

Full Length Research Paper

Spectrophotometric determination of acetaminophen content of different brands of paracetamol tablets from South-West Nigeria

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The quantitative determination of acetaminophen in paracetamol tablets manufactured in Nigeria by five different manufacturers was done in this study. The results obtained from the mean percentage analysis of paracetamol tablets (%) containing 500 mg of acetaminophen showed that the mean percentage determined in three replicate analyses is more than the claimed amount by the manufacturers. Paracetamol with resorcinol gave azodye and the concentration of paracetamol was investigated spectrophotometrically at wavelength (λ_{max}) of 505 nm. The azodye formed with resorcinol as coupling agent follows Lambert Beer's law in the range of 2 to 10 $\mu\text{g/ml}$ ($r^2 = 0.9991$) of paracetamol with detection limits of 0.192 $\mu\text{g/ml}$ and 0.640 $\mu\text{g/ml}$ limit of quantification respectively. The method developed by the authors was found to be precise and accurate for qualitative and quantitative determination of paracetamol. The precision was obtained as repeatability; relative standard deviation ranges from 0.53 to 1.37 %, standard error also ranges from 1.41 to 2.49 while standard deviation ranges from 2.78 to 7.22 respectively. The accuracy results of 75 %, 100 %, 125 % by replicate analysis ($n = 3$) were 101.85 % to 102.35 % respectively. Validation study reveals that the methods are specific, accurate, precise, and reproducible. Validation studies are statistically significant as all the statistical parameters are within the acceptance range (% RSD < 2.0 and S.D. < 2.0) for both accuracy and precision study. High recovery and low % RSD reveals the reliability of the method for quantitative study of three drugs in tablet formulation. The proposed method is simple, rapid, accurate, precise, reproducible and economical and can be used for routine quantitative analysis of paracetamol.

Keywords: Paracetamol, Acetaminophen, Resorcinol, UV/Visible Spectrophotometry, Validation.

INTRODUCTION

Paracetamol is a pharmaceutical compound widely used as analgesic and antipyretic (Martindale, 1996). It belongs to the class of drugs, known as aniline analgesics. It is commonly used for the relief of headache, other minor aches, pains, inflammations and a major ingredient in numerous cold and flu remedial

combination drugs (British Pharmacopeia, 1999). While generally safe for use at a recommended dose, toxicity of paracetamol is the foremost cause of acute gastro intestinal problems (Sarg *et al.*, 2007). Paracetamol is considered to be the inhibitor of cyclooxygenase (COX), and recent findings suggest that it is highly selective for COX-2. While it has analgesic and antipyretic properties comparable to those of aspirin or other NSAIDs, its peripheral anti-inflammatory activity is usually limited by several factors, one of which is high level of peroxides present in inflammatory lesions (Tripathi, 2004). It could be considered as one in Non-Steroidal Anti Inflammatory

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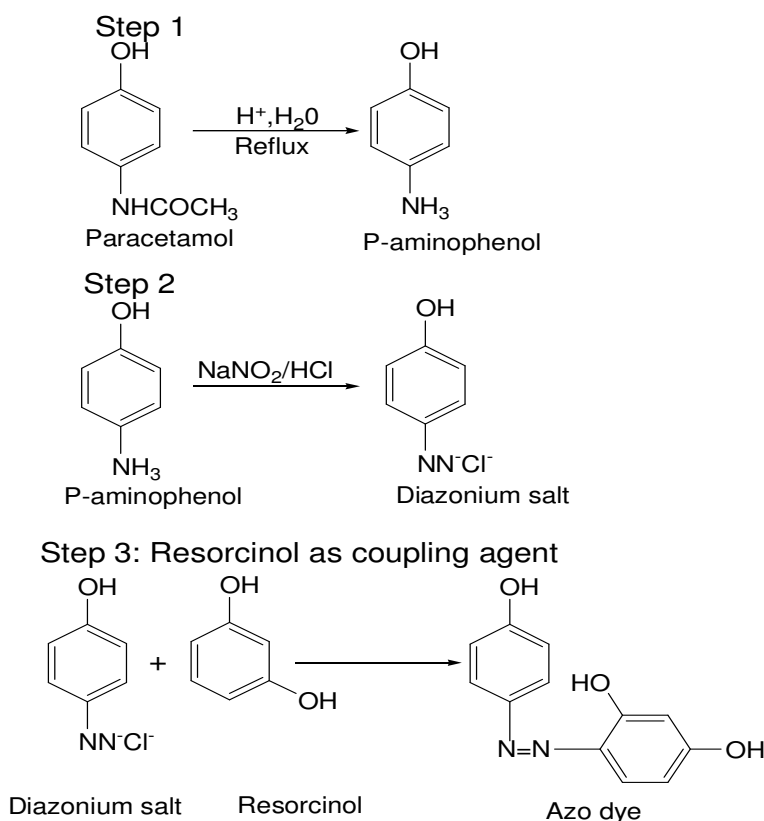


Figure 1. Reaction of Paracetamol and resorcinol as coupling agent

Drugs (NSAID).

Many methods for its determination have been described in literature, including chromatography (RP - HPLC) (Joshi and Sharma, 2008), (Carnevale, 1983), (Sa'sa and Rashid, 1984), (Suzen *et al.*, 1998), chemometric-assisted spectrophotometric (Wafaa, 2008), spectroscopy (Garg *et al.*, 2007), (Karla *et al.*, 2009), (Narayan *et al.*, 2009), Spectrophotometry (Bouhsain *et al.*, 1996), titrimetry (Kumar *et al.*, 1997) and electrochemistry (Altria, 1994). In the standard method, paracetamol is determined titrimetrically with Ce (IV) in acidic medium, using ferroin as indicator. The titration is performed in cold conditions and hence the estimation takes long time with limited accuracy (British Pharmacopeia, 1999). Hence a quicker and accurate method needed.

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

Thus, hydrolysis of paracetamol to gives p-aminophenol and coupled with coupling agents to yield

azo dye followed by Diazotization of aromatic amine and coupling the product with phenols or aromatic amines is a famous Griess reaction which has been extensively used to estimate nitrate in water, soil vegetables, meat products etc (Fox, 1985), (Clesceri *et al.*, 1998). Surprisingly very little work has been done to estimate paracetamol using Griess reaction (Sethi, 1993). Thus the present study on paracetamol estimation becomes relevant for accuracy, simplicity and specificity, which may find a wide range of application in quick estimation.

The above Griess reaction mechanism is assumed to be followed during the present study. (Figure 1)

MATERIAL AND METHODS

Reagents: All reagents used were of analytical grade and supplied by sigma incorporated, USA.

Tested Samples

Pure sample of paracetamol obtained from Eco-med Phama Nigeria Limited was used as standard and

Table 1. Sample labelling by location

Location	Sample code
Lagos	PT-1
Lagos	PT-2
Oyo	PT-3
Kwara	PT-4
Ogun	PT-5

Table 2. Results of Regression Analysis from the Absorbance of Standard Solution

Compound	Concentration ($\mu\text{g/ml}$)	Absorbance Reading	Slope	Intercept	r^2
Acetaminophen	2	0.352	0.1786	0.0183	0.9991
	4	0.692			
	6	1.043			
	8	1.389			
	10	1.789			

paracetamol tablets (500 mg) manufactured by different manufacturer were purchased from retailers, and labelled as shown in Table 1.

Instrumentation

Apparatus

An JENWAY- SPEC/6400, 520 × 330 × 180 mm :Rs 232 output, band width of 5 nm Scanning Visible Spectrophotometer with recording unit and matched set of 1 cm. glass or quartz cuvettes was used for recording the spectra. All the weighing measurements were made by a Shimadzu-AUX-220 model digital electronic balance.

Preparation of Standard Stock Solution

Accurately 250 mg of pure authentic sample (standard) of paracetamol was weighed out and then refluxed with 20 ml of 4 M HCl with 30 ml of distilled deionised water for about 30 minutes to prepare a standard solution. The content was appropriately diluted and required aliquots were taken for preparation of calibration curve.

Calibration Curve

Accurately pipette volumes of 2 ml, 4 ml, 6 ml, 8 ml and 10 ml respectively of the acetaminophen stock were taken in 25 ml volumetric flasks. To this aliquot, 0.6 ml of 4 M Hcl and 1 ml of 0.1 % w/v solution of sodium nitrite were added for diazotization. 1 ml of 0.5 % w/v solution of

ammonium sulphamate was added after 3 minutes to destroy excess nitrous acid and then left for 2 minutes. Then, 1.5 ml of 0.55 w/v solution of resorcinol in 4 M sodium hydroxide was added as coupling agent. The absorbance of this azo dye was measured at 505 nm (Buddha and Raja 2009).

Assay of Paracetamol Tablets (Samples)

Ten tablets of paracetamol of each pharmaceutical firm under study were weighed and ground to a fine powder. From this, a sample of 250 mg of paracetamol was weighed out and exactly same process for hydrolysis and colour development was carried out as was carried out for standard. Absorbance was measured at appropriate wavelength and paracetamol was estimated from calibration curve.

Statistical Analysis

The values were expressed as mean \pm SEM (Standard Error Measure). The Pearson values ($p < 0.05$) were considered significant using Statistical Package for Social Sciences (SPSS) version 18.

Validation Procedure

Method validation is the process of documenting or proving that an analytical method provides analytical data acceptable for the intended use. The first step in method development and validation is setting minimum requirements, which essentially are the specifications of

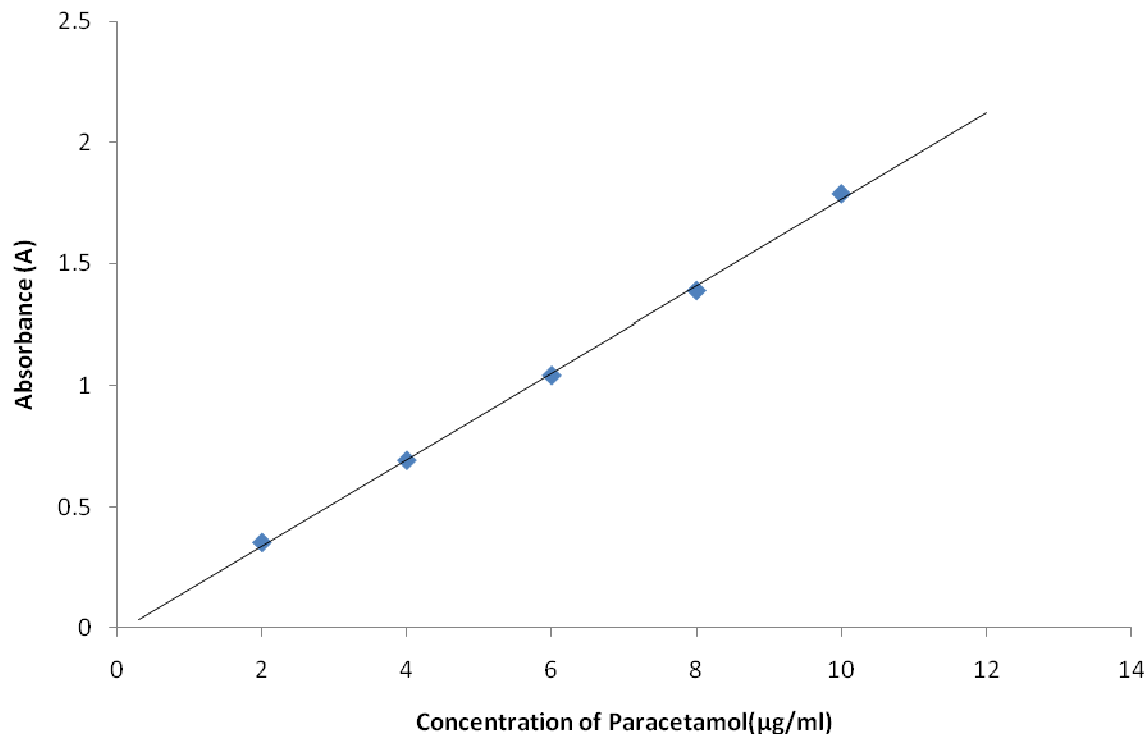


Figure 2. Standard calibration curve of acetaminophen

the method for the intended purpose e.g. how accurate and purpose does it have to be? What is the target concentration?

Accuracy

Accuracy was confirmed by recovery study as per ICH norms (International conference on harmonisation, 1996) at three different concentration levels 75 %, 100 %, 125 % by replicate analysis ($n = 3$). Here to a preanalysed sample solution, standard drug solutions were added and then percentage of drug content was calculated. The result of accuracy study was reported in Table 5. From the recovery study it is clear that the method is accurate for quantitative estimation of paracetamol in tablet dosage form as the statistical parameters are within the acceptance range (S.D. < 8.0).

Precision

Precision was determined as repeatability.

Repeatability

Repeatability result indicates the precision under the same operating conditions over a short interval of time.

The standard deviation, relative standard deviation and standard error were calculated. Repeatability was performed for three times with tablets formulation. The results of statistical evaluation are given in Table 3.

Linearity

Appropriate dilutions of standard stock solutions were assayed as per the developed methods. The Beer-Lambert's concentration range is 2-10 µg/ml. The linearity is presented in Table 2 and Figure 1 respectively.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of standard acetaminophen by proposed method were determined using calibration standards. LOD and LOQ were calculated as $3.3 \sigma/S$ and $10 \sigma/S$ respectively, where S is the slope of the calibration curve and σ is the standard deviation of response. The results of the same are shown in Table 3.

RESULTS

The quantitative determination of acetaminophen content using UV-visible spectrophotometric techniques in

Table 3. Linearity and Sensitivity for the evaluated UV/Visible Spectrophotometer

Method	Calibration Line	r^2	LOD ($\mu\text{g/ml}$)	LOQ ($\mu\text{g/ml}$)
Ultra/Violet Visible Spectrophotometer	$y=0.1786 C+ 0.0183$	0.9991	0.192	0.640

Table 4. Spectrophotometric Analysis result of five different brands of paracetamol tablet (mg/tablet)

Assay	PT-1	PT-2	PT-3	PT-4	PT-5
1	520.50	529.60	539.18	634.10	537.19
2	517.80	520.50	534.48	636.87	534.42
3	523.35	534.76	530.56	628.57	540.28
Sum	1561.65	1584.86	1604.22	1899.54	1614.89
Mean	520.55	528.62	534.74	633.18	537.96
$\pm\text{SD}^a$	2.78	7.22	4.32	4.23	2.93
$\pm\text{SEM}^b$	1.60	1.59	2.49	2.44	1.41
RSD	0.53	1.37	0.81	0.67	0.54

Note: SD^a = standard deviation, SEM^b = standard error of the mean

Table 5. Results obtained from the mean percentage analysis of paracetamol tablets (%) containing 500 mg of acetaminophen

S/N	Sample No.	Mean Percentage (%) Determined	Relative Standard Deviation (95% Confidence)
1	PT-1	104.11	0.56
2	PT-2	105.66	1.44
3	PT-3	106.95	0.87
4	PT-4	126.64	0.84
5	PT-5	107.46	0.59

paracetamol by four different manufacturers in the south-west region of Nigeria was carried out in this study.

In general, the mean assay of acetaminophen in three replicate analyses was found to be more than the claimed quantities of the active by the manufacturers. Before the commencement of the analyses, the weight of each samples were determined. PT-4 paracetamol have the highest weight (670 mg), the reason being that it is a caplet not a tablet. Caplet needs to be strong so as to avoid breaking of the tablet during blistering process. PT-1, PT-2, PT-3, PT-5 are 560 mg, 580 mg, 560 mg and 570 mg respectively. Similarly, Table 5, showed the mean percentage of acetaminophen is high in PT-4 paracetamol compared with the standard range of percentage of acetaminophen in 500 mg tablet of paracetamol which is about 95 – 105 % according to British Pharmacopeia (2010). The mean percentage of other brands of paracetamol was also shown in Table 4.

Standard calibration curve was constructed using known amount of acetaminophen in the concentration range of 2 $\mu\text{g/ml}$ to 10 $\mu\text{g/ml}$ with a regression analysis

($r^2 = 0.9991$). The value of intercept was 0.0183 and the slope was 0.1786 (Figure 2.). Thus, the linearity study of this method verifies that the response is linearly proportional to the analyte (acetaminophen) concentration in the concentration range of sample solutions.

The sensitivity of any analytical instrument is also related to the limit of detection because high sensitivity often gives a low limit of detection, thus the sensitivity of the spectrophotometer used was estimated by determining limit of detection (LOD) and limit of quantification (LOQ) which were 0.192 $\mu\text{g/ml}$ and 0.640 $\mu\text{g/ml}$ respectively (Table 3.) low LOD and LOQ indicates good sensitivity.

The accuracy of proposed method was assessed by recovery studies. The percentage recovery ranged from 101.85 % to 102.35 % of paracetamol tablets at all three levels of the recovery analysis and the percentage of relative standard deviation values for each level, ranged from 0.370 % to 1.74 % (Table 6), thus sample recoveries are in good agreement with British

Table 6. Accuracy i.e. recovery data of standard concentration solution of acetaminophen R.S.D. is relative standard deviation, a is n=3

Standard Concentration ($\mu\text{g/ml}$)	Amount Added (μg)	Found ($\mu\text{g/ml}$)	% Recovery	% Mean ^a recovery	R.S.D. (%)
2	75	77.60	103.47	102.35	1.74
		77.46	103.28		
		75.22	100.29		
100	100	102.25	102.25	102.15	0.130
		102.20	102.20		
		102.00	102.00		
125	125	127.60	102.08	101.85	0.370
		126.78	101.42		
		127.58	102.064		

Pharmacopoeia (1999).

Precision was determined by studying the repeatability. Repeatability result indicates the precision under the same operating conditions over a short interval time. The standard deviation, coefficient of variance and standard error are calculated for paracetamol (Table 4). The standard deviation ranges from 2.78 to 7.22, standard error also ranges from 1.41 to 2.49 and relative standard deviation ranges from 0.53 to 1.37 % respectively. Thus the relative standard deviation is less than 2.0 indicating good intermediate precision.

DISCUSSION

The excess acetaminophen contents obtained in the spectrophotometric results obtained in this result may be due to the effect of interference i.e. the excipients used in formulation. Buddha and Raja (2009) stated that if any ingredients added to paracetamol formulation contain aromatic amine, it is likely to interfere in the determination of active in the paracetamol tablet. The commonly used preservatives in the formulation of paracetamol in most of the pharmaceutical companies in Nigeria are methyl and propyl paraben. Other additives may include Talcum powder to give shining and smoothness to the tablet, starch as binding agent, magnesium stearate as lubricant, gelatine and lactose.

CONCLUSION

The quantitative determination of acetaminophen in paracetamol tablets manufactured in Nigeria by five different manufacturers was done in this study. The results obtained are tabulated in Table 3. The results obtained from the mean percentage analysis of paracetamol tablets (%) containing 500mg of acetaminophen is shown in Table 4 with relative standard

deviation ranging from 0.56 % to 1.44 % at 95.5 % confidence level. In general, the mean percentage determined in three replicate analyses is more than the claimed amount by the manufacturers (500 mg) Table 4.

Thus, the proposed method is simple, rapid accurate, precise, reproducible, and economic and can be used for routine quantitative analysis of paracetamol in pure and tablet dosage form.

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