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RESEARCH ARTICLE

COMPARATIVE STUDIES ON THE CONTENT OF ACTIVE INGREDIENTS OF CONTAMINATED AND NON-CONTAMINATED ASPIRIN TABLETS SOLD IN PATENT MEDICINE STORES IN CALABAR

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ABSTRACT

A comparative assessment of the content of active ingredients of genuine and degraded expired and unexpired acetylsalicylic acid(Aspirin) was investigated using the high performance liquid chromatography (HPLC) method. The results obtained were compared with known standard references' peaks observed on the chromatograms. The results showed reduction in the concentration of Aspirin in samples contaminated when compared with the control. The concentration of Aspirin in samples contaminated by *Bacillus subtilis* (ASP) and *Staphylococcus aureus* (ASP_2) were 18.58µg/ml and 20.32ug/ml respectively, while the control was 34.13µg/ml. The peak area in the chromatogram for the control (ASPo) was 2430.07mAU^2, whereas that of ASP_3 was 30.44mAU^2. The ASP_3 was completely degraded to a new metabolite with a peak area of 290.32mAU^2 and a retention time of 3.40minutes. From the results, Aspirin was contaminated during storage and its active ingredients degraded by certain spoilage organisms present in the products. Good manufacturing practice and regular batch inspection should always be carried out on drugs. Most importantly, good storage conditions should be maintained for the safety of Aspirin.

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INTRODUCTION

Pharmaceutical products used in the prevention, treatment and diagnosis of disease; contain a wide variety of ingredients, often in quite complex physico-chemical states (Bloomfield *et al;* 1996, and Mugoyela and Nwambete; 2010). Such product must not only meet current pharmaceutical Good manufacturing practice (GMP) requirements for quality, safety, and efficacy, but also must be stable and sufficiently elegant to be acceptable to patients (Brooks *et al;* 2002 and Ghulam *et al*; 2008).

According to Hugo *et al;* (1992), Mendie *et al*; (1993) and later Aulton (2002), some products sometimes fail to meet high microbiological specifications such as sterile or for non-sterile drugs, a minimal microbial population at the time of product release. The formulation of an elegant, efficacious medicine which is both stable and acceptable to the patient may necessitate the use of a wide variety of ingredients in a complex physical state (Booth, 2001 and Mehdi *et al;* 2004).This condition has been shown to create conducive environment for survival and even extensive replication of contaminants, that may enter the product during manufacture or with use, by the patient or medical staff (Takon *et al* 2013). The consequences of such contamination may be serious and far-reaching on several accounts, particularly, if contaminants have had the opportunity to multiply to high levels (Brooks *et al*; 2002).

The metabolic versatility of microorganisms is such that any tablet ingredient from simple sugar to complex aromatic molecules may undergo chemical modification by suitable spoilage organisms. This constitutes a potential health hazard (Takon, *et al* 2013). When the product is spoilt it renders it unfit for use through chemical and physico-chemical deterioration of the formulation (Ghulam, *et al* 2008).

Aspirin or O-acetyl salicylic acid is an analgesic or antipyretic agent which contains not less than 99.5 percent and not more than 101.0percent of $C_9H_8O_4$, as active ingredient, calculated with reference to the dried substance. It is characteristically, colorless crystals or white, crystalline powder, odourless or almost odourless. It melts at about 143^{o}C. It is slightly soluble in water, soluble in 7 parts of ethanol (96%), or in 17 parts of chloroform and in 20 parts of ether (British Pharmacopoeia 1988). It is used typically in the treatment of aches and pains (Obuekwe *et al;* 2000).

Aspirin should be kept in an airtight container. It is stable in dry air but in the presence of moisture, aspirin had been shown to decompose by molecular hydrolysis to acetic acid and salicylic acid (Smith *et al;* 1983).

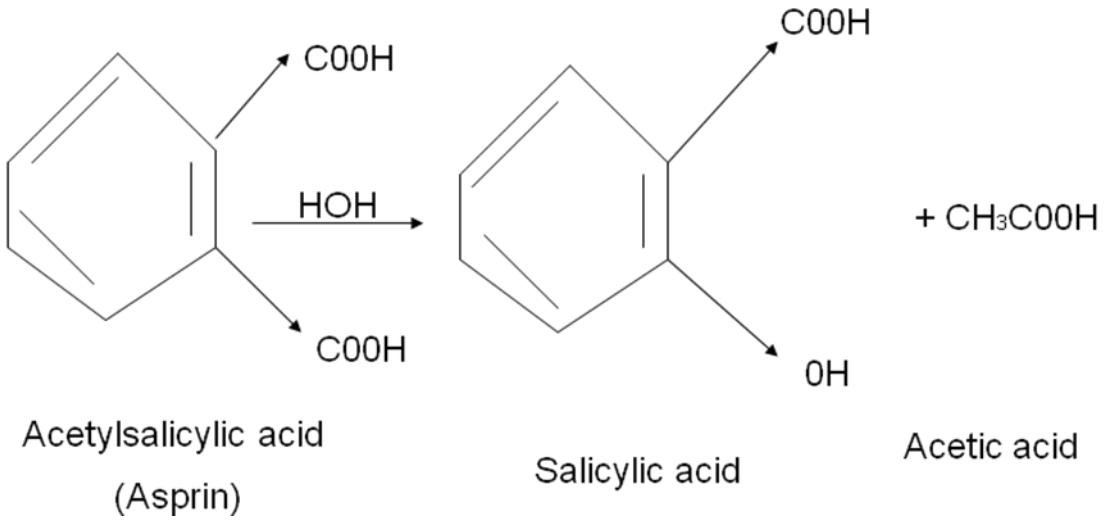

Fig. 1: Structure of Aspirin showing hydrolysis of Aspirin to salicylic acid and Acetic acid

Source: Smith *et al* (1983).

According to Mugoyela, V.and Mwambete K. (2010), Aspirin tablets stored under damp conditions supported growth of *Aspergillus niger* and utilized the active ingredient, as source of nutrient.

MATERIALS AND METHODS

SAMPLE COLLECTION

The aspirin or acetylsalicylic acid was obtained from some pharmacies and drug stores in Calabar, while the unexpired samples were bought from the same pharmacies and drug stores. The aspirin were of different trade name based on their manufacturers.

CHEMICAL REAGENTS USED

All chemical reagents employed in this study were of analytical grade and were products of Sigma chemical company, St. Louis, Missouri, USA and BDH Chemicals, Poole, England. They were of HPLC grade.

APPARATUS

High Performance liquid chromatography apparatus model Agilent 110 series with silica steel column (Coulter, USA), measuring 46mm x 25cm and 5µm ultrasphere ODS system, USA, was used for the analysis. It consists of a solvent delivery system, a means of introducing the sample, a chromatographic column and a detection and recording system.

METHOD:

High performance liquid chromatography is a method of separation in which the stationary phase is contained in a column, one end of which is attached to a source of pressurized liquid eluent (Mobile phase).

SOLUTION 1

This was the reference standard Aspirin solution prepared by dissolving 1.0g of acetylsalicylic acid (Sigma) in 25 flask containing 9.0ml ethanol (96%) - HPLC water mixture in the ratio of 1.9. The solution was sonicated in water bath for 3 minutes for proper mixing. Various concentrations were then prepared from the stock solution by dilution.

SOLUTION 2

A measured quantity (100µl) of contaminated Aspirin tablet solution was mixed with 1.0ml of ethanol (HPLC grade) and 9.0ml of HPLC water. The final volume was sonicated for 10 minutes and 3.0ml portion of the well-mixed solution filtered into micro centrifuge tubes, labeled and votex-mixed using ultratech 524 votex mixer for 5minutes. Non-contaminated Aspirin tablets solutions were treated in the same way and they served as control.

SOLUTION 3 (MOBILE PHASE)

The mobile phase was water ethanol mixture in the ratio of 80volumes of HPLC water to 20 volumes of ethanol. Twenty microlitres (20µl) of the different concentrations of the reference standard were injected in triplicates into the HPLC injection port and the amount of Aspirin determined at the detection wavelength of 254mm at a flow rate of 1.0ml per minute. Eluting peaks were plotted and quantified by the HP56 laser jet 1000 computer and HP laser jet 1200 printer. Average peak height, peak area, and retention time of the reference standard were recorded. A standard curve of average peak area versus concentration was plotted and the linearity (r) determined. The contents of Aspirin (X) in the contaminated and non-contaminated Aspirin tablets solutions were determined using the

Formula $Y = 65.821x$; where $r = 0.9990$.

This formula is derived from the equation of a straight line.

$Y = Mx + c$, based on the values of average peak area and concentration.

RESULTS

The result of the HPLC analysis of contaminated and non-contaminated Aspirin tablets is presented in table 3.1. There were reductions in the concentration of Aspirin (ASP) in the contaminated samples when compared with the control (ASPo). The concentrations of Aspirin (ASP) in samples contaminated by *Bacillus subtilis (*ASP_1) and *Staphylococcus aureus* (ASP_2) were 18.58 and 20.32µg/ml respectively, while the control had 34.13µg/ml of Asprin. A very small concentration 0.14µg/ml of Aspirin was recorded in the sample contaminated with *Aspergillus niger* (ASP_3) *Pseudomonas aeruginosa's* presence in sample (ASP_4) did not affect the concentration of Aspirin (34.06µg/ml) when compared with control.

Similarly, the differences in peak areas of contaminated and non-contaminated Aspirin tablets in the chromatogram are also reported in table 3.1. The average peak area of control (ASPo) was 2430.07mAU^2 whereas that of ASP_3 was 30.44. This sample ASP_3 was completely degraded to a new metabolite with peak area of 290.32 mAU^2 and a retention time of 3.40 minutes.

DISCUSSION, SUMMARY AND RECOMMENDATION

The comparative analysis of the active ingredients of contaminated and non-contaminated acetylsalicylic acid (Aspirin) has shown that most drugs were contaminated with spoilage organisms and a few pathogens such as *Bacillus subtilis, Pseudomonas aeruginosa, Staphyloccus aureus, Candida albicans and Aspergillus niger.* This is in agreement with the work of Obuekwe *et al;* (2000), who reported that Aspirin tablets stored in damp conditions supported growth of *Aspergillus niger,* which utilized the active ingredients, as a source of nutrient.

From the above studies, it could be inferred that biodeterioration of Aspirin was due in part to microbial utilization of active ingredients as substrate. The results from the chromatogram showed a steady drop in peak area size in Aspirin contaminated by *Pseudomonas aeruginosa*, Staphylococcus *aureus*, especially the one contaminated by *Aspergillus niger,* with a peak area of 30.44mAU^2 as compared with the control, 34.13mAU^2. It was degraded to a new metabolite. This result is in agreement with that obtained by Brooks *et al*; (2002), where it was observed that, some microorganisms were able to transform some drugs upon degradation to novel metabolite or even break these drugs to their primary constituents. These inferences raise health concern. It is therefore recommended, that amendments to existing product formulation standards be made to eliminate possible contaminants by creating non-supportive substrates as drugs ingredients. Good manufacturing practice and proper storage and dispensing methods should be encouraged to eliminate moisture which is the main problem of Aspirin deterioration and stability.

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TABLE 3.1 QUANTITATIVE HPLC ANALYSIS OF CONTAMINATED AND NON-CONTAMINATED ASPIRIN TABLETS (ACETYLSALICYLIC ACID)

Sample Code	Contaminating Microorganism	Average peak height (mAU)	Average peak area $(mAU)^2$	Concentration (µg/ml)	Retention Time (min)
ASPo	None	356.35	2430.07	34.12	3.02
ASP_1	*Bacillus subtilis*	218.70	1477.77	18.58	3.02
ASP_2	*Staphylococcus aureus*	230.64	2130.44	20.32	3.02
ASP_3	*Aspergillius niger*	4.58	30.44	0.14	3.40
ASP_4	*Pseudomonas aeruginosa*	342.55	1516.03	34.06	3.02
ASP_5	*Candida albicans*	318.40	2400.24	29.55	3.02

Legend:

ASPo = Control and non-contaminated Aspirin

ASP_1 = contaminated samples of Aspirin

Chromatograms of HPLC Analysis of Asprin(Acetylsalicylic Acid)