Comparative Chromatographic and Spectrophotometric Methods for Quantitative Estimation of Paracetamol in Analgesic Tablet Dosage Forms

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Abstract: Poor quality drugs pose a challenge to many countries especially the developing countries. This present study is a scientific approach to further combat medicines counterfeiting as well as the validation of claimed drug specification on labels. This was achieved by comparing two analytical methods viz; spectrophotometric (UV-Vis) and Chromatographic (HPLC) techniques for quantification of Paracetamol (4-acetamidophenol) in selected analgesics marketed in Lafia, Nigeria. The statistical validation parameters such as linearity and methodic error (accuracy, precision) tests were verified and reported in terms of deviation and significance. The tagged 500 mg/tab Paracetamol concentrations for procured Para-P, X, Y and Z were estimated to range between 505.62-570.70 mg/tab and 459.42-512.58 mg/tab using UV-Visible and HPLC respectively. It was reported that experimental paracetamol concentration compares well with the claimed label specification and are within limits prescribed by existing legislation. From results in this study, deviations in API's concentration values between the two techniques are reasonable but of no statistical significance.

Keywords: Analgesic, Paracetamol, Label, Chromatographic, Lafia, API, HPLC, UV-Visible

1. Introduction

The challenge of proliferation of substandard and adulterated drugs within our society is evidenced by well-informed reports [1], with cited cases like"1990, "Paracetamol syrup disaster" that occurred when 109 children died in Ibadan and Jos, after taking Paracetamol syrup produced with the toxic Ethylene Glycol solvent instead of Propylene Glycol. In 2002, 3 patients reacted adversely to infusions manufactured by a Nigerian company.

A report [2] confirmed the non compliance of manufactured drugs found in Nigerian market with a study of 27 different drug samples collected from Lagos and Abuja. The regulatory agency NAFDAC, has been making concerted efforts at fighting the incidence of fake and adulterated drugs manufactured or imported into the country since 1993. In 1998 the "counterfeit and fake drug (miscellaneous provisions) decree No. 21" prohibiting the "sale and distribution of counterfeit, adulterated, banned, and fake drugs or poisons in open markets and without a license of registration [3].

Analgesics refer to a group of drugs used to temporarily relieve pain. They are sometimes known as painkillers. They block pain signals by changing how the brain interprets the signals and slowing down the central nervous system. The common analgesics are acetaminophen or paracetamol, aspirin and ibuprofen. However, commercially prepared analgesics usually contain a binder often starch, micro crystalline cellulose and silica gel [4].

It has been revealed that combination of analgesic from different classes may provide addictive analgesic effects with fewer side effects than when a single therapeutic drug is used [6]. Paracetamol (4-acetamidophenol) is a popular anti-pyretic and analgesic agent. It is the most widely used over

the counter analgesic (pain reliever) and anti-pyretic (pain reducer). It is the most used medicine as alternative to aspirin (acetylsalicylate). Paracetamol as a non-narcotic CNS agents used for treatment of headaches, neuralgia, and pains of muscular-skeleton origin. It has no anti-inflammatory action in usual doses. Paracetamol reduces fever by direct action on the hypothalamus heat-regulating center with consequent peripheral vaso-dilation, secretion, and dissipation of heat [7]. Some recent studies have discovered that the combination of acetaminophen and Ibuprofen improves the pain relieve action during the first 48 hours after oral surgery than those using the same daily dosage of either of the agents alone [8]. Combination of Acetamophine and ibuprofen therefore presents better synergistic effect than Acetamophine alone. Studies also revealed that the combination of 200 mg of Ibruprofen and 500 mg Paracetamol provide effective pain relieve [9]. However, it has been reported that using Paracetamol and Ibruprofen together increases the risk of renal and hepatic toxicity [10].
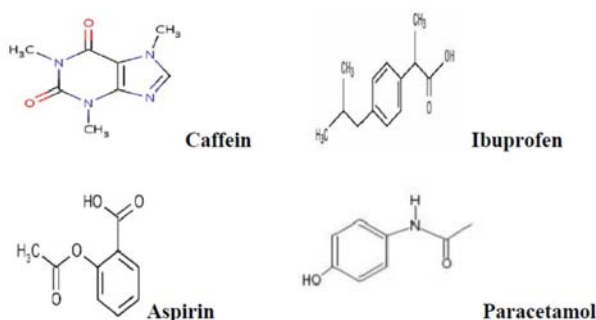

Figure 1. *Structure of some Analgesics [5].*

Spectrometry relies on producing, differentiating, and detecting ions in the gas phase, the development of the atmospheric pressure ionization interface (electrospray and chemical ionization) has enabled the direct coupling of solution introduction of compounds, through HPLC, to the mass analyzer. The drive for using HPLC for measurement and validation using low per milliliter circulating concentrations. A number of strategies have been reported for sample preparation and ways to automate these processes with solid-phase extraction and 2-dimensional chromatography. Despite the disadvantages of HPLC such as initial cost of equipment and availability of suitably skilled manpower, HPLC method presents high sensitivity, specificity, small sample requirements, minimal sample preparation, rapid throughput, and simultaneous measurement.

With the recent rise in the production and importation of counterfeit drugs, the need for spontaneous and on the spot check of drugs in the market became necessary and the government made available, hand held spectrophotometers [11]. This instrument allows the authentication of drugs even at points of sale. This only ensures that drugs are registered with NAFDAC but does not ensure the maintenance of the quality of the drug particularly at a consistent existence of supply over time.

There is no existing 'on the spot assessment of drugs' standards and adulteration. These result into observed increase in drug resistance and consequent death of the consumers. The need to validate claims by Pharmaceutical companies with respect to product composition and assay has posed great challenge to Regulatory Agencies. Several studies have reported low content of active ingredient. Gaudiano *et al*. [12] reported one sample as having low quantity of active ingredient in a study carried out in Congo, Burundi and Angola. Ogwal-Okeng *et al*. [13] found that 39% of chloroquine in Uganda failed the active ingredient content test with 11% having sub-normal and 28% having supranormal amounts. The aim of this study is to evaluate comparatively the effectiveness of using chromatographic (HPLC) and Spectrophotometric (Uv-Vis.) methods in the quantitative estimation of paracetamol in different analgesics as a quality control practice. This study is expected to validate the rapid HPLC method as it compares to UV-Vis method for the estimation of Paracetamol and it is hoped that our findings will present to relevant bodies, a drug validation within the operative framework of the Regulatory Agency with consequent standardization of common medication within our society.

2. Materials and Methods

In this study, we randomly procured brands of analgesic tablets from pharmaceutical shops and local market. Certified reference standards supplied by pharmaceutical company was used. NAFDAC registration status, manufacturing and expiring date were also reported. Pure and analytical grade of PCM was supplied by Lobal Chemicals, India and the brands of analgesic tablets used are coded as Para-P, Para-X, Para-Y and Para Z were randomly obtained from local market at Onitsha, Anambra state Nigeria.

Table 1. *Sample codes and sampling information.*

s/n	SAMPLE CODE	Manuf. Date	Expiry. Date	Reg. Status
1	Para-X	02/2015	02/2018	Registered
2	Para-Y	01/2014	12/2018	Registered
3	Para-Z	01/2015	01/2018	Registered
4	Para- P	12/2014	12/2017	Registered

2.1. Estimation of Paracetamol Using UV-Vis. Method

In the UV-Spectrophotometric Methods; Perkin Elmer lambda 35 UV-Vis spectrophotometer was used. Standard stock solutions of PCM was prepared [14] by dissolving 100 mg PCM in 50 mL double distilled water, sonicated for 10 minutes and made up to volume 100 mL. Then 0.03 mg/mL of the stock solution of acetaminophen was further diluted in 50 mL double distilled water to get a concentration of 30 µg/mL.

For Preparation of calibration curve, the stock solution of acetaminophen was appropriately diluted with double distilled water to obtain a concentration range of 2-16 µg/mL which was measured at 243 nm. The absorbance of solutions of pure Paracetamol was measured at the predetermined wavelength and a calibration curve was designed.

In the assay of marketed formulation, ten tablets of each of the sample were weighed and crushed to a fine powder. Equivalent of 60 mg PCM was accurately weighed using Eqn. 1

$$Actual weight = \frac{Weqv}{Str.} \times Avr.Wt \qquad (1)$$

Equivalent weight (Weq) is the amount of ground tablet dissolved in 100 mL Volumetric flask, Strength is the Amount of active ingredient claimed by the manufacturer on the label and Average weight (Wav) is the Mean weight of the individual tablets

$$\% \text{ Content} = \frac{Concentration}{Strenght} \times 100$$

$$\text{or } \frac{Calculated amount}{Claimed amount} \times 100 \qquad (2)$$

Each weight of the various samples was transferred into a 100 mL volumetric flask to give a concentration of 600 ppm (0.6 µg/mL). Approximately 20 mL of de-ionized water was added in each of the volumetric flask and sonicated for 16minutes. The volume was made up to the mark (100 mL) with de-ionized water and then filtered with what man filter paper no. 1 [3, 14].

The filtrate was further diluted to get final concentration of 6 µg/mL of PCM. Absorbance of each of the samples was measured and their concentration determined.

To establish Linearity, the calibration curve was constructed with concentrations ranging from 2-16 µg/mL.

2.2. Estimation of Paracetamol Using HPLC Method

The HPLC (Hitachi elite lachrome L-2300) system comprised of Perkin-Elmer Series 200 (pump, L=2420 UV-Vis detector, auto sampler and vacuum degasser) with a chromatography interface Series connected to a computer loaded with Chromatography software. A suitable mixture of methanol and de-ionized water in the ratio of 20-80 v/v was prepared as mobile phase. It was passed through a filter paper having finer porosity and sonicated for 20 minutes.

2.2.1. Preparation of 1000 PPM Standard Stock Solution

The stock solution (1000 ppm) was prepared as reported [14] with slight modification in mass and volumes measured.

2.2.2. Preparation of Calibration Curve

From the stock solution, different dilution were made to obtain a concentration of 25 -100 ppm for HPLC analysis. Ten tablets of each of the drugs was weighed and crushed into powder. An accurately quantity equivalent to 10mg of Paracetamol was transferred into 100 ml volumetric flask; 50 mL of distilled deionized water was added and stirred for 15 minutes. The volume was made up to the mark and then filtered with whatman filter paper no. 1. This gave a concentration of 0.1 mg/mL [15].

2.3. Optimization of Chromatographic Conditions

The chromatography was performed using HPLC system on a Hitachi elite Lachrome, equipped with a model l-2300 for column, L-2200 auto sampler, and L- 2420 UV-Vis detector. The samples and stock standard solution of 20µl were injected into LichchrospherRF 18-5µ-100A, 150X4.5 mm column with a flow rate of 2.0 mL/min using a mobile phase consisting of methanol and water (80:20) (v/v). The UV/Vis detector was set at a predetermined wavelength of 254 nm. Equal volume of standard and the samples were injected into the chromatograph. The chromatograms were recorded and responses for the major peaks were measured [16].

3. Results and Discussion

The assay in this study shows that both chromatographic and spectrophotometric methods are linear and sensitive for determination of Paracetamol content in tablet dosage form as also supported [17].

3.1. Spectrophotometric (UV-Vis.) Method

The result of the analysis was satisfactory. The absorbance response of standard acetaminophen was significantly linear from 2-16 µg/mL, according to the determination coefficient (r^2=0.995). The concentration of the samples was extrapolated from the graph using linear equation. The unknown concentration of PCM samples using UV-Vis method was found to be 6.268, 6.212 and 5.599 µg/mL for Para –X, Para-Y and Para- Z respectively.
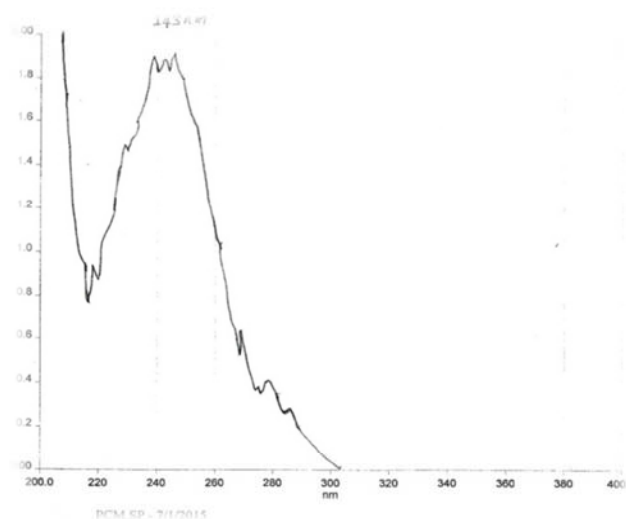

Figure 2. Paracetamol (acetaminophen) spectra using UV-Vis spec.

Table 2. UV –Vis Experimental Results for PCM Standards.

Sample	Abs. (A₁)	Abs. (A₂)	Mean Abs.	Concentration (µg/mL)
Para-X	0.461	0.467	0.465	6.268
Para-Y	0.459	0.462	0.461	6.212
Para-Z	0.410	0.435	0.423	5.599

3.2. Chromatographic (HPLC) Method

Results from this study shows that Paracetamol was successfully separated on C18 HPLC column, using

acetonitrite and methanol 80:20 as mobile Phase. The flow rate of mobile phase plays an important role in resolving the paracetamol, as the flow rate increases the retention time decreases [17]. The retention time was found to be 2.253 and 2.257 minutes and the flow rate is 2 μmL/min. The method gave a linear response to acetaminophen standard within the concentration range of 0.025 mg/mL-0.20 mg/mL with r^2 = 0.9992.

The unknown concentration of each sample was estimated from standard calibration plot. The result showed that the unknown concentration of Para - X, Para-Y and Para - Z using HPLC method was 0.101 mg/mL, 0.108 mg/mL and 0.103 mg/mL respectively.

Table 3. *HPLC experimental results for pure PCM standard.*

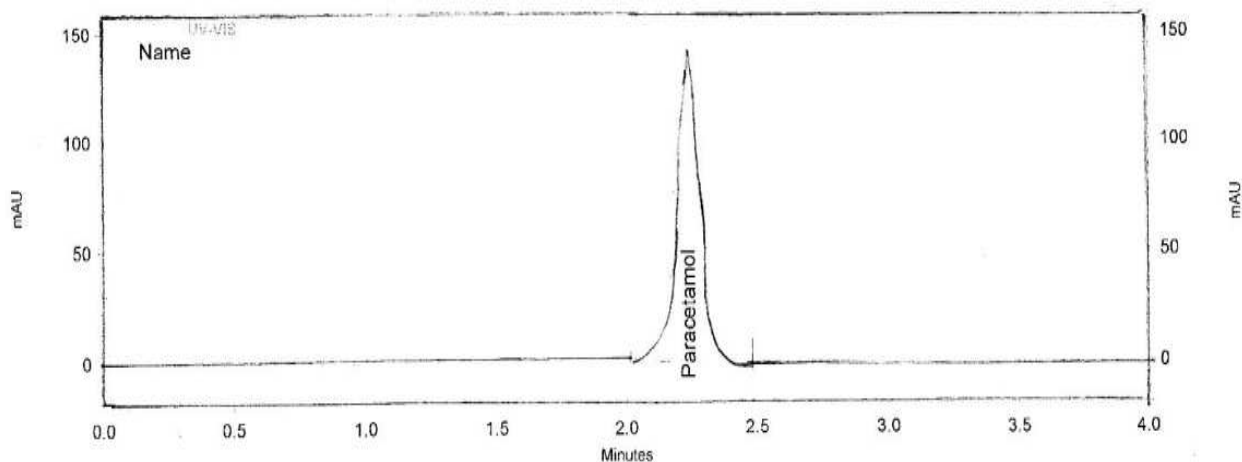| Concentration (mg/mL)	Peak area (A₁)	Peak area (A₂)	Peak area (A₃)	Peak area Mean
0.025	3351171	3326824	3328477	3335491
0.05	6547138	6551240	6545218	6547865
0.10	12911832	12883317	12915341	12903497
0.20	25812063	25785173	25880187	25825808

Figure 3. *HPLC Chromatogram for 0.025 mg/mL PCM standards.*

Table 4. *Concentration of PCM in Analgesic Samples.*

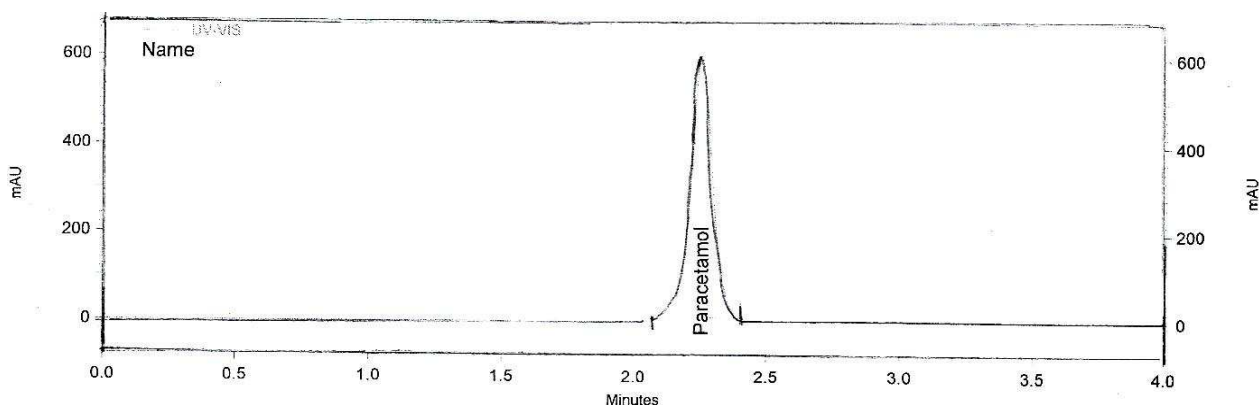| Sample	Peak area (1)	Peak area (2)	Peak area (3)	Mean peak area	Concentration (mg/mL)
Para-X	13604085	13095099	1314562	13292709.33	0.101
Para- Y	13975964	13967000	13957467	13966681.03	0.108
Para-Z	13085257	13132481	13017710	13066022. 00	0.101

Figure 4. *HPLC Chromatogram for Para-P.*

3.3. Comparative Study

The two methods obey the Beer –Lambert's Law. The UV-Spectrophotometric method obeys this Law over the concentration range of 2-16 μg/ml which was measured at a wave length of 243 nm. The HPLC method obeys the Beer's Lambert Law over the concentration range of 0.025 mg/mL-0.20 mg/mL. From the above result and data it may concluded that the study under review is simple, cost effective and suitable for daily Laboratory practice. Thus, the calibration curve of all the two methods shows linearity.

Concentration of tablet samples between 2.0-16.0, gave mean absorbance between 0.7-1.1 and these results are within the standard for Paracetamol tablets according to British Pharmacopeia. Consequently a spot check with a hand held spectrophotometer with absorbance values within this range

should be accepted as meeting quality standard for

Paracetamol tablets in the open market.

Table 5. Comparative Concentrations of PCM in Analgesic using HPLC and UV-Vis. Techniques.

Sample	PCM (mg/tab) Using HPLC	PCM (% Assay) compared to label claim	PCM (mg/tab) Using (UV-Vis)	PCM (%Assay) compared to label claim	% Deviation, UV-VIS from HPLC
Para-X	514.24	102.8	500.85	100.17	2.6
Para-Y	540.70	108.1	512.58	102.52	5.5
Para-Z	505.62	101.1	459.42	91.88	9.1

HPLC operate in elution mode. In elution mode all the analysis have the same migration distance but different migration time. HPLC is quicker and has wide range of applicability than spectrophotometry. The measured quantity of PCM found in Para-X, Y and Z (mg/tab) was 514.24, 540.7 and 505.62 respectively, while the amount of PCM found in Para-X, Y and Z using UV-visible was found to be 500.85 mg/tab, 512.58 mg/tab and 459.42 mg/tab respectively, Generally, assays and content consistency techniques employ the use of UV detection, because no elaborate sample preparation takes place, typical specifications range from 90-110% assay on dried basis of the label claim. From the analysis of the measured values, the sampled products meet the required standard. The result of the absolute errors obtained from using the individual techniques shows that values for HPLC (0.001, 0.001 and 0.008), are lower than those of UV-Vis (0.0211, 0.027 and 0.040) for Para-XYZ respectively. This implies that the HPLC technique is more precise and specific than the UV-Vis spec. technique in the analysis of PCM.

The statistical t-test analysis result, gave a two tail t-test values of 0.905 for UV-Vis and 0.192 for HPLC. With the alpha value of 0.05 or 95% confidence interval, there is no significant difference between the results from the two techniques, considering also that the P-value 0.243 is larger than statistical alpha value of 0.05 at 95%. Unlike the reports in this study, Kibwage *et al*. [18] reported that about 45% of drugs sampled on the Kenyan market and analysed at the Daru quality control laboratory on a routine basis were of substandard quality in terms of the content of the active ingredient. Shakoor *et al*. [19] reported both fake and substandard drugs on Thai and Nigerian markets where 36.5% of 89 samples failed in the assay determination.

4. Conclusion

In this study PCM in selected analgesics were successfully resolved and quantified using HPLC and UV-Visible spectrophotometric techniques. From results, there is deviation in API's concentration values between the two techniques, which is reasonable but of no statistical significance. This shows that analysis PCM in analgesic using HPLC is more accurate than that from UV-VIS spec. nonetheless UV-Vis can still be used for randomized on the spot check for quality standards of PCMs in the market. On the general, the label acclaimed concentrations of PCM in analgesics from Lafia vendors is in good agreement with the analytical results investigated in this work and with those of

the exiting Nigerian legislation as proven by the one way ANOVA.

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