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Comparative Accuracy of Titrimetry and Spectroscopic Methods for the Quantitative Assay of Various Commercially Available acetaminophen Products in Nigeria

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Abstract: *Paracetamol (Acetaminophen) is an analgesic and antipyretic used in the treatment of pain and fever in adults and children. Titrimetric and UV spectrophotometric methods were employed to determine the weight of acetaminophen (per tablet and per 5ml of syrup) in nine (9) selected brands of acetaminophen, viz; Four (4) syrup brands (PS1- PS4), some of which were 125mg/5ml and others 120mg/5ml and Five (5) 500mg tablet Brands (PT1- PT5). Pure acetaminophen was purchased and used as standard to compare the results obtained. The titrimetric methods involved refluxing the acetaminophen in an acidic medium and then titrating against standard ammonium cerium (IV) sulphate solution. The UV spectrophotometric method involved the use of methanol: water (50:50 v/v) as the blank to prepare both the samples and the standards with their absorbance read from the spectrophotometer at a wavelength of 245nm. The titre values obtained from the titrimetric method were multiplied with a factor (7.56mg) since each ml of ammonium cerium (IV) sulphate is equivalent to 7.56mg of acetaminophen. In the UV spectrophotometric method, the absorbance obtained from the various concentration of standard were used to plot a calibration curve (Absorbance against Concentration) from which the concentration of the various samples were determined. The result of the analysis shows that the amount of acetaminophen in some of the brands investigated (syrups and tablets) was lower than the manufacturers claimed strength. The British pharmacopeia specifies that the content of acetaminophen in any pharmaceutical formulation should not be less than 95% and not more than 105% of the total acetaminophen content claimed by the manufacturer, also the United State pharmacopeia specified minimum content as 90% and maximum as 110%. The percentage content of acetaminophen in all nine samples analyzed using the UV method ranges from 87.5 to 102.5% and 89 to 99.79% using the titrimetry method. From the results obtained not all samples (formulation) passed the specification stated by both pharmacopeia, some of the brands went below both the B.P and U.S.P specifications. The content of acetaminophen in PT1, PT2, PT3 though below manufacturers claim of 500mg/tablet, passed both the British pharmacopeia and the United State pharmacopeia specification in both methods i.e. UV and titrimetry, PT5 went slightly above manufacturers claimed strength but fell within the specification by both pharmacopeias, PT4 fell below manufacturers claimed strength and did not pass the specification by both pharmacopeia. The content of acetaminophen per 5ml of syrup in PS1 is approximately the same as the manufacturers claim of 125mg/5ml, the result for the sample PS2 was below the manufacturer claim of 120mg/5ml but passed both pharmacopeia's specification, the results for the sample PS3 is below the manufacturers claim (125mg/5ml), PS3 passed the specification by the United State pharmacopeia but did not pass the specification by the British pharmacopeia. The results obtained for the acetaminophen content of sample PS4 is more than the manufacturers claim although it passed the specification of both pharmacopeias. Although the similarity in the results obtained for both methods indicates both methods can be used alternatively for routine analysis of drugs containing acetaminophen, but the UV method proved more simple, rapid, economic, and reproducible with values that better estimate the acetaminophen content of the different formulations.*

1. INTRODUCTION

1.1. Background to Study

Acetaminophen is part of the drugs known as aniline analgesic. It is the only such drugs still in use today (Bertolinia et al., 2006). Acetaminophen or N-(4-Hydroxyphenyl)-acetamide is one of the most popular over-the-counter analgesic and antipyretic drugs. It is the most widely used analgesic and antipyretic (Budavaris, 1996) The first observations about the analgesic and antipyretic properties of

acetaminophen were made back in the late nineteenth century when alternative compounds were being sought to reduce fever in the treatment of infections.

Acetaminophen is available in different dosage form: tablet, capsules, drops, elixirs, suspension and suppositories. Dosage form of acetaminophen and its combinations with other drugs have been listed in various pharmacopoeias. Acetaminophen is an odourless, white crystalline powder with a bitter taste, 4-hydroxyacetanilide or N-acetyl-p-aminophenol and in the US Pharmacopoeia it is known as acetaminophen as shown in figure (1.1). It is soluble in 70 parts of water, 7 parts of alcohol (95%), 13 parts of acetone, 40 parts of glycerol, 9 parts of propylene glycol, 50 parts of chloroform or 10 parts of methyl alcohol. It is also soluble in solutions of alkali hydroxides. It is insoluble in benzene and ether. A saturated aqueous solution of acetaminophen has a pH of about 6 and is stable (half-life over 20 years) but stability decreases in acid or alkaline conditions, the acetaminophen being slowly broken down into acetic acid.

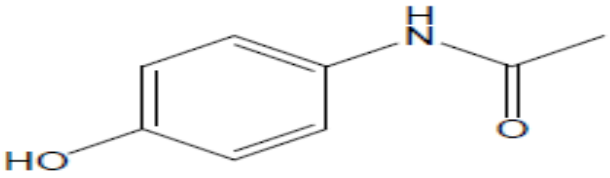


Figure1.1. *Chemical structure of Acetaminophen*

Acetaminophen is 4-acetamidophenol and can be represented by the following formula ($C_8H_9NO_2$), with a molecular weight (151.2), pKa (9.5). A number of papers in literature have described the assay of Acetaminophen and its combination in pharmaceuticals or biological fluids. Determination of Acetaminophen using electrical method has been reported, UV-Spectrophotometry, titration method, high performance liquid chromatography (HPLC) e.t.c.

In present study a qualitative assay for acetaminophen from different manufacturing sources in NIGERIAN market was investigated. Acetaminophen gives relief for all kinds of mild to moderate pain including headache and rheumatic pain (Martin, 1996), it is also used in bringing down high temperature associated with some illness, since the discovery of the drug in the late 19th century (Royal Society of Chemistry, 2004), constant use has registered a permanent acceptance of the drug in the mind of dispensers and users. Over the years it has been available in different forms to suit the need of the various age groups, for example pediatrics oral suspension for children, tablets/capsules for adults and also in combination with other drugs .

Many method has been used for the estimation of this compound in pharmaceutical preparation; some include titrimetric (Blake and Shumaker, 1973), chromatography (Caroll et al, 1981), flourimetry (Oztune, 1982) and various modes of electrochemistry (Ozkan et al , 2003). Methods adopting less complicated equipment are however used in this part of the world. The analysis of acetaminophen in syrups and tablets should fall within a specified range of 95-105% (British Pharmacopeia, 2000). In the standard method acetaminophen is determined titrimetrically with Ce(iv) in acidic medium, using ferroin as indicator. The titration is performed in cold condition and hence the estimation takes longer time with limited accuracy (British Pharmacopeia, 1999) hence a quicker and accurate method is needed.

There are many spectrophotometry methods of determining acetaminophen contents in drugs formulation especially tablets, some of which are based on hydrolysis of acetaminophen to p-aminophenol and the latter is reacted with specific reagent to produce coloured substances and the absorbance of which is measured in visible region at appropriate wavelength (Xu and Li, 2004).

Ultraviolet spectrophotometer procedure have been adopted by the British Pharmacopeia and US national formulary XI for the determination of acetaminophen in tablets. Although the official ultraviolet spectrophotometer assay for acetaminophen is fast and simple, its accuracy is greatly influenced by active substance or common excipients such as diluents and binders in tablets or colouring matter, sweetening agents and preservatives in syrups and drops. For the determination of acetaminophen in multi-component pharmaceutical preparation , computer controlled instrumentation and multivariant calibration methods are playing a very important role (Mot et al., 2010).

Various colour reaction have been proposed for the determination of acetaminophen , including indophenol dye and schiff's base formulation, nitrosation and subsequent chelation, oxidation, oxidative coupling. Most of these method require lengthy treatment and lack the simplicity needed for routine analysis.

The majority of published spectrophotometric method are based on the preliminary hydrolysis of acetaminophen to P-aminophenol and coupling of the latter with various phenolic reagents (Cekic et al., 2005). Although these methods are rather selective but both procedures are slow. The hydrolysis of acetaminophen in acid solution is completed after 0.5-2 hours of boiling. In addition, coupling reaction is also further slowing down the determination and needing additional 10-15 minutes for finishing. Reaction for the formation of schiff's base is fast but again, preliminary long hydrolysis stage is necessary (Mohammed Khairand Al-Shwaiyat, 2013).

Figure1.2. *acetaminophen capsule and tablet pack*

Acetaminophen is available in tablet, capsule, liquid suspension, suppository, intravenous, intramuscular and effervescent form. The common adult dose 1000mg. the recommended maximum daily dose for adults is 4000mg. in recommended doses, acetaminophen is generally safe for children and infants as well as for adults, (Montvale and Thompson, 2009) although rare cases of acute liver injury have been linked to amounts lower than 2500mg per day (Charles et al., 2009)

1.2. Chemistry of Acetaminophen

Table1.2.1. *General properties*

1	**Molecular formulae**	C8H9NO2	Encyclopedia of chemical, drugs and Biologicals, 2006
2	**Molecular weight**	151.16 g/mol	Encyclopedia of chemical, drugs and Biologicals, 2001
3	**Colour/form**	White crystalline powder/Large monoclinic prism form	Encyclopedia of chemical, drugs and Biologicals, 2001
4	**Odour**	Odourless	Harley's Condensed chemical Dictionary, 2001
5	**Taste**	Slightly bitter	Harley's Condensed chemical Dictionary, 2001
6	**Melting point**	169-170.5 °c	Encyclopedia of chemical, drugs and Biologicals, 2001
7	**Density/specific gravity**	1.263g/cm3 / 1.293 at 21°c	An encyclopedia of chemical Drugs and Biologicals, 2001
8	**Dissociation**	PKA= 9.5	Dastmalchi et al., 1995

	constant		
9	**PH**	Saturated aqueous solution 5.5-6.5	Harley's Condensed chemical Dictionary, 2001
10	**Solubility**	Slightly soluble in cold water (7.21g/kg of water at 0°c, 8.21g/kg of water at 5°c, 10.97g/kg of water at 15°c), considerably more soluble in hot water (12.78g/kg of water at 20°c), soluble in methanol , ethanol, acetone, ethyl acetate, practically insoluble in petroleum ether, pentane and benzene.	An encyclopedia of chemical Drugs and Biologicals, 2001
11	**Vapour pressure**	6.29x10-5 mmHg at 25°c	Taylor and Francis, 1989

1.2.1. Structure and Reactivity

Paracetmol consists of a benzene ring core, substituted by one hydroxyl group and the nitrogen atom of an amide group in the para 1,4 pattern. (Bales et al., 1985). The amide group is acetamide (ethan amide). It is an extensive conjugated system, as a lone pair, the P orbital on the carbonyl carbon and the

lone pair on the carbonyl oxygen is all conjugated. The presence of two (2) activating groups also makes the benzene ring highly reactive towards electrophilic aromatic substitution. As the substituents' are -ortho-, para- directing and para with respect to each other, all position on the ring are more or less equally activated. The conjugation also greatly reduces the basicity of the oxygen and the nitrogen, while making the hydroxyl acidic through delocalization of charge developed on the peroxide anion.

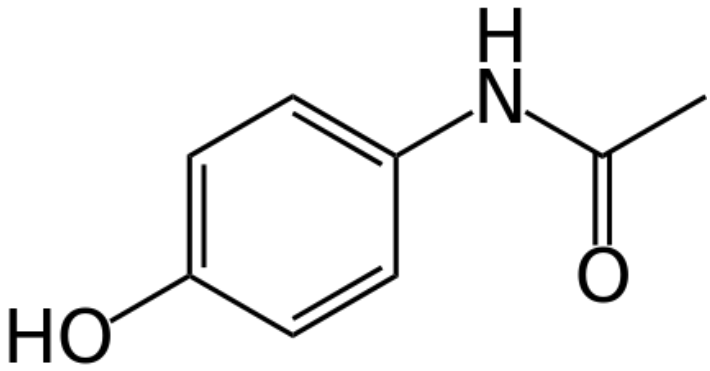


Figure1.3. *Acetaminophen structure*

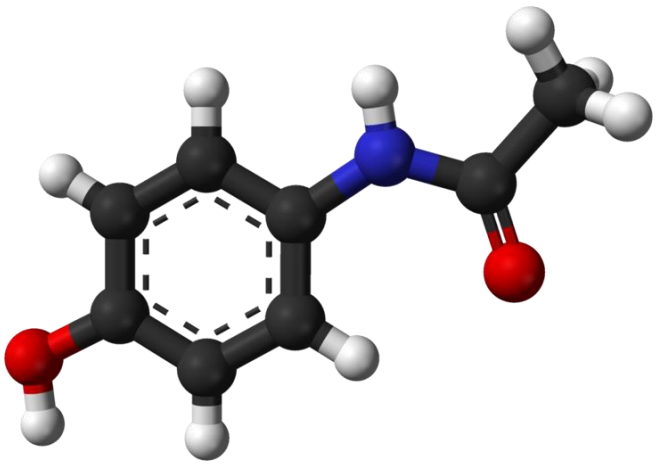


Figure1.4. *(Ball-and-stick model of the acetaminophen molecule, C8H9NO2. X-ray crystallographic data from A. Parkin, S. Parsons and C. R. Pulham (December 2002)*

1.3. Clinical Data

Trade Names:

Tylenol, Anacin, Aspirin free, Apra, Feverall, Genapap (USA), Crocin, Aeknil (india), Panadol (UK, Australia and New Zealand), Panodil (denmark), Efferalgan, Doliprane, Dafalgan (France), Alvedon (Sweden). (Wikipedia the free encyclopedia)

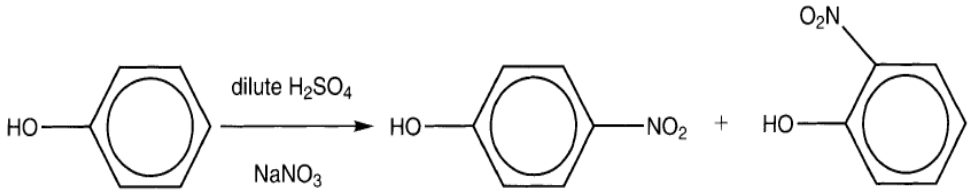
The amide group is acetamide (ethan amide). The acetaminophen molecule above is an extensively conjugated system, as the lobe pair of the hydroxyl oxygen, the benzene pie-electron cloud, the nitrogen lone pair, the p-orbital on the carbonyl carbon and the lone pair on the carbonyl oxygen are all conjugated and the presence of two activating groups (OH, $NHCOCH_3$) also makes the benzene ring highly reactive towards electrophilic aromatic substitution. As the substitution are ortho-para directing all position on the ring are more or less equally activated. The conjugation greatly also reduces the basicity of the oxygen and the nitrogen while making the hydroxyl (OH) group acidic through delocalization of the charge developed on the peroxide anion.

1.4. Reactions

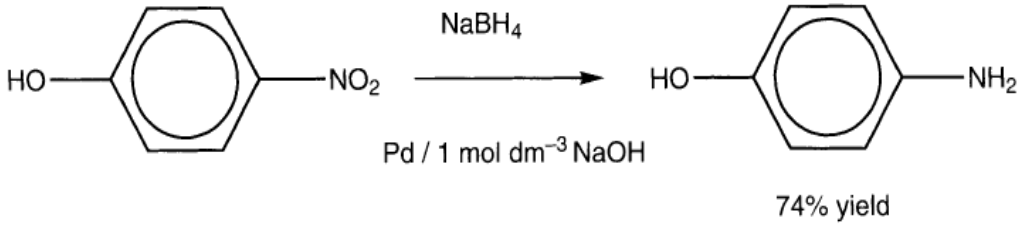
4-aminophenol may be obtained by the amide hydrolysis of acetaminophen. 4-aminophenol prepared this way and related to the commercially available metol, has been used as a developer in photography by hoppyists. (Henny and Dudley, 1939), this reaction is also used to determine acetaminophen in urine samples, after hydrolysis with hydrochloric acid, 4-aminophenol reacts in ammonia with a phenol derivative e.g salicylic acid to form an indophenol dye under oxidation by air. (Novotny and Elser, 1984).

1.4.1. Synthesis of Acetaminophen

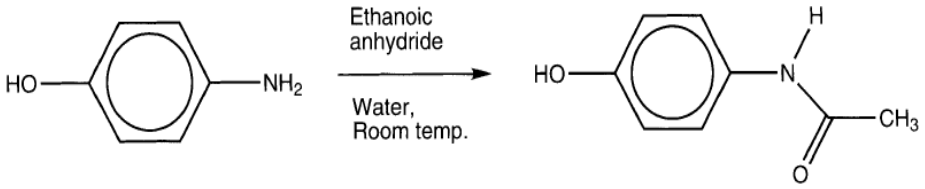
In a small scale laboratory, acetaminophen is prepared by a three reaction sequence. First, nitration of phenol with sodium nitrate gives a mixture of two isomers from which the wanted 4-nitrophenol (bp. 93°c) can easily be separated by steam distillation. In this electrophilic aromativ substitution reaction, the phenol oxygen is strongly activating thus the reaction requires only mild condition as compared to nitration of benzene itself, the nitro group is then reduced to an amine, giving 4-aminophenol. This reaction can be accomplished using sodium borohydride. Finally, the amine is acetylated with acetic anhydride. (Ellis and Frank, 2002). The industrial process is analogous but hydrogenation is used instead of sodium borohydride reduction. (Anthony and Travis, 2007) (Elmar et al., 2005).



Step1



Step2



Step3

An alternative industrial synthesis developed by Hoechst-Celanese involves direct acylation of phenol with acetic anhydride catalysed by HF, conversion of the ketone to a ketoxime with hydroxylamine followed by the acid catalysed Beckmann arrangement to give the amide. (Elmar et al., 2005) (Kenneth et al., 1985). Demand for acetaminophen in the United State was estimated at 30-35 thousand tones per year in 1997, equal to the demand from the rest of the world. (IARC Monograph,73).

1.4.2. Metabolism

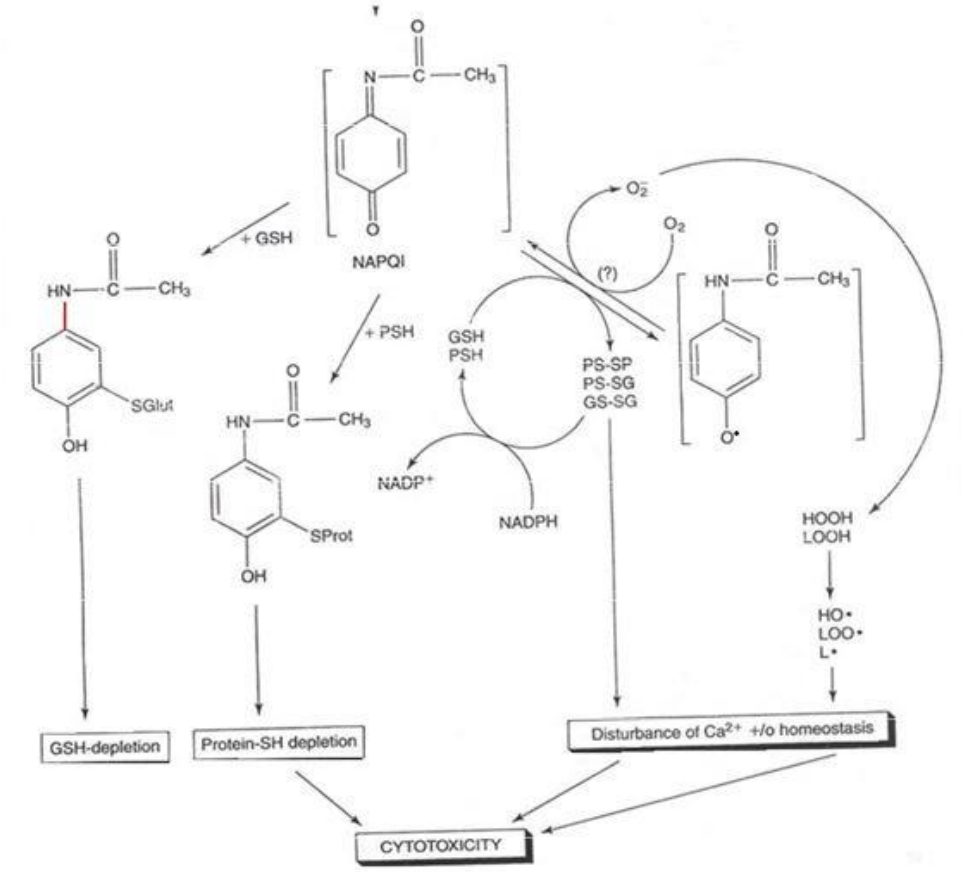


Figure1.5. *Main path ways of acetaminophen metabolism .(Source: Dahm and Jones 1996).*

Three metabolic pathways are notable (Mehta and Sweety, 2012).

- Glucuronidation is believed to account for 40% to two-third of the metabolism of acetaminophen. (Hendrickson et al., 2006)
- Sulfation (sulfate conjugation) may account for 20-40%. (Hendrickson et al., 2006).
- N-hydroxylation and rearrangement, GSH (Glutathione) conjugation, account for less all three pathways yields final products that are inactive, non-toxic and eventually excreted by the kidneys. In the third pathway, however, the intermediate product NAPQI is toxic. It is normally produced only in small amounts, and then almost immediately detoxified in the

liver. However, under some conditions in which NAPQI is not effectively detoxified (usually in case of acetaminophen overdose), it causes severe damage to the liver. This becomes apparent 3–4 days after ingestion and may result in death from fulminant liver failure several days after the overdose.

Production of NAPQI is primarily due to two enzymes of cytochrome P450, vis CYP2E1 and CYP1A2. The P450 gene is highly polymorphic, however, and individual differ than 15%`the hepatic cytochrome P450 enzyme system metabolizes acetaminophen, forming a minor yet significant alkylating metabolite known as NPAQI (N-accetyl-p-benzo-quinone imine) (also known as N-accetylimidoquinone) (Mehta and Sweety, 2012), (Borne and Ronald 1995)

1.5. Spectrophotometer

Spectrophotometry is designed to measure the degree of absorption of light by a substance (in this case acetaminophen syrup some are 120mg/5ml while others are 125mg/5ml) in a definite and narrow wavelength range .the absorption spectrum in the visible and ultraviolent region of a substance in a solution is characteristics depending on its chemical structure .therefore, spectrophotometry is used to identify a substance by measuring the absorbance at various wavelength this method is applicable to identification test , purity test and assays tests in which the absorbance of a solution with a certain concentration is usually measured at the wavelength of the maximum assumption (Xmax) or the minimum absorption (X min). When monochromatic light passes a substance in a solution, the ratio of the transmitted light intensity (l) to the incident light intensity (lo)is called transmittance (T) while the common logarithm of the reciprocal of transmittance (T) is called absorbance.

T=l/lo

A=loglo= -log T

The absorbance (A) is proportional to the concentration (C) of the solution and the length (L) of the layer of solution which the light passes.

A=KCL where K= constant

Calculated on the basis that L is 1cm and C is 1%w/v solution, the absorbance is called specific absorbance is called molecular extinction coefficient (E). the molecular extinction coefficient at the wavelength of the maximum absorption is expressed as E_{max} which ranges from 0-10^6 , values above 10^4 are termed high intensity absorption while values below 10^3 are low intensity absorption.

In the measurement of the absorbance performed for a solution using specific solvent, the appropriate concentration of the solution is one in which the measured absorbance ranges between 0.2-0.7, if the absorbance of the solution indicates a higher value than described above, the solution should be diluted with the solvent to a suitable concentration and measured against $E^{1\%}$ 1cm or E_{max} can be obtained using the formula below

$E^{1\%}$ 1cm= a/c(%) x L, E_{max} =a/c(mol) x L where

L= length of solution layer (cm)

A= measured absorbance

C(%)= concentration of solution (% w/v)

C (mol)= molarity of solution (mol/l).

1.5.1. Scope of the Work

The scope involves the determination of the dosage strength specified by manufacturers with the actual amount of acetaminophen determined in some available brands (in the form of liquid suspension/syrup and tablets/caplets). As well as to confirm the suitability of the two methods used for this determination. This was carried out by carefully verifying the actual amount of acetaminophen active ingredient in each specific variety of analgesic and antipyretic drugs commonly marketed and consumed in Nigeria.

This study was necessary due to recent emphasis via public awareness in the toxic effect of over-dosage of acetaminophen as a cause of liver damage. Also to ascertain the level of efficacy of acetaminophen administration as analgesic/antipyretic due to the effect of under-dosage of active acetaminophen in the brands available around us.

2. MATERIALS AND METHODS

2.1. Experimental (Apparatus and Material used)

2.1.1. Apparatus

- Reflux condenser
- Round bottom flask
- Conical flask
- Standard volumetric flask (100ml, 250ml, 500ml)
- Measuring cylinder (100ml, 250ml)
- Reagent bottles
- Burette (50ml)
- Pipette (1ml and 20ml)
- Retort/clamp stand
- Funnel and spatula, filter paper (whattman No. 41)
- UV-Visible spectrophotometer C715 UV/VIS Spectrophotometer Jenway (single beam)
- Jenway electric hotplate/stirrer
- OHAUS electric weighing balance (0.001-600g)
- Sonicator Bandelin Sonorex super RK 514 BH

2.1.2 Materials (reagents)

- Analytical grade methanol
- Raw acetaminophen powder (BP) specification, manufacturing date;- 04/2016, expiring date;- 07/2019,purity 98%, was purchased from laider pharmaceuticals (W.A) limited, oshodi, Lagos state.
- Brands of acetaminophen formulation used , these were in the form of tablets (500mg) and syrup (125mg/5ml and 120mg/5ml) and are listed in the appendix.
- Hydrochloric acid:- A general purpose hydrochloric acid of BDH grade.
- Tetraoxosulphate VI acid:- ANALAR grade tetraoxosulphate VI acid was used for acidification.
- Ammonium cerium IV sulphate ($(NH_4)_2Ce(SO_4)_2$) of BDH grade was used without further purification
- Ferroin sulphate solution:- A general purpose 1,10-phenanthroline iron ii sulphate was used as an indicator.
- Distilled water

2.2. Methods

Titrimetry and spectrophotometry method were employed.

Five commonly used tablets and four (4) syrups were obtained from pharmacy stores close to University of Benin.

The syrup brand packs were labeled 120mg/5ml and 125mg/5ml and the tablets were labeled 500mg per tablets.

TITRIMETRY method recommended by the British pharmacopeia 2000 was used and a standard practical spectrophotometric method was employed for the UV determination.

Practical Procedure

2.3. Titrimetry Method

2.3.1. Sample Pre-treatment of Tablet

Ten tablets were weighed individually and the mean of the weights was calculated in order to obtain a representative weight of a tablet, the tablets were grounded to fine powder and a weight equivalent to 0.3g (300mg) of acetaminophen was dissolved in 30ml of 1M H_2SO_4 and 10ml of distilled water.

The solution was refluxed for one hour and allowed to cool. It was then diluted to 100ml with distilled water in a 100ml standard flask. To 20ml of the solution was added 40g of ice chips, 40ml of distilled water and 15ml of 2M HCl.

2.3.2. Sample Pre-treatment of Syrup

5ml of syrup was weighed repeatedly and the average weight was determined to give a representative weight of 5ml of the syrup (120mg/5ml and 125mg/5ml).This was then dissolved in 30ml 1M H_2SO_4 and 10ml of distilled water, the resulting mixture was then refluxed for one hour and allowed to cool, the resulting solution was filtered and the filtrate was diluted to 100ml in a 100ml volumetric flask. To 20ml of the mixture, 40g ice chips, 40ml distilled water and 15ml 2M HCl was added.

2.3.3. Pre-treatment of Acetaminophen Standard

0.3g (300mg) of powered acetaminophen raw material was dissolved in 30ml of 1M H_2SO_4, 10ml distilled water and was refluxed for one hour and then allowed to cool. The resulting solution was diluted to 100ml in a 100ml volumetric flask (standard flask).

To 20ml of the above solution was added 40g ice chips, 40ml distilled water and 15ml of 2M HCl.

2.3.4. Titration

The resulting solution from the above sample pre-treatment in each case i.e tablet, syrup and standard served as the titrand and was titrated against 0.1M ammonium cerium IV sulphate (as titrant), using 0.1ml of ferroin solution (1,10 phenanthroline iron ii sulphate solution) as the indicator.

A colour change from orange to yellow indicator the end point of the reaction.

The above procedure was repeated without the test material (acetaminophen) being present. The difference between the titration figures represent the amount of ammonium cerium IV sulphate required. Each cm^3 or ml of $0.1moldm^{-3}$ ammonium cerium IV sulphate is equivalent to 0.007560g or 7.56mg of acetaminophen.

2.3. Spectrophotometric Method

2.3.1. Sample Pre-treatment of Tablet

10 tablets were weighed and powered , powered tablets equivalent to 100mg of acetaminophen was weighed and taken into 100ml volumetric flask, then 50ml of methanol was added and shaken well to dissolve it, after that 50ml of distilled water was added to adjust the volume upto 100ml, the resulting mixture was sonicated for 30 minutes to ensure proper dissolution of the test sample, from that 1ml solution was withdrawn and taken in 100ml volumetric flask and the volume was adjusted with diluents up to 100ml mark to obtain a concentration of 10ug/ml (10ppm)

2.4. Diluent Preparation

Methanol and water (50:50 *v/v*) used as diluents.

2.4.1. Sample Pre-Treatment of Syrup

5ml of the syrup was added to 50ml of methanol in a 100ml standard flask to this 50ml of distilled water was added and shaken vigorously. 1ml of the resulting solution was taken into a 100m standard flask and the volume made upto the mark with the diluents (Methanol and water (50:50 *v/v*).

2.4.2. Sample Pre-Treatment of Standard

100mg of the raw acetaminophen was dissolved in 50ml methanol and was shaken well, then 50ml of distilled water was added to it to adjust the volume upto 100ml(concentration of 1mg/ml), the resulting solution was sonicated for 30 minutes to ensure proper dissolution of the standard raw material. This from which various working concentration of 50, 75, 100, 125, 150 ug/ml of the standard were gotten.

2.4.3. UV Spectrophotometer Measurement

The UV- spectrophotometer was put at zero by running a baseline between 200-400nm using a mixture of methanol: water (50:50 *v/v*).

2.4.4. Determination of Absorption Maximam

A UV absorption maxima was determined by scanning 150ug/ml and 10ug/ml solution of acetaminophen standard between 200-400nm by using UV- visible spectrophotometer.

2.5. Preparation of Calibration Curve

The standard solution for the drug having concentration of 50, 75, 100, 125 and 150ug/ml was prepared with the diluents from the stock solution. The absorbance of solution of pure acetaminophen drug were measured at 245nm and a calibration curve was plotted between absorbance and concentration to get the linearity and regression equation from which the concentration of the analyte in the sample matrix can be determined

The concentration can also be calculated using the Beer and Lambert's law formula according to British pharmacopeia 2007. The percentage content of the different brands were also calculated using their absorbance.

2.6. Aim and Objectives

2.6.1. Aim of the Study

The aim of this study is to compare the accuracy of TITRIMETRY and UV-VISIBLE methods in the assay of acetaminophen content of different acetaminophen brands available in Nigerian market, and to ascertain if the obtained amounts agree with the prescribed standards of British Pharmacopeia/United State Pharmacopeia

2.6.2. Objectives

- Estimating the acetaminophen content per 500mg tablets and per 5ml syrups.
- Comparing the content of acetaminophen in the brands studied if it is within acceptable standards (British Pharmacopeia and United State Pharmacopeia.).
- Comparing the TITRIMETRY and UV-VISIBLE methods using statistical tools (F-test and T-test)

3. RESULTS AND DISCUSSION

3.1. Results

PS= Paracetamol Syrup

PT= Paracetamol Tablet

Table3.1. *shows acetaminophen standard absorbance at different concentration*

Concentration ug/ml	***Absorbance (A)***
0	0
50	0.246
75	0.338
100	0.456
125	0.582
150	0.672

Graph of the linear relationship is as follow i.e. a plot of absorbance against concentration.

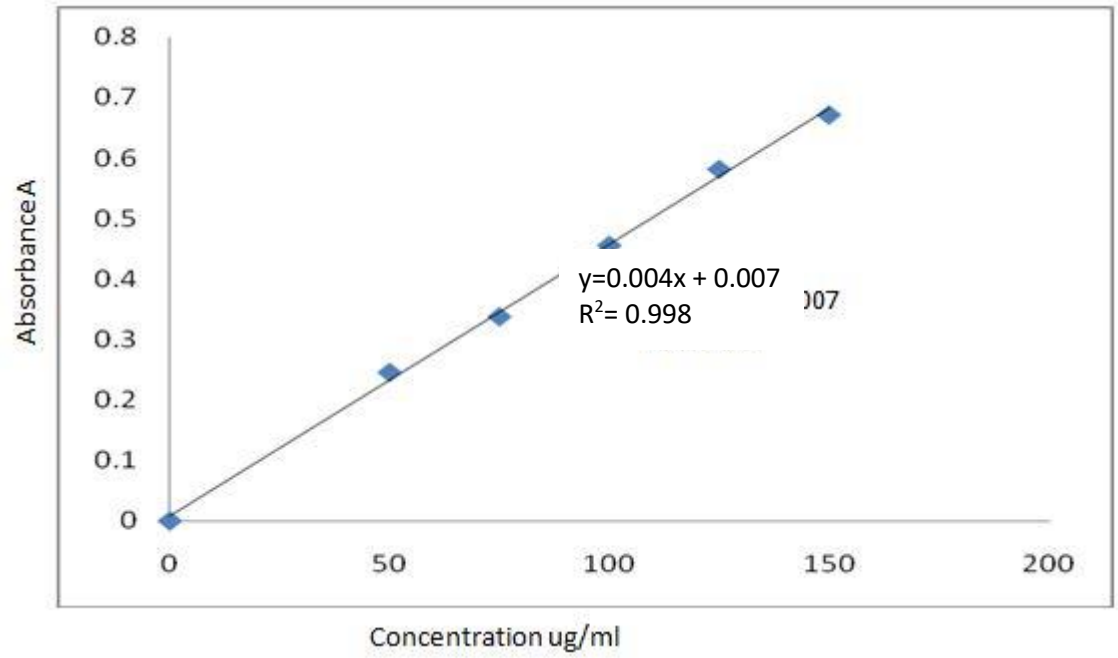


Fig3.1. *Calibration curve of Acetaminophen standard*

Table3.2 *shows the absorbance and mg content per 500mg tablet of samples using the UV method*

Sample	***Absorbance***	***Mg content/500mg***
PT1	0.046	487.5
PT2	0.045	475.0
PT3	0.044	462.5
PT4	0.042	437.5
PTS	0.048	512.5

Table3.3. *shows percentage content of acetaminophen in various brands of tablet*

Sample	***% content***	***Intent 500mg/ml***
PT1	97.50	487.5
PT2	95.00	475.0
PT3	92.50	462.5
PT4	87.50	437.5
PT5	102.5	512.5

Table3.4. *shows the absorbance and content of acetaminophen in syrup brand*

Sample	***Absorbance***	***Intent mg/5ml***
PS1	0.057	125.0
PS2	0.054	117.5
PS3	0.054	117.5
PS4	0.058	127.5

Table3.5. *shows percentage content of acetaminophen per 5ml of syrup*

Sample	***% content***	***Content per 5ml (mg)***	***Intent mg/5ml***
PS1	100.0	125.0	125
PS2	97.5	117.5	120
PS3	94.0	117.5	125
PS4	102.0	127.5	125

Table3.6. shows *the mean, variance, standard deviation and coefficient of variance of the UV method for tablets.*

Sample	***Mg content***	***x- $\bar{x}$***	***$(x- \bar{x})^2$***
PT1	487.50	12.50	156.25
PT2	475	0	0
PT3	462.5	-12.5	156.25
PT4	437.5	-37.5	1406.25
PT5	512.5	37.5	1406.25
			$\sum(x- \bar{x})^2 = 3125$
MEAN $\bar{x}= \sum x/N$	VARIANCE (s^2)	SD=$\sqrt{s^2}$	CV= (SD/ $\bar{x}$) X 100
475	781.25	27.95	5.88

Table3.7. *shows the mean, variance, standard deviation and coefficient of variance of the UV method (for syrup).*

Sample x	***Mg content***	***x- $\bar{x}$***	***$(x- \bar{x})^2$***
PS1	125	3.12	9.73
PS2	117.5	4.38	19.18
PS3	117.5	4.38	19.18
PS4	127.5	-5.62	31.58
			$\sum(x- \bar{x})^2 = 79.67$
MEAN $\bar{x}= \sum x/N$	VARIANCE (s2)	SD=$\sqrt{s2}$	CV= (SD/ $\bar{x}$) X 100
121.88	26.56	5.15	4.23

Table 3.8, 3.9 and 3.10 shows the result for the different brands of acetaminophen using titrimetric determination with 0.1M ammonium cerium IV sulphate.

1ml of 0.1M ammonium cerium IV sulphate used = 7.56mg of acetaminophen (B.P.)

Blank= 0.70ml (without the test substance)

Table3.8. *Result for Titration of Acetaminophen Standard*

Burette readings/ml	***Rough***	***1st***	***2nd***
Final reading	8.40	8.50	8.5
Initial reading	0.00	0.00	0.00
Titre volume	8.40	8.50	8.5

Recovery factor= 0.98

Table3.9.

Brand (tablet)	*Volume of titrant (ml)*	*Amount of acetaminophen per 500mg tablet.*
PT1	8.40	485.11
PT2	8.20	472.51
PT3	8.00	460.00
PT4	7.80	447.00
PT5	8.70	497.71

Table3.10. *(syrup)*

Brand	***Volume of titrant (ml)***	***Amount of acetaminophen/5ml***
PS1	4.00	124.74
PS2	3.80	117.18
PS3	3.70	113.40
PS4	4.10	128.52

Table3.11. *shows the percentage content of acetaminophen in tablet formulations*

Brand	% content	Intent mg
PT1	97	500
PT2	95	500
PT3	92	500
PT4	89	500
PT5	99.54	500

Table3.12. *shows the percentage content of various brands of acetaminophen syrup*

Brands	***% content***	***Intent mg/5ml***
PS1	99.79	125
PS2	97.64	120
PS3	90.72	125
PS4	102.82	125

Table 3.13 shows the mean, variance, standard deviation and coefficient of variance of the titrimetric method for tablet formulations

Brand	***Mg content***	***x- $\bar{x}$***	***(x- $\bar{x}$)^2***
PT1	485.11	12.64	159.7696
PT2	472.51	0.04	0.0016
PT3	460.00	-12.47	155.5009
PT4	447.00	-25.47	648.7209
PT5	497.71	25.24	637.0576
			$\sum(x- \bar{x})^2$ = 1601.0506
MEAN $\bar{x}= \sum x/N$	VARIANCE (s2)	SD=$\sqrt{s2}$	CV= (SD/ $\bar{x}$) X 100
472.47	400	20.00	4.23

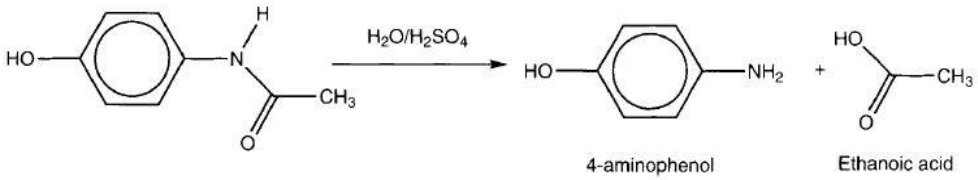
Table 3.14 shows the mean, variance, standard deviation and coefficient of variance of the titrimetric method for syrup formulations.

Brand	***Mg content***	***x- $\bar{x}$***	***(x- $\bar{x}$)^2***
PS1	124.74	3.78	14.29
PS2	117.18	-3.78	14.29
PS3	113.40	-7.56	57.15
PS4	128.52	7.56	57.14
			$\sum(x- \bar{x})^2$ =142.88
MEAN $\bar{x}= \sum x/N$	VARIANCE (s2)	SD=$\sqrt{s2}$	CV= (SD/ $\bar{x}$) X 100
120.96	47.63	6.90	5.70

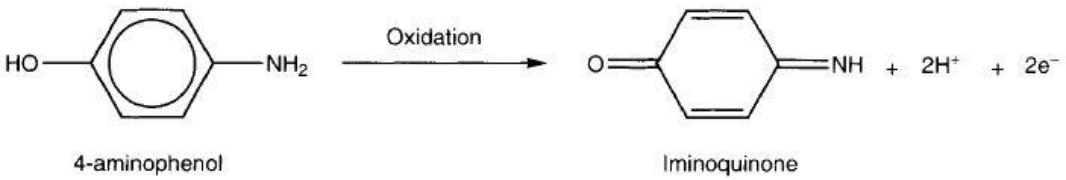
3.2. Discussion

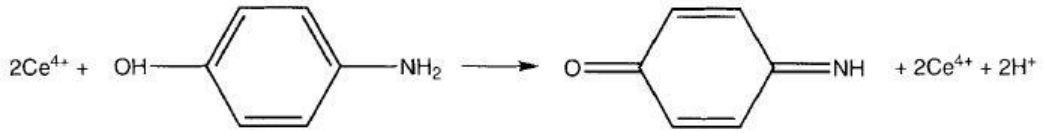
All of the tested tablets were within the range of "Maximum % difference allowed". In determining the amount of acetaminophen present in different pharmaceutical formulations two methods were employed vis titrimetry and spectrophotometry (UV visible).

In the titrimetry method, refluxing with 1M sulphurc acid and later on diluting with 2M HCl is a straight forward acid catalysed hydrolysis of an amide to an amine and carboxylic acid, the 4-aminophenol which is formed is then titrated with an oxidizing agent ammonium cerium IV sulphate using ferroin as indictor, the first reaction is as follows:-

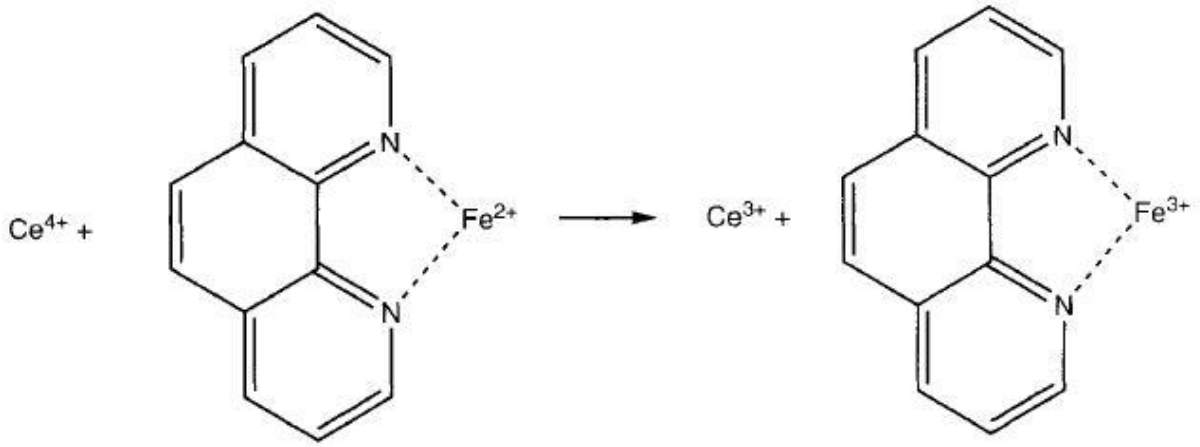


4-aminophenol can easily be oxidized as follows:- to iminoquinone using cerium IV sulphate





After all the 4-aminophenol has been oxidized to iminoquinone, the ferroin indicator is oxidized thus:



Ferroin (red) Ferriin (blue)

Gives a greenish yellow end point with a transition to pale blue.

Ammonium cerium IV sulphate exhibits high stability over a long period and also in the presence of high volume of sulphuric acid(10-40ml) unlike other strong oxidizing agent like potassium tetraoxomanganate VII $KMnO_4$ which reduces readily under strong acidic medium.

The amount of acetaminophen determined in each brand of pharmaceutical formulation (syrup/tablet) using titrimetry indicates directly the amount of hydroxylanaline (4-aminophenol) hydrolysed by the acid (HCl and H_2SO_4) to form the iminoquinone. These amounts are tabulated in tables 3.9 and 3.10 above.

From the table of results a blank titration (without the test substance) gave a titre value of 0.70ml .this indicates the volume of cerium IV sulphate required to oxidized the ferroin indicator.

On titrating the standard acetaminophen solution (0.3g pure acetaminophen powder) the recovery factor was found to be 0.98, this indicate substantial recovery rate and also indicates the validity of the titrimetry method.

From the results obtained PT_5 and PS_4 tablet and syrup respectively have the highest active acetaminophen while PT_4 and PS_3 have the lowest amount of active acetaminophen in tablet and syrup respectively.

In the spectrophotometric method, the prepared standard for different brands of acetaminophen (tablets and syrup) absorbs maximally at 245nm as shown in table 3.1, 3.2 and 3.3 above. At this wavelength of maximum absorption the absorbance of the different concentration of standard acetaminophen solution was read from which the calibration curve depicted in figure 3.1 above was plotted, which shows a linear relationship indicating the absorbance to be directly proportional to the concentration and hence obeys Beer-Lambert's law i.e intensity of a solution of the acetaminophen was proportional to the concentration of acetaminophen in a particular brand.

A linear model was deduced from the calibration curve from which the concentrations of active acetaminophen in the different test samples were determined.

According to the British pharmacopeia 2007 (B.P 2007) the content of acetaminophen in any pharmaceutical formulation should not be less than 95% and not more than 105% of the total

acetaminophen content claimed by the manufacturer, also the United State pharmacopeia 2007 (U.S.P 2007) specified minimum content as 90% and maximum as 110%.

The percentage content of acetaminophen in all nine samples analyzed using the UV method ranges from 87.5 to 102.5% and 89 to 99.79% using titrimetry method (i.e. 5 tablet brand and 4 syrups brand), from the results obtained not all samples (formulation) passed the specification stated by both pharmacopeia (B.P and U.S.P), some of the brands went below both the B.P and U.S.P specification.

The content of acetaminophen in PT_1, PT_2, PT_3 though below manufacturers claim of 500mg/tablet passed both the British pharmacopeia 2007 and the United State pharmacopeia 2007 in both method vis UV and titrimetry, PT5 went slightly above manufacturers claimed strength but fell within the specification by both pharmacopeia, PT_4 fell below manufacturers claimed strength and did not pass the specification by both pharmacopeia.

The content of acetaminophen per 5ml of syrup in PS_1 is approximately the same with manufacturers claim of 125mg/5ml, the result for the sample PS_2 was below the manufacturers claim of 120mg/5ml but passed both pharmacopeia's specification, the results for the sample PS_3 is below the manufacturers claim (125mg/5ml), PS_3 passed the specification by the United State pharmacopeia 2007 but did not pass the specification by the British pharmacopeia 2007.

The results obtained for the acetaminophen content of sample PS_4 is more than the manufacturers claim although it passed the specification of both pharmacopeia.

The mean standard deviation and coefficient of variance obtained by analyzing both syrup and tablets using different methods i.e titrimetry and spectrophotometry are 121.88±5.15, CV=4.23 for syrups and 475±27.95, CV=5.88, the slight deviation comparing the SD of both method indicates that they can be used alternatively for routine analysis of drugs containing acetaminophen, although the UV method proved more simple, rapid, economic and reproducible with slightly more accurate results.

Despite the deviation of ±5.15 and ±27.95 the results obtained still fell within the specification by the United State pharmacopeia 2007 though not all fell within the British pharmacopeia.

3.3. Findings

Quantitative analysis was carried out to study the different samples of commercially available drug containing acetaminophen as their active pharmaceutical ingredient (API). It was found out from the analysis that the total amount of API which is present in the testing sample has sufficient quantity, and the amount is approximately similar to the company's recommended or claimed value, with exception of PT_4, PT_5 contains the highest amount of acetaminophen followed by PT_1 (for tablets) and PS_4 contains the highest amount of acetaminophen followed by PS_1 (for syrups) and also results obtained using both methods (titrimetry and UV spectroscopy) showed no significant difference at 0.05 significance level using F-test and T-test, but due to convenience UV spectroscopy method is recommended for the routine analysis of acetaminophen as API in its various formulation. (I.e. tablets, syrups, suppositories, capsule e.t.c.)

4. CONCLUSION

In this research, the content of acetaminophen in different formulations both syrups and tablets brands present in Nigeria market were determined using Titrimetry and UV spectrophotometric methods.

The results obtained showed that the percentage content of acetaminophen in both syrups and tablets analyzed fell within either the British Pharmacopeia or United State Pharmacopeia specification.

More so, for the routine analysis of drugs to check the level of concordance of manufacturers claim to the actual content, proper choice of method should be guided by the instrument sensitivity, precision and accuracy, reagent availability, ease of sample preparation, cost of equipment, level of exposure to hazard and specialized training for personnel (analyst).

Although there is no significant difference between the methods employed for assay, UV spectrophotometric method is therefore recommended for the analysis of acetaminophen in different pharmaceutical formulation due to the advantages of being sensitive with convenience of sample preparation e.t.c.

REFERENCES

[1] Acetadote. "Highlight of Prescribing Information" Retrieved (2014).Acetaminophen linkedto rare fatal skin reaction. FDA says.

[2] Ali, H.M.; Homeida, M.M. A.; Ford, J.; Truman, C.A.;. Roberts, C.J.C; and Badwan., A.A. (1988). Int. J. Pharm., 42,155-159

[3] Altinez, M.A Korkmaz, R. And NF- Kappa, B (2004) Macrophage "migration inhibitory and cyclo oxygenase as likely mechanism behind the acetaminophen and NSAIDs prevention of ovarian cancer "Neoplasma 51(4); 239-247

[4] Anton. J.M De-Craen, Giuseppe D. G. Angela, J.E.M., Schoenmaeckers, L., Kessel,, G.H. and Jos K.(1996). "Analgesic efficacy and safety of acetaminophen-codeine combination versus acetaminophen alone: a systematic review BMJ 313(7053); 321-324

[5] Ashutosh K., : Medicinal Chemistry revised 3rd edition New Age International Publication (2006).

[6] BBC news, "Baby acetaminophen asthma concern" September 19, 2008 retrieved September 19, 2008.

[7] Bertolli, A., Guerzoni, S., Tacchi , R. and Leone, S. (2006). "acetaminophen; New vistas of an old drug (PDF) CNS drug review 12 (3-4): 250-275

[8] Blake M.I. and Shumaker L.B. (1973), Journal of As s o c i a t i o n o f Of f i c i a l Analytical Chemistry, Vol. 56: 653 655.

[9] Bray G.P (1993). Liver failure induced by acetaminophen BM: 2:484-1043

[10] British Pharmacopoeia (2000), Vol. II: Pg 2147, A102 Appendix IB, HM Stationery Office, London.

[11] British Pharmacopoeia (2000), Vol. I: Pg 1170 - 1171, HM Stationery Office, London.

[12] British Pharmacopoeia (2007), Vol. III: Pg 2814 - 2815, HM Stationery Office, London.

[13] Budavaris, S. (2006). The Merck index: on encyclopedia of chemicals, drugs and biological, 12th ed. RAHWAY, New Jersey, Merck and Co Inc.

[14] Carroll M.A., White E.F. and Zarembo J.E. (1981), *"Over the Counter DrugAnalysis with HPLC AnalyticalChemistry* " Volume 53: 1111A 1114A

[15] Cekic,, S.D., Filik, H. and Apak,, R. (2005). Journal of analytical chemistry 60:1019-1023

[16] Chan,.H.K and Grant., D.J.W. (1989). Int J. Pharm., **57,** 117-124

[17] Charles., G., Gerald., M.D., Dal Pan, M.D. and Bob-Rappaport., M.D., may 22, 2009, "Acetaminophen Overdose and Liver Injury- Background and Options for Reducing Injury". Retrieved July 8, 2010.

[18] Cheng, L., Ming-liang, H., Lars, B., Is COX-2 a perpetrator or a protector? Selective COX-2 inhibitors remain controversial, *Acta Pharmacologica Sinica*, 26: 926-933, 2005.

[19] Daly, F.F., Fountain, J.S., Murray, L., Graudins, A. and Buckley, N.A. 2008). Guidelines for the management of acetaminophen poisoning in Australia and New Zealand- explanation and elaboration. A consensus statement from clinical toxicologists consulting to the Australian poisonous information centres" Med J Aust 188 (5) 296-301

[20] Delay, W., (2009) acetaminophen molecule polar surface area. Orientation Journal of chemistry, 28:1517-1529

[21] Dogan, H.N. (1996). Pharmazie, 51 (10), 773-774.

[22] Ellis and Frank (2002), Cambridge, Royal society of Chemistry Pg 1.

[23] Elma.,F.I, Thomas, C., and Helmut, B. (2005), "Analgesic and Antipyretic", Ul.lmann's encyclopedia of Industrial Chemistry, Weinheim Wiley-VCH

[24] El-Obeid, H.A. and Al-Badr.., A.A. (1985).Analytical Proles of Drug Substances", Vol.14,pp.551-596, American Pharmaceutical Association.

[25] *Erik., N. and F. Onur.,* A.A. *(1997). Anal.Lett., 30 (6), 1201-1210* European Pharmacopoeia (1997).3rd edition, pp. 748-749, Convention on the Elaboration of a European. Parmacopoeia (European Treaty Series No. 50), Strasbourg, (1996).

[26] FDA limits acetaminophen in prescription combination products; requires liver toxicity warnings" (press release). US food and Drug Administration (FDA). January 13, 2011. Retrieved January 13, 2011.

[27] FDA Acetaminophen doses over 325mg might lead to liver damage." Garcia, Roderiguez, L.A and Hernandez- Diaz , (2000) "the riskof upper gastrointestinal complications associated with nonsteroidal anti-inflammatory dugs, glucocorticoids, acetaminophen and combination of these agents" *Arthritis Research Therapy* 3(2): 98-10

[28] Hawkins, L C, Edwards, J.N and Dargan, P.I (2007). "impact of restricting acetaminophen pack sizes on acetaminophen Poisoning in the United Kingdom: a review of the literature" *Drug saf30(6) : 465-79*

[29] Jenkins, C. (2000). Recommending analgesic for people with Asthma, J. therapy, PP 55-61

[30] Kanita T., Nipon T., and Duncan T. (2005), KMITL Science and Technology Journal Vol.5: 547, 555.

[31] Khashab M., Tector, A.J and Kwo, P.Y (2007). "epidemiology of acute liver failure" *currGastroenterol Rep 9*(1): 66-73

[32] *Lau , O.V.; Luk, S.F. and Cheung., Y.M. (1989). Analyst, 114, 1047-1051*

[33] Laurence, T., Sheena, D.R., Andrew. and Henry, J.M. (2009). "Single dose oral acetaminophen (acetaminophen) with codeine for post operative pain in adults" *Cochrane database of systematic review*(1)

[34] Martindale the Extra Pharmacopoeia(1996) 31 Edition, Pg.83, Royal harmaceutical Society.

[35] MEspinosa., M. Bosch, A.J. Sanchez, F.Sanchez Rojas & C.Bosch Ojeda : Determination of acetaminophen :Historical evolution

[36] Mehta, Sweety (August 25, 2012), metabolism of paracetanol (acetaminphen), acetanilide and Phenacetin, pharmaxchange.info

[37] MHossain, J., and Ayres, J. W. (1996). Int. J. Pharm., 133, 223-235. AJPS, 2011, Vol. 10, No.2 151

[38] National Drug Formulary Volume 2, 25 Edition, Pg 1267, United States Pharmacopoeia.*"Analysis of Pharmaceuticals and Biological Fluids usingModern Electroanalytical Techniques"*

[39] Novotny, P.E and Elser, R.C (1984). "indophenols method for determination of acetaminophen in serum examined" *Clin chem. 30 (6):884-6*

[40] Orsi , D.D; Gagliardi, L.; Bolasco A. and Tonelli, D. (1996). Chromatographia, 43(9/10), 496-500

[41] Ozkan S.A., Uslo B. and Aboul E. (2003) Critical review in Analytical Chemistry. Vol. 33: 155 181.

[42] Oztunc A. (1982) , ". Analyst 107: 77587. *"Acetaminophen: AC u* r r i c u l um R e s o u r c e "" C y c l i c Vo l t a m m e t r i cD e t e r m i n a t i o n o fAcetaminophen in AcetaminophenTablets""Fluor imet r icD e t e r m i n a t i o n o *fAcetaminophen as its DansylDerivative* st

[43] Acetaminophen: Arthritis research UK .Retrieved October 16, 2013.Reynolds, J.E.F. (1996) . Martindale The Extra Pharmacopoeia", 31*st* ed., pp. 27-28, Pharmaceutical Press, London.

[44] Ricardo, P. ; Hermı´nio P. Diogo ;Manuel E. Minas da Piedade Thermochemistry of Acetaminophen Research Scapes / Volume-I, Issue- II, April-June-2012/ ISSN:2277-7806J Therm Anal Calorim DOI 10.1007/s10973-009-0634-y

[45] Royal Society of Chemistry. Pain Relief from Coal Tar to Acetaminophen (2005), Pg 1,4. United States Pharmacopoeia (2007) 30 Edition.

[46] Seth, K.D (2009). Some common form of acetaminophen in Nigeria.*African journal of pharmacy and pharmacology; 6:1332-1336*

[47] Sisco, W.R.; Rittenhuse, C.T.; Everhart, L.A. and McLaughlin**,** A.M. (1986).J.Chromatogr., 354, 355-366.

[48] SMITH, M.J.H. (1953). Some recent advances in the pharmacology of salicylates. J. Pharm.(Lond.), 5, 81-93

[49] Somezeet., P.N.S. Choudhury , K., Jagannath., V.P, Dipti K.P., Goutam., K.J. Analgesic, Antipyretic and Anti-inflammatory Effect of the Whole Plant Extract of *Desmostachya bipinnata Staff* (Poaceae) in Albino Rats; *Somezeet Panda et al. / Drug InventionToday 2009,1(2),150-153*

[50] The American Society of Health System Pharmacists "Acetaminophen" Retrieved 3 April 2011.

[51] The United States Pharmacopoeia, (2000). 24*th* revision, pp. 17-39, U.S. Pharmacopeial Convention, Rockville, MD .

[52] The United states Pharmacopoeia and the National Formulary, 30th edition. USP 30-NF25, (2007)

[53] *The Wealth of India*, Council for Scientific and Industrial Research, New-Delhi, India, I, 81-87, 1976.

[54] Tripathi., K.D. Pharmacology 5th edition Jaypee Brothers Medical Pub (2003)

[55] US FDA .January 16, 2014.Acetaminophen Information Page accessed February 23, 2014 United States Pharmacopeia Vol. IV 5th Ed

[56] United State pharmacopeia (2007). The official compendia of standards, 2 p. 1269

[57] Vale, J.A and Proudfoot, A.T (1981). Treatment of acetaminophen poisoning: use of oral methionine. *Arch intern med 141, 394-396*

[58] *Webmd.com. 2009-07-01.*Retrieved 2016-08-20

[59] Wikipedia, Acetaminophen the free encyclopedia (http:len.wikipedia.org/wikilacetaminophen)

[60] Woodbury D.M., Fingl E: Analgesic-Antipyretic and Antiinflammatory Agents and Drugs Employed in the Treatment of Gout. *In* Hardman J.G., Gilman AG, Limbird, L.E. (eds): Goodman's & Gilman's The Pharmaceutical Basis of Therapeutics, 5th ed. New York, Mcgraw-Hill, 1970:325-58

[61] Xu, C. and Li, B (2004).Spectrochem.Aceta A. Molecular Biomolecular Spectroscopy. 60:1861

[62] Yuen, K.H.; Peh, K.K.; Quah, Y.L. and Chan, K.L. (1997).; Drug Dev. Ind. Pharm., 23(2), 225-228.

[63] ZED, P.J and Krenzelok, E.P. (1999) Treatment of Acetaminophen over dose. Am J Health SystemPharm; **56** 1081-9

APPENDIX 1

Preparation of Reagent Stock Solution

2M Hydrochloric Acid Solution

86.21ml of concentrated Hydrochloric acid (36.5% w/w, SP 1.16g/ml) was added to an initial 100ml distilled water and subsequently made up to 500ml mark with more water to produce a 2M HCl solution.

1M tetraoxosulphate (vi) acid solution

27.32ml ofconcentrated sulphuric acid (95% w/w, SP 1.83g/ml) was measured and added to an initial volume of distilled water and made up to the 500ml mark in a standard volumetric flask with more distilled water. This gives a 1M H_2SO_4 solution.

0.1M ammonium cerium IV Sulphate Solution

63.30g of powdered Ammonium cerium (iv) sulphate was weighed and carefully dissolved in 100ml of distilled water and then the clear solution was made up to the mark in a 1000ml standard volumetric flask.

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