Comparative quantitative analysis of different brands of 300mg aspirin tablet marketed in Maiduguri metropolitan council

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ABSTRACT

In the last few years, aspirin has become a life saver against cardiovascular accident. This study was carried out to determine possible dose variations in the amount of active constituent (acetylsalicylic acid) in the various brands of aspirin drugs marketed in Maiduguri metropolitan council. In this study, ten different brands of 300mg aspirin tablet were randomly sampled from different pharmacy shops; identification test was performed on each sample to confirm the presence of the active drug (acetylsalicylic acid), and each of these samples was subjected to a rigorous quantitative analysis using HPLC and UV-spectrophotometer to determine the amount of the active drug. From the analysis, out of the ten brands of aspirin worked on, only Bond aspirin(41.7%) has less than 90% of active drug in UV-spectroscopy and Odesprin(112%) has more than 110% while Biopharma(99.77%), Kp(90%), Kunimed(100%), Maxprin(105%), Michelleaspirin(95%), Nemeprin(100%), Propon(93%) and Stop-eke aspirin(105%) passes the USP specification. In HPLC analysis, both Bond aspirin(41%) and Propon(57%) have less than 90% while Odesprin(112.6%) still has more than 110% and Biopharma(99.9%), Kp(95.5%), Kunimed(100%), Maxprin(105%), Michelle(95.5%), Nemeprin(100%), and Stop-eke aspirin(105%) passes the official specification. At the end of the study, it is convincingly seen that quantitative variation often exists among drugs of different product. However, despite the variation most drug products are within the official limit

Keywords: Asprin, HPLC, UV Spectrophotometer.

INTRODUCTION

Pharmaceuticals are an integral component of health care system world wide. Thus regulating weakness in the governance of pharmaceutical system can negatively impact health outcome especially in developing countries [1]. Nigeria is one of the number of countries whose pharmaceutical system has been impacted by corruption and has struggled to curtail the production and trafficking of substandard drugs [2]. In 2001 the national Agency for food and drug administration (NAFDAC) underwent organizational restructuring resulting in reforms to reduce counterfeit drugs and better regulate pharmaceutical [2].despite these changes there is still room for improvement.
The WHO defined counterfeit drugs as “medicine which is deliberately and fraudulently mislabeled with respect to identity and or source”. According to WHO, counterfeiting can apply to both branded and generic product and counterfeit product may includes product with correct ingredient or with wrong ingredients, without active ingredient or with fake packaging. (WHO department of essential drugs and other medicine)

Substandard drugs are genuine drug product which does not meet quality specification set for them. The term substandard is used to describe the quality status of genuine drug produced by legitimate manufacturer. Normally, manufacturers used specifications laid down by official pharmacopoeias such as British pharmacopoeia (BP), united state pharmacopoeia (USP) and European pharmacopoeia (EP) for each drug that they produced, if a drug fails to meet the pharmacopoeias specifications used for its formulations, The drug is classified as substandard [3]

There are also cloned drug with some quantity of active ingredient as the original drug. Cloning is hiding behind a fast moving registered product to rake up profit with out the associated liabilities and it is solely driven by financial motives. For Example panadol by GSK containing 500mg of Paracetamol powder was cloned by some criminals to contain the same 500mg paracetamol powder as original. What this frauds stars fails to understand is that minimal effective blood concentration which determines the efficacy of drug, is not only dependent on the quantity of active ingredients but also on the quality, excipient and formulation technique [3]

The big question however is who take responsibility when there are adverse effects of cloned drugs, Drugs without full name and address of the manufacturer expired drugs or drugs without expiry dates, toxic herbal preparation mixed with or orthodox medicine etc.?

As such, it is the responsibility of the pharmacist to study various brands of drug in his care before dispensing them to their patient.

Aspirin /Acetyl salicylic acid is a derivative of salicylic acid, is a derivative of salicylic acid that is a mild, non-narcotic analgesic usefully in the relief of headache and muscle and joint aches [4]

Salicylic acid was tough on stomach and a means of “buffering” the compound was searched for, the first person to do so was fredric Gerhardt. In 1853 Gerhardt neutralized salicylic acid by buffering it with sodium salicylate and acetyl chloride [5]. Since no structural theory existed at that time, Gerhardt called the compound he obtained “salicylic acetic anhydride” Gerhardt product worked but he had no desire to market it and abandoned his discovery. In 1859, Von Gilm obtained analytically pure acetyl salicylic acid (which he called acetylated salicylic acid) by a reaction of salicylic acid and acetyl chloride [6]. In 1869, Schroder, prinzhorn and kraut concluded that both reaction gave the same compound, “Acetyl salicylic acid.” they were the first to assign to it the correct structure with the acetyl group connected to the phenolic oxygen. [7]

In 1899, a German chemist named Felix Hoffmann, rediscovered Gerhardt’s formulary. Hoffmann made some of the formulae and gave it to his father who was suffering from the pain of arthritis, with good result. Hoffmann then convinced Bayer to market the new wonder drug. Aspirin was patented on February 27, 1900 [4].

The folk at Bayer came up with the name Aspirin, it comes from the “A” in Acetyl chloride, the “spir” in spiraea ulmaria (the plant from which salicylic acid was derived) and the “in” was a then familiar name ending for medicine [4].

Aspirin was first sold as a powder. In 1915, the first aspirin tablets were made. The popularity of aspirin grew over the first half of the 20th century, spurred by it supposed effectiveness in the wake of the Spanish flu pandemic [8].

Since its market introduction under the trade mark aspirin in the year 1899, it has profitably led to fierce competition and proliferation of aspirin brands and products, especially after the American patent held by Bayer expired in 1917 [9].

The popularity of aspirin declined after the market release of paracetamol in 1956 and ibuprofen in 1969.
CHEMICAL PROPERTIES
Aspirin, an acetyl derivative of salicylic acid, is a white crystalline, weakly acidic substance, with a melting point of 150°C (pharmaceutical codex 11th edition). Acetyl salicylic acid decomposed rapidly in solutions of ammonium acetate or of the acetates, carbonates citrates or hydroxides of metals [10].

Acetylsalicylic acid is stable in dry air but in contact with moisture with the great degrades by hydrolysis to acetic acid and salicylic acid. Hydrolysis is catalyzed by H⁺ and by OH⁻. Sodium ions can also catalyze hydrolysis.

In aqueous suspensions, then PH of maximum stability is 2-3. Aqueous Suspensions shows appreciable decomposition after storing for only a few days. (Pharmaceutical codex 11th edition)

Degradation in tablet is increased in the presence of stearates used as lubricants (Pharmaceutical codex 11th edition), the acid dissociation constant (Pka) for aspirin is 3.5 at 25°C (77°F). [10].

CHEMISTRY
Aspirin also known as acetylsalicylic acid has chemical formula of C₉H₈O₄ and a chemical structure as [4].

![Chemical Structure of Aspirin](image)

There are various salt of aspirin developed i.e. the Calcium or Aluminum Salt (Soluble aspirin) lysine acetyl salicylate (Aspegic) and acetylsalicylic acid sodium glycerol phosphate (ivepirine). Aspirin slowly hydrolyses in the presence of moisture into acetic acid and salicylic acid; Decomposition of aspirin is detected by the appearance of a violet color when the product is treated with ferric chlorides solutions, practically all salt of aspirin except those of calcium and aluminum are unstable for pharmaceutical use[11].

EXPERIMENTAL SECTION
Liquid Chromatography (LC) equipped with 280nm detector and 4.0mm X 30cm column packed with silica gel.

Plastic test tube
Distilled water

REAGENTS
Acetonitrile
Brand of 300mg of Aspirin tablet
Glacial acetic acid
Formic acid
Pure aspirin (USP) Tablet
Sodium -1-heptane sulfonate
0.1N HCL

IDENTIFICATION TEST
To 200mg of each of the powered drug sample, 4ml of dilute sodium hydroxide solutions was added, boil for 3minutes; cooled 5ml of dilute sulphuric acid was then added and filter; the residue, after washing with water and drying at 105°C melt at about 158°C. A portion of the residue was dissolved in water and ferric chloride solution was added; a purple colure was produced. This confirmed that all the brands contain the active drug (Acetyl salicylic acid).

The filtrate obtained produced an ethyl acetate odor on heating with 2ml of alcohol and 2 ml of sulphuric acid.
SAMPLE AND SAMPLE SIZE
Aspirin is used as a case study of this work; and the study is limited to ten different brands of aspirin [12].

SAMPLING METHOD
10 Different brands were randomly obtained from different pharmacy shop to ensure that each product or sample is obtained on the basis of chance [13].

UV METHODOLOGY
10 tablet of each brands of the 300mg aspirin were randomly weighed and there average weight was determined, 3 tablets from each of the brands were powered and an equivalents weight of 100mg aspirin tablet is weighed and dissolved in 100ml 0.1N GCL to give a solution equivalent to 1.0mg/ml, 1ml of this solution was pipetted and diluted with 99ml of 0.1N HCL to give a solution equivalent of 0.01 mg/ml.

The solution of each brand equivalent to 0.01mg/ml pure aspirin was then place in UV-spectrophotometer to determine their absorption at wave length of 229nm (E1%, 1cm= 434).

The Sample concentration, percentage content and the milligram content of each brand was obtained as followed.

\[ C = \frac{A}{E_L} \]

where
\[ C = \text{Sample Concentration} \]
\[ A = \text{Absorbance} \]
\[ E = \text{Molar Absorption} \]
\[ L = \text{Path Length} \]

Percentage content (%) = \( \frac{\text{Sample Conc.}}{\text{Working Conc.}} \) \times 100

Milligram content (mg) = \( \frac{\text{percentage content}}{100} \) \times 300mg

The USP 2007 official compendia of standard volume 2, stated that Aspirin Tablet should contain not less than 90.0% and not more than 110% of the labeled amount of aspirin (C9H8O4).

CHROMATOGRAPHIC SYSTEM
The LC is equipped with a 280nm detector and a 4.0mm X 30cm column packed with silica gel. The flow rate is about 1ml/min. The tailing factor is not greater than 2.0 and the relative standard deviation is not more than 2.0% [14].

HPLC METHODOLOGY
MOBILE PHASE PREPARATION
2g of sodium -1- heptane sulfonate was dissolved in a mixture of 550ml of water and 150ml of acetonitrile, and adjusted with glacial acetic acid to a pH of 3.4.

DILUTING SOLUTION
A mixture of acetonitrile and formic acid was prepared at a ratio of 99:1.

STANDARD PREPARATION
100mg of pure aspirin (USP aspirin) was accurately weighed and dissolved in diluting solution to obtain a solution having a known concentration of 0.01mg/ml.

SAMPLE PREPARATION
Not fewer than 10tablet of each brand is weighed and powered. An accurately weighed quantity of the powder equipment to about 100mg of aspirin tablet is then transferred into a 100ml volumetric flask, 20ml of diluting
solution was added and shaken for 10 minutes and centrifuge (Stock solution). 1ml of the stock solution is measured and diluted with 9ml of the diluting solution (Assay preparation) [13].

PROCEDURE

Equal volume (about 10uL) of the standard preparation and the sample preparation were separately injected into the chromatograph. The chromatogram is recorded and the major peak responses were measured.

The percentage content and the milligram content of aspirin in each brand are obtained as followed.

Percentage Content (%) = \frac{\text{Area peak of Sample}}{\text{Area peak of standard}} \times 100

Milligram Content (mg) = \frac{\text{Percentage content}}{100} \times 300mg

The USP 2007 official compendia of standard volume 2, stated that: Aspirin tablet should contains not less than 90.0% and not more than 110% of the labeled amount of Aspirin (C_9H_8O_4).

RESULTS

INFORMATION ABOUT THE SAMPLES

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>BRANDS</th>
<th>BATCH NUMBER</th>
<th>EXPIRY DATE</th>
<th>NAFDAC NUMBER</th>
<th>STRENGTH</th>
<th>MANUFACTURE ADDRESS</th>
<th>MANUFACTORY DATE</th>
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<td>Bond aspirin</td>
<td>1100a</td>
<td>04/14</td>
<td>04-1619</td>
<td>300mg</td>
<td>Adesakin layout, Awe, oyo state, Nigeria</td>
<td>04/2011</td>
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<tr>
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<td>Biopharma aspirin</td>
<td>A28AI</td>
<td>08/14</td>
<td>A4-2081</td>
<td>300mg</td>
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<td>09/2011</td>
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<td>KP aspirin</td>
<td>29AS</td>
<td>06/15</td>
<td>04-8441</td>
<td>300mg</td>
<td>Off KM 15 Enugu road Ogidi Anambra</td>
<td>07/2010</td>
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<tr>
<td>Kunimed pharmaceutical ltd</td>
<td>Kunimed aspirin</td>
<td>AK94</td>
<td>08/14</td>
<td>04-3231</td>
<td>300mg</td>
<td>1, Adelawa street, valley Estate, Dopemu, ikeja, lagos Nigeria</td>
<td>08/2011</td>
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<tr>
<td>Michelle laboratory ltd</td>
<td>Michelle aspirin</td>
<td>A134</td>
<td>11/13</td>
<td>A4-2081</td>
<td>300mg</td>
<td>Plot 23, Block 2, Thinkers corner industrial layout, Enugu, Nigeria</td>
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<tr>
<td>Charzmax pharmaceutical ltd</td>
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<td>CAS927</td>
<td>11/13</td>
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<td>Richy gold international ltd</td>
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<th>S/No</th>
<th>SAMPLE</th>
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<th>MAXIMUM WEIGHT</th>
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<td>350</td>
<td>336</td>
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<td>9</td>
<td>Propon</td>
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<td>608</td>
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<tr>
<td>10</td>
<td>Stop-eke</td>
<td>420</td>
<td>440</td>
<td>428</td>
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</table>
UV-SPECTROPHOTOMETRY CALCULATION OF PERCENTAGE AND MILLIGRAM CONTENT

**Bond Aspirin**
\[ \text{% content} = \frac{0.181 \times 100}{0.434} = 41.7\% \]
Mg content = 41.7×300mg = 125mg

**Biopharma Aspirin**
\[ \text{% content} = \frac{0.433 \times 100}{0.434} = 99.8\% \]
Mg content = 99.8×300mg = 299mg

**Kp Aspirin**
\[ \text{% content} = \frac{0.391 \times 100}{0.434} = 90\% \]
Mg content = 90×300mg = 270mg

**Kunimed Aspirin**
\[ \text{% content} = \frac{0.434 \times 100}{0.434} = 100\% \]
Mg content = 100×300mg = 300mg

**Maxiprin**
\[ \text{% content} = \frac{0.456 \times 100}{0.434} = 105\% \]
Mg content = 105×300mg = 315mg

**Nemepirin**
\[ \text{% content} = \frac{0.434 \times 100}{0.434} = 100\% \]
Mg content = 100×300mg = 300mg

**Odesprin**
\[ \text{% content} = \frac{0.486 \times 100}{0.434} = 125\% \]
Mg content = 125×300mg = 376mg

**Propon**
\[ \text{% content} = \frac{0.404 \times 100}{0.434} = 93\% \]
Mg content = 93×300mg = 279mg

**Stop-ek**
\[ \text{% content} = \frac{0.456 \times 100}{0.434} = 105\% \]
Mg content = 105×300mg = 315mg

**TABLE 3: SHOWING UV-SPECTROMETRY RESULT**

<table>
<thead>
<tr>
<th>SAMPLES</th>
<th>ABSORBANCE(A)</th>
<th>PERCENTAGE CONTENT (%)</th>
<th>MILLIGRAM CONTENT(Mg)</th>
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<tr>
<td>Bond aspirin</td>
<td>0.181</td>
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<td>Biopharma aspirin</td>
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<td>Kunimed</td>
<td>0.434</td>
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<td>300</td>
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<tr>
<td>Michelle aspirin</td>
<td>0.413</td>
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<td>Maxprin</td>
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<td>Nemepirin</td>
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<td>Propon</td>
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<tr>
<td>Stop-ek</td>
<td>0.456</td>
<td>105</td>
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HPLC GRAPHICAL PRESENTATION

Analyst: LAB MANAGER
Sample ID: ASPIRIN STD 0.05 151211  Vial: 200  Injection Volume: 20

**UV-VIS Results**

<table>
<thead>
<tr>
<th>Name</th>
<th>Retention Time</th>
<th>Area</th>
<th>Area Percent</th>
<th>Integration Codes</th>
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<tbody>
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<td>ASPIRIN</td>
<td>7.627</td>
<td>668702</td>
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<td></td>
<td>668702</td>
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</table>

Analyst: LAB MANAGER
Sample ID: BOND 0.05 151211  Vial: 190  Injection Volume: 20

**UV-VIS Results**

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<tr>
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### UV-VIS Results

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**Totals**

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### UV-VIS Results

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**Totals**

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**UV-VIS Results**

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**Analyst:** LAB MANAGER  
**Sample ID:** KUNIMED 0.05 151211  
**Volume:** 20

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**UV-VIS Results**

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<td>ASPIRIN</td>
<td>7.543</td>
<td>702661</td>
<td>100.000</td>
<td>MM</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td></td>
<td>702661</td>
<td>100.000</td>
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**Analyst:** LAB MANAGER  
**Sample ID:** MAXPRIN 0.05  
**Vial:** 197  
**Injection Volume:** 20
<table>
<thead>
<tr>
<th>UV-VIS Results</th>
<th>Name</th>
<th>Retention Time</th>
<th>Area</th>
<th>Area Percent</th>
<th>Integration Codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASPIRIN</td>
<td>7.567</td>
<td>638610</td>
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<tr>
<td><strong>Totals</strong></td>
<td></td>
<td>638610</td>
<td>100.00</td>
<td></td>
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</tr>
</tbody>
</table>

**Analyst:** LAB MANAGER  
**Sample ID:** MICHELLE ASPIRIN 0.05 151211  
**Vial:** 200  
**Injection Volume:** 20

---

<table>
<thead>
<tr>
<th>UV-VIS Results</th>
<th>Name</th>
<th>Retention Time</th>
<th>Area</th>
<th>Area Percent</th>
<th>Integration Codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASPIRIN</td>
<td>7.627</td>
<td>668702</td>
<td>100.00</td>
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<td></td>
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<tr>
<td><strong>Totals</strong></td>
<td></td>
<td>668702</td>
<td>100.00</td>
<td></td>
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</tbody>
</table>

**Analyst:** LAB MANAGER  
**Sample ID:** NEMEPHRIN 0.05 151211  
**Vial:** 200  
**Injection Volume:** 20

---
Analyst: LAB MANAGER
Sample ID: ODESPRIN 0.05151211  Vial: 140  Injection
Volume: 20

UV-VIS Results
Name | Retention Time | Area   | Area Percent | Integration Codes
---|----------------|--------|--------------|------------------
ASPIRIN | 7.557 | 753587 | 100.000 | MM

Totals: 753587 100.000

Analyst: LAB MANAGER
Sample ID: PROPON 0.05 151211  Vial: 170  Injection
Volume: 20

UV-VIS Results
Name | Retention Time | Area   | Area Percent | Integration Codes
---|----------------|--------|--------------|------------------
ASPIRIN | 7.543 | 383423 | 100.000 | MM

Totals: 383423 100.000
Analyst: LAB MANAGER  
Sample ID: STOP-EKE 0.05 151211  
Injection Volume: 20

<table>
<thead>
<tr>
<th>UV-VIS Results Name</th>
<th>Retention Time</th>
<th>Area</th>
<th>Area Percent</th>
<th>Integration Codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASPIRIN</td>
<td>7.310</td>
<td>708841</td>
<td>100.000</td>
<td>MM</td>
</tr>
</tbody>
</table>

| Totals              | 708841         | 100.000|              |                    |

HPLC CALCULATION

**Bond aspirin**

\[
\% \text{ content} = \frac{276863}{668702} \times 100 = 41.4\% \\
\text{Mg content} = \frac{41.5 \times 300}{100} = 124 \text{mg}
\]

**Biopharma aspirin**

\[
\% \text{ content} = \frac{668701}{668702} \times 100 = 99.9\% \\
\text{Mg content} = \frac{99.9 \times 300}{100} = 299.7 \text{mg}
\]

**Kp aspirin**

\[
\% \text{ content} = \frac{605036}{668702} \times 100 = 90.5\% \\
\text{Mg content} = \frac{90.5 \times 300}{100} = 271.5 \text{mg}
\]

**Kunimed aspirin**

\[
\% \text{ content} = \frac{668702}{668702} \times 100 = 100\% \\
\text{Mg content} = \frac{100 \times 300}{100} = 300 \text{mg}
\]

**Maxprin**

\[
\% \text{ content} = \frac{702661}{668702} \times 100 = 105\% \\
\text{Mg content} = \frac{105 \times 300}{100} = 315 \text{mg}
\]
Michelle aspirin
% content = $\frac{665358 \times 100}{668702} = 95.5\%$
Mg content= $\frac{95.5 \times 300}{100} = 286.5mg$

Nemeprin
% content = $\frac{668702 \times 100}{668702} = 100\%$
Mg content= $\frac{100 \times 300}{100} = 300mg$

Odesprin
% content = $\frac{753587 \times 100}{668702} = 112.6\%$
Mg content= $\frac{112.6 \times 300}{100} = 337.8mg$

Propon
% content = $\frac{383423 \times 100}{668702} = 57\%$
Mg content= $\frac{57 \times 300}{100} = 171mg$

Stop-eke
% content = $\frac{708841 \times 100}{668702} = 105\%$
Mg content= $\frac{105 \times 300}{100} = 315mg$

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>PEAK AREA</th>
<th>PERCENTAGE CONTENT (%)</th>
<th>MILLIGRAM CONTENT(mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bond aspirin</td>
<td>276863</td>
<td>41</td>
<td>124</td>
</tr>
<tr>
<td>Biopharm aspirin</td>
<td>668701</td>
<td>99.9</td>
<td>299.6</td>
</tr>
<tr>
<td>Kp aspirin</td>
<td>605036</td>
<td>95.5</td>
<td>271.5</td>
</tr>
<tr>
<td>Kupmed aspirin</td>
<td>668702</td>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td>Michelle aspirin</td>
<td>638610</td>
<td>95.5</td>
<td>286.5</td>
</tr>
<tr>
<td>Maxprin</td>
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<td>315</td>
</tr>
<tr>
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<tr>
<td>Odesprin</td>
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<td>337.8</td>
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<tr>
<td>Propon</td>
<td>383423</td>
<td>57</td>
<td>172</td>
</tr>
<tr>
<td>Stop-eke</td>
<td>702661</td>
<td>105</td>
<td>315</td>
</tr>
</tbody>
</table>

**DISCUSSION**

To ensure that drugs produce the require therapeutic effect as well as avoiding toxicities due to overdose, it is very important that the content of the drugs meet the specification limit as stated in the monograph

According to USP, the percentage content of aspirin should fall within the range of 90%-110%. Out of the ten brands of aspirin analyzed, only BOND(41.7%) aspirin has less than 90% of the active drug [acetylsalicylic acid] in UV-spectroscopy while ODESPRIN(112%) has more than 110%, and the remaining brands pass the USP specification. In HPLC analysis, both BOND aspirin (41%) and PROPON (57%) have less than 90% while ODESPRIN (112.6%) has more than 110% and the remaining brands pass USP specification.

The failure to meet the specification could be due to poor preparation techniques during formulation and subsequent manufacturing, incorrect weighing and incorrect storage condition.
In summary ten different product of 300mg aspirin tablet were randomly sampled from different pharmacy shop, identification test was perform to confirm the presence of aspirin (acetylsalicylic acid) in each sample and each of the sample were rigorously subjected to quantitative analysis using HPLC and UV-spectroscopy and the result were instantly recorded.

CONCLUSION

In conclusion quantitative variation often exists among drugs of different product and these variation can have significant influence on the drug activity; low level of active drug below the official recommendation often result in treatment failure, while high level of the active drug may predispose patient to drug toxicity.

However, despite the quantitative variation, most drug products are within the official specification.

REFERENCES

[5] (German) Gerhardt C (1853) “Untersuchungen Uberdie wasserferien und organischen säuren” Anna Len der chemie und pharmacie 18:149-179
[7] (German)SchrOder, Prinzhorn, Kraut K (1869)”Uber salicylverbindungen” Anna Len der chemie und pharmcie 150(1):1-20
[9] Bayer patent aspirin-History.com (This day in history-03/06/1899)