7.017	68.680	No

4. Stability Study:
Aspirin tablets (Label claim 325mg) removed from original blister packing and crushed it well using mortar pestle. Transferred quantitatively powdered of tablet in to plastic container and glass container (figure 12). Kept both containers into stability chambers at two different conditions i.e. 25°C ± 5°C, 65% RH ± 5% RH and 40°C ± 5°C, 75% RH ± 5% RH. This stability study of powdered drug product was performed to check the compatibility of storage container in different form of

drug product. Crushed form of drug product interacts with plastic and glass container which may generate the impurities. To quantify these impurities at different time points interval, samples were analysed for related substances by reverse phase high performance liquid chromatography and also performed assay test as per Indian Pharmacopeia 2018.

Figure 12. Glass and plastic containers

5. Additional study:

The crushed sample kept in glass container heated at 100°C in oven for 30min. Allowed to cool to room temperature. Performed assay and related substances test.

6. Result and discussion:

Samples kept in glass and plastic container were observed for physical appearance and it found opaque white powder. Stability study performed from initial, 3M (ACC & CRT), 6M (ACC & CRT) and 12M (CRT) for assay and related substances test by HPLC for glass and plastic containers. The results are shown as below table 2 and table 3. Additional study of high temperature performed which shows in the table 4.

Table 2: Observation for glass container

Sr. No.	Stability time points	Maximum unknown Impurity (NMT 0.5%)	Total Impurity (NMT 1.0%)	%Assay (95.0% to 105.0%)
1	Initial	0.03	0.06	99.94
2	3M (ACC)	0.09	0.11	99.53
3	3M (CRT)	0.07	0.11	99.85
4	6M (ACC)	0.12	0.18	99.24
5	6M (CRT)	0.11	0.13	99.43
6	12M (CRT)	0.15	0.22	99.25

Table 3: Observation for plastic container

Sr. No.	Stability time points	Maximum unknown Impurity (NMT 0.5%)	Total Impurity (NMT 1.0%)	%Assay (95.0% to 105.0%)
1	Initial	0.03	0.06	99.94
2	3M (ACC)	0.07	0.12	99.88
3	3M (CRT)	0.08	0.17	99.83
4	6M (ACC)	0.09	0.15	99.85
5	6M (CRT)	0.09	0.21	99.79
6	12M (CRT)	0.12	0.28	99.54

Table 4: Observation for sample kept at 100°C for 30min.

Sr. No.	Stability time points	Maximum unknown Impurity (NMT 0.5%)	Total Impurity (NMT 1.0%)	%Assay (95.0% to 105.0%)
1	Initial	0.03	0.06	99.94
2	100°C for 30 min	0.23	0.42	98.03

All the results found satisfactory results for both the assay and related substances test. Figure 13 shows the trend of maximum unknown impurity in glass as well as plastic containers up to 6M for both the ACC and CRT conditions. There is no significant change in the result of glass container and plastic container at 3M and 6M time points for both the conditions. While additional stress study performed at 100°C was also found less than 0.5% level.

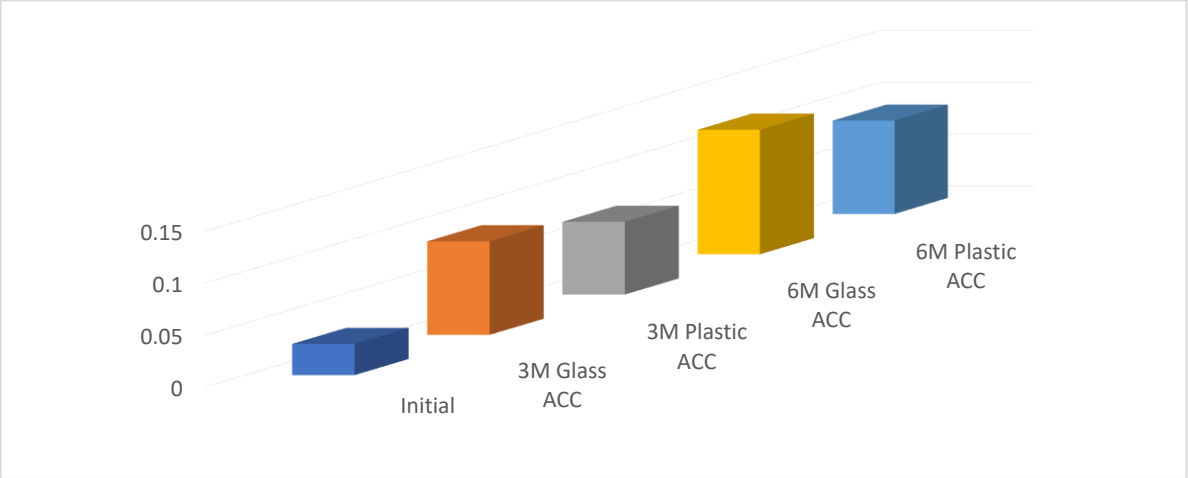

Figure 13. Trend of maximum unknown impurity

7. Conclusion:

In this current research article, all types of degradations were performed in which maximum degradation observed in the alkali degradation. Also, the impurity level observed in ACC conditions were found less than 1.0%. Stability study performed up to 12M and found satisfactory results. To support the stability data, additionally stress was study performed which also found less than 1.0%. Along with this impurity quantification, the assay test also performed and found between 95.0% and 105.0%. This study shows the storage compatibility of the aspirin crushed tablet in glass container as well as plastic container. Based on the data of crush condition, both the containers i.e. glass and plastic are suitable for the storage of Aspirin tablet.

8. Acknowledgement:

We wish to express our gratefulness to Dr. Manoj Singh Z. Nacrum Bio-Sciences, Makarpura GIDC, Vadodara-390010 for the research topic selection to the idea of analytical activities. We wish to acknowledge ACE Laboratories, Ankleshwar GIDC, Ankleshwasr-393002 to support of analytical activities.

9. Conflict of Interest:

The author has no conflict of interest in the presented research.

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