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Quantitative Analysis of Some Analgesics Used in Maiduguri Metropolis Using Ultra Violet Spectrophotometry

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Abstract

Paracetamol and diclofenac sodium are common over-the-counter analgesics which are accessible to the general population. The aim of this work was to assay paracetamol and diclofenac sodium content in samples selected across the Maiduguri metropolis using UV spectrophotometric method. 15 samples each of paracetamol tablet, paracetamol syrup and diclofenac sodium tablet were randomly selected from three different sources; hospital pharmacy, community pharmacy and drug patent stores. Wavelength of 257 nm was adopted for paracetamol samples assay while 276 nm was adopted for diclofenac sodium samples. Percentage mg content for paracetamol tablets ranged from 37-73%, 18-104% for paracetamol syrups and 77-95% for diclofenac sodium tablets. According to the USP none of the paracetamol tablet samples passed, 12 out of 15 paracetamol syrup samples passed and 8 out of 15 diclofenac sodium tablet samples passed (USP acceptance range is 90-110%). Statistical significance was observed between class of drug and percentage mg content (p-value < 0.05) but no significance between source of drug and percentage mg content (p-value > 0.05) when the findings were subjected to ANOVA.

1. Introduction

Pharmaceutical analysis is a set of analytical technique used to evaluate drug substances, products and additives to ensure quality and safety from development through consumption by the end user [1,2].

1.1 Paracetamol

Paracetamol is part of the class of drugs known as aniline analgesics, it is the only such drug still in use today[3].Paracetamol(Fig 1) is a white or almost white, crystalline powder. Sparingly soluble in water, freely soluble in alcohol, very slightly soluble in methylene chloride [4].It is the most used medicine after acetylsalicylic acid in many countries as an alternative to aspirin and phenacetin. Paracetamol is also known as acetaminophen (N-acetyl-p-aminophenol, 4-acetamidophenol); it is a major ingredient in numerous cold and flu medications and many

prescription analgesics. It is remarkably safe in standard doses, but, because of its wide availability, deliberate or accidental overdoses are common [5].

1.1.1 Assay

USP has described an analytical method to quantify paracetamol as a bulk drug by spectrophotometry at 244 nm using water as blank. Paracetamol tablets are assayed by liquid chromatography using a mixture of water and methanol (3 :1) as mobile phase and solvent system [4]. The British Pharmacopoeia also have spelt out methods [5]. Different paracetamol analysis methods published in over 50 years were summarized in a review article [6].

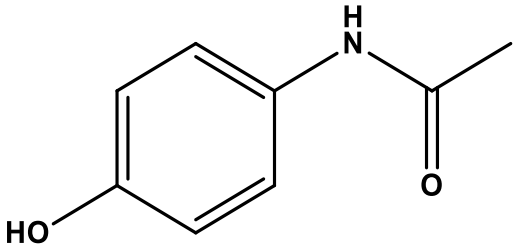

Fig. 1 Paracetamol

1.2 Diclofenac

Diclofenac (Fig 2)belongs to a group of NSAIDs that inhibit both COX-1 and COX-2 enzymes. The binding of NSAIDs to COX isozymes inhibits the synthesis of prostanoids (i.e., prostaglandin [PG]-E 2, PGD 2, PGF 2, prostacyclin [PGI 2], and thromboxane [TX] A 2). PGE 2 is the dominant prostanoid produced in inflammation, and the inhibition of its synthesis by NSAIDs is believed to be the main mechanism of the potent analgesic and anti-inflammatory properties of these agents [7].It is an effective anti-inflammatory, analgesic and antipyretic agent which is commonly used in the treatment of acute and chronic pain, rheumatoid and osteoarthritis. Diclofenac sodium was found to be the most commonly used off-label medicines in UK paediatric surgical wards[8].

1.2.1 Assay

Different methods of assay for diclofenac are available; titrimetry, UV spectrophotometry, HPLC among others as described in the USP, BP and some other journals [2,4,5,9].

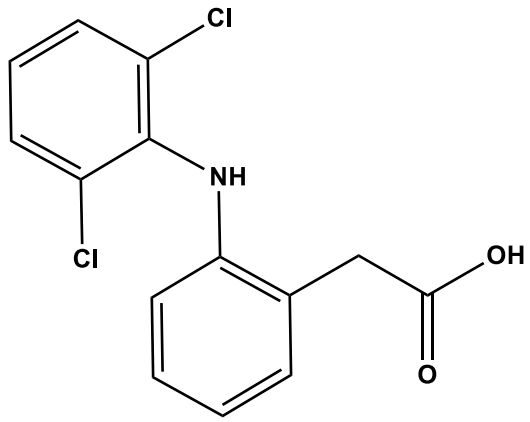

Fig. 2 Diclofenac (2-((2,6-Dichlorophenyl)aminobenzeneacetic Acid)

2.Materials and Method

2.1 Instruments

Metertech UV-Vis spectrophotometer SP8001(Taiwan) and mechanical shaker.

2.2 Reagents

Standards: paracetamol and diclofenac sodium (Sigma Aldrich, France).Solvents: methanol (Sigma Aldrich, France) and distilled water. Substances: sodium hydroxide (BDH Chemicals, UK). Materials: quartz cuvette.

2.3 Sampling

The sample were selected from the different pharmacy premises as well as the patent stores. Different dosage forms were used; tablets and syrups. The pharmacies and the patent stores of each area were selected randomly.

2.3 Sample Size

The samples were randomly selected from three different sources; hospitals, community pharmacies and patent drug stores. 300 tablets and 15 syrups of paracetamol produced by 5 different companies were randomly selected. 300 tablets of diclofenac sodium produced by 5 different companies were randomly selected. Different doses and types were randomly selected.

2.4 Data analysis

Data analysis was carried out by using statistical package of social science (SPSS) program version 21 [10]. Data coding and entry, statistical examination such as frequency Crosstabs, distribution, ANOVA and Post Hoc tests was carried out and the level of significance was performed at $p \leq 0.05$.

2.5 Paracetamol Tablets Assay

20 tablets were weighed and powdered using pestle and mortar after noting the mean weight. A quantity of the powder containing 0.15g of Paracetamol was added to 50ml of 0.1M sodium hydroxide, diluted with 100ml of water, shaken for 15 minutes andsufficient water was added to produce 200ml.This was mixed and filtered. 10ml of the filtrate was diluted to 100ml with water. 10ml of the resulting solution was added to 10ml of 0.1M sodium hydroxide, diluted to 100ml with water and the absorbance of the resulting solution was measured at the maximum at 257nm. To calculate the content of $C_8H_9NO_2$, 715 was taken as the value of A (1%, 1 cm) at the maximum at 257nm. Blank solution was prepared by taking 20ml of 0.1M sodium hydroxide and diluting to 100ml with distilled water. This solution was used as blank to put the UV spectrophotometer at zero before taking the absorbance of the samples [5].

2.6 Paracetamol Syrup Assay

2.6.1 Paracetamol Standard

0.1g of standard paracetamolpowder was accurately weighed and dissolved in 5ml methanol. 1ml of this solution was diluted with methanol to 20ml. 1ml of the solution was transferred into 100ml volumetric flask and diluted with methanol. The concentration of this standard solution is 0.01mg/ml. Using this paracetamol standard solution (0.01mg/ml) a series of different

concentrations to estimate the calibration curve was prepared. 4.0, 2.0, 1.0, 0.5ml of the paracetamol standard solution would be diluted with the same solvent in 5ml volumetric flask. The resulting concentrations are 8.0, 4.0, 2.0 and 1.0µg/ml, respectively[6].

Commercial paracetamol syrup has a concentration of 125mg/5ml. 1ml of the preparation was transferred to 25ml volumetric flask and diluted to volume with methanol and filtered. 1ml of the last solution was further diluted to 100ml with the same solvent. 3ml of the solution wasdiluted in 5ml volumetric flask with methanol, followed by measurement of absorbance at 257nm after putting the UV spectrophotometer at zero by running a base line using methanol as blank[6].

2.7 Quantitative Determination of Diclofenac Sodium

This was conducted as described by Naveed and Qamar[8]. Diclofenac sodium standard solution: 10mg of standard diclofenac sodium was dissolved in 100ml of distilled water.

The mean weight of the tablets from each sample was determined using twenty (20) tablets and then powdered using pestle and mortar.By calculation, powder containing 10mg equivalent of diclofenac sodium was weighed and transferred into a volumetric flask containing 10ml of distilled water. This solution was sonicated for 5 min and more distilled water was added to make up to 100ml. The absorbance of the standard solution and the samples was measured at 276nm after putting the UV spectrophotometer at zero by running a base line using distilled water as blank.

The % content and mg content was determined as:

$$\text{content} = \frac{\text{Absorbance of sample} \times 100\ \%}{\text{Absorbance of standard}} \quad (1)$$

$$\text{Mg content} = \frac{\%\ \text{content} \times \text{Manufacturer's claim}}{100} \quad \ldots\ldots\ldots\ldots(2)$$

3. Results and Discussion

3.1 Quantitative Determination of Paracetamol

3.1.1 Calibration Curve of Paracetamol Standard

The absorption of paracetamol standard solutions was measured at 257 nm. Microsoft Excel 2016 program was used to obtain the regression line. It has the equation

$$y = 0.0685x - 0.055 \quad (3)$$

and a correlation factor of $R^2 = 0.9924$. The data were plotted and shown in Fig.3

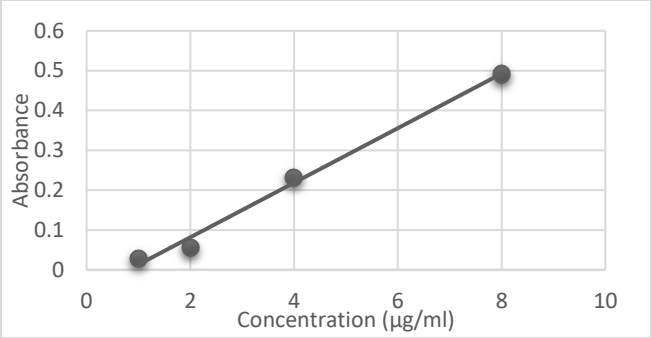

Fig. 3: Calibration curve of paracetamol standard

3.1.2 Paracetamol Content in Tablets

Paracetamol tablets of different available manufacturers were analyzed quantitatively. The percentage weight of paracetamol lies between 37-73%. The results are expressed as percentage (w/w) and are listed in Table 1

Table 1: Paracetamol content in tablets using UV spectroscopy

Sample ID	Percentage Content (w/w)	Mg content (mg)
PTH-1	67	335
PTH-2	66	330
PTH-3	39	195
PTH-4	63	315
PTH-5	72	360
PTCP-1	73	365
PTCP-2	65	325
PTCP-3	64	320
PTCP-4	37	185
PTCP-5	51	255
PTPS-1	66	330
PTPS-2	42	210
PTPS-3	54	270
PTPS-4	65	325
PTPS-5	56	280

3.1.3 Paracetamol Content in Syrup

Paracetamol syrups of different available manufacturers were analyzed quantitatively. The percentage weight of paracetamol lies between 18-104%. The results are expressed as percentage (w/w) and are listed in Table 2.

Table 2: Paracetamol content in syrups using UV spectroscopy

Sample ID	Percentage Content (w/w)	Mg content (mg/5ml)
PSH-1	18	22.5
PSH-2	92	115
PSH-3	97	121.25
PSH-4	100	125
PSH-5	90	112.5
PSCP-1	104	130
PSCP-2	99	123.75
PSCP-3	105	131.25
PSCP-4	96	120
PSCP-5	95	118.75
PSPS-1	95	118.75
PSPS-2	101	126.25
PSPS-3	44	55
PSPS-4	129	161.25
PSPS-5	94	117.5

3.1.4 Quantitative Determination of Diclofenac Sodium

Diclofenac sodium tablets of different available manufacturers were analyzed quantitatively. The percentage weight of diclofenac sodium lies between 77-95%. The results are expressed as percentage (w/w) and are listed in Table 3.

Table 3: Diclofenac sodium content in tablets using UV spectroscopy

Sample ID	Percentage Content (w/w)	Mg content (mg)
DSTH-1	93	46.5
DSTH -2	89	89
DSTH -3	93	46.5
DSTH -4	95	47.5
DSTH -5	86	43
DSTCP-1	92	46
DSTCP -2	93	93
DSTCP -3	88	88
DSTCP -4	94	47
DSTCP -5	87	43.5
DSTPS -1	77	38.5
DSTPS -2	86	43
DSTPS -3	92	46
DSTPS -4	90	90
DSTPS -5	88	43

3.1.4 The Relationship Between Analyzed and Reference Content of the Drug Samples

ANOVA tests were applied to test if the difference between the drug samples and sources of the drugs in relation to the percentage mg content is significant. The p-value was 0.000003 as illustrated in tables 5for drug sample versus percentage mg content, and 0.783 for sources of drug versus percentage mg content, this means that there is a statistical significance between drug samples and percentage mg content but not between sources of drug and percentage mg content.

Table 4: Descriptive statistics of the drugs versus percentage content

	N	Mean	Std. Deviation	Std. Error
PCM Tabs	15	58.6667	11.67823	3.01530
PCM Syrup	15	90.6000	26.31349	6.79412
Diclofenac Na Tabs	15	89.5333	4.56488	1.17865
Total	45	79.6000	22.23981	3.31531

Table 5: Drugs versus Percentage mg content one-way ANOVA

Source of Variation	Sum of Squares	Degree of freedom	Mean Square	F	Sig.
Between Groups	9868.133	2	4934.067	17.422	0.000003
Within Groups	11894.667	42	283.206		
Total	21762.800	44			

Table 6: Descriptive statistics of the sources versus percentage content

	N	Mean	Std. Deviation	Std. Error
Hospital Pharmacy	15	77.3333	23.52709	6.07467
Community Pharmacy	15	82.8667	20.28675	5.23802
Patent Dug Store	15	78.6000	23.90397	6.17198
Total	45	79.6000	22.23981	3.31531

Table 7: Sources versus Percentage mg content one-way ANOVA

Source of Variation	Sum of Squares	Degree of freedom	Mean Square	F	Sig.
Between Groups	252.133	2	126.067	.246	.783
Within Groups	21510.667	42	512.159		
Total	21762.800	44			

3.2 The Relationship between Drug Samples

Post Hoc Test was applied to make multiple comparisons between drug samples. LSD test was performed. There was a statistical significance between PCM tabs and PCM syrup, Diclofenac Na tabs and PCM tabsas p-value < 0.001. The results are listed in Table 8.

Table 8: Drugs versus Percentage mg content one-way ANOVA post hoc: LSD

(I) Drug	(J) Drug	Mean Difference (I-J)
PCM Tabs	PCM Syrup	-31.93333*
	Diclofenac Na Tabs	-30.86667*
PCM Syrup	PCM Tabs	31.93333*
	Diclofenac Na Tabs	1.06667
Diclofenac Na Tabs	PCM Tabs	30.86667*
	PCM Syrup	-1.06667

*The mean difference is significant at the 0.05 level.

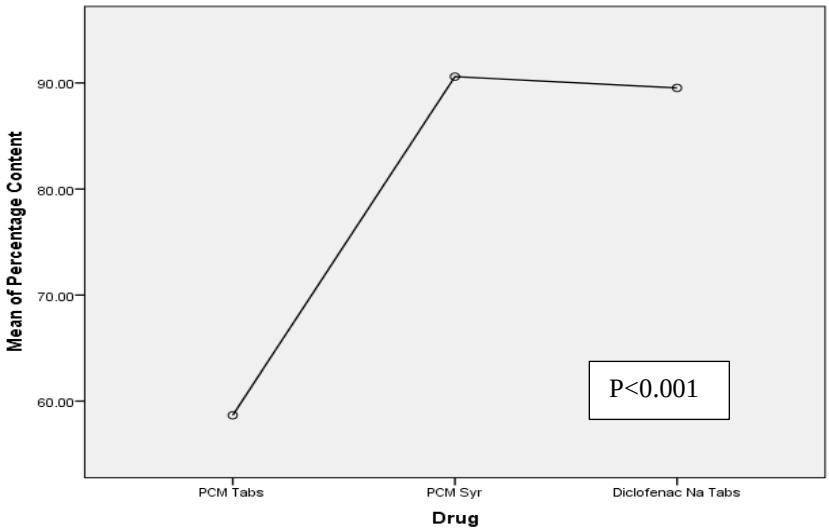

Fig. 4:Plot of the means of the drugs versus percentage mg content

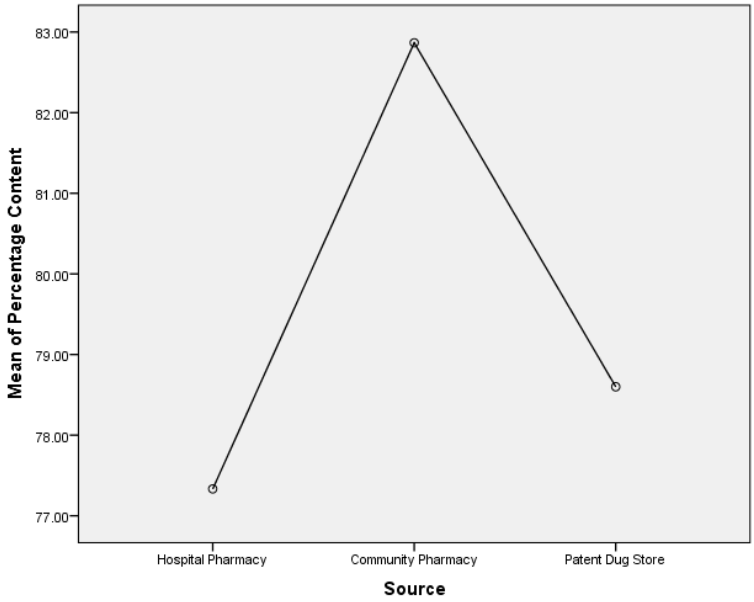

Fig. 5:Plot of the means of the sources versus percentage mg content

3.3 The Relationship Between Analyzed and Reference Content of the Drug Samples

Two-way ANOVA test was applied to test if the difference between the drug samples and sources of the drugs in relation to the percentage mg content is significant. The p-value was 0.000008 as illustrated in Table 10for drug sample versus percentage mg content, 0.658 for sources of drug versus percentage mg content and 0.537for the drug*source interaction versus percentage mg content. This means that there is a statistical significance between drug samples and percentage mg content but not between sources of drug, drug*source interaction and percentage mg content.

Table 9: Descriptive statistics of the drugs versus sources versus percentage mg content

Drug	Source	Mean	Std. Deviation	N
PCM Tabs	Hospital Pharmacy	61.4000	12.93445	5
	Community Pharmacy	58.0000	14.14214	5
	Patent Drug Store	56.6000	9.73653	5
	Total	58.6667	11.67823	15
PCM Syrup	Hospital Pharmacy	79.4000	34.55141	5
	Community Pharmacy	99.8000	4.54973	5
	Patent Drug Store	92.6000	30.68061	5
	Total	90.6000	26.31349	15
Diclofenac Na Tabs	Hospital Pharmacy	91.2000	3.63318	5
	Community Pharmacy	90.8000	3.11448	5
	Patent Drug Store	86.6000	5.81378	5
	Total	89.5333	4.56488	15
Total	Hospital Pharmacy	77.3333	23.52709	15
	Community Pharmacy	82.8667	20.28675	15
	Patent Drug Store	78.6000	23.90397	15
	Total	79.6000	22.23981	45

Table 10: Drugs versus sources versus percentage mg content two-way ANOVA

Source	Sum of Squares	Degree of Freedom	Mean Square	F	Sig.
Drug	9868.133	2	4934.067	16.603	0.000008
Source	252.133	2	126.067	0.424	0.658
Drug * Source	944.133	4	236.033	0.794	0.537
Error	10698.400	36	297.178		
Total	21762.800	44			

3.4 The Relationship Between Drug Samples

Post Hoc Test was applied to make multiple comparisons between drug samples. Bonferroni test was performed. There was a statistical significance between PCM tabs and PCM syrup, Diclofenac Na tabs and PCM tabsas p-value < 0.001. The results are listed in Table 11.

Table 11: Drugs versus sources versus percentage mg content two-way ANOVA post hoc:Bonferroni

(I) Drug	(J) Drug	Mean Difference (I-J)
PCM Tabs	PCM Syrup	-31.9333*
	Diclofenac Na Tabs	-30.8667*
PCM Syrup	PCM Tabs	31.9333*
	Diclofenac Na Tabs	1.0667
Diclofenac Na Tabs	PCM Tabs	30.8667*
	PCM Syrup	-1.0667

*The mean difference is significant at the .05 level.

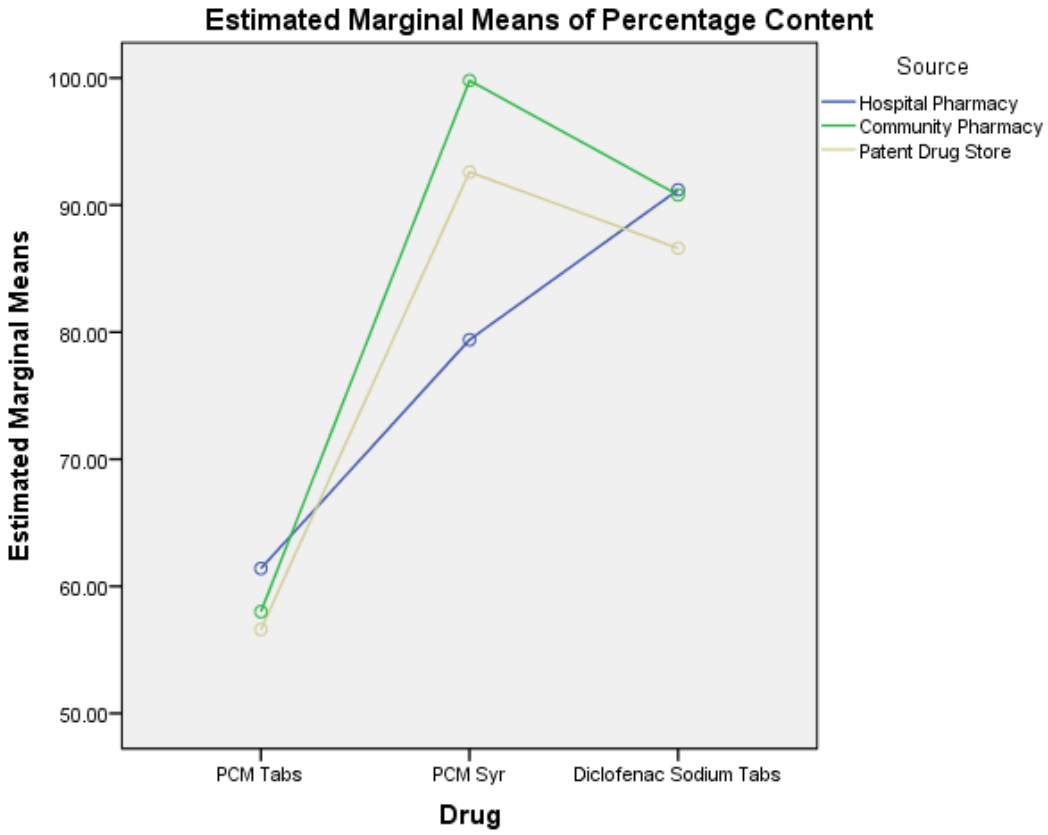

Fig. 6:Plot of the means of the drugs versus sources versus percentage mg content (UV)

Several methods are available for the quantification of the drug samples used in the study; ranging from titrimetric, chromatographic and spectroscopic methods[4,5,6,8].

Paracetamol tablet samples have a paracetamol content range from 37-73%, this means that all paracetamol tablet samples were rejected according to the criteria of the USP which describes an acceptable range for paracetamol content 90-110% for tablets. For paracetamol syrup samples (USP acceptance range 90-110%), the range was found to be 18-104% which means while some were accepted some others were rejected. For diclofenac sodium tablets (USP acceptance range 90-110%) the range was 77-95% which also means certain samples were accepted and some were rejected [4].

Upon subjecting the results to statistical analysis, it was found that there was significant difference between class of drugs and mg content of the drugs in question as p-value was <0.001 ($p = 0.000003$). Post-hoc analysis (LSD) was conducted and it was found that there was statistically significant difference between paracetamol tablet samples and the syrup samples, as well between paracetamol tablet samples and the diclofenac sodium tablet samples. This can be clearly demonstrated as the mean percentage content for paracetamol tablet samples was 58.7% (which is low) compared to that of paracetamol syrup and diclofenac sodium tablet samples 90.6% and 89.5% respectivelyas illustrated in Fig. 4 which represents the mean plot of percentage mg content in relation to the classes of drug involved. The paracetamol syrup and diclofenac sodium tablet samples are comparable. However, there was no significant difference between sources of the drugs and mg content of the drugs as the p-value was >0.05 ($p = 0.783$). Figure 5 best explains this as the plot of the mean percentage mg content versus sources showed that the means are within close range 77.3%, 82.9% and 78.6% for hospital pharmacy, community pharmacy and patent medicine stores respectively.

Similar results were obtained upon conducting two-way ANOVA test; there was significant difference between class of drugs and mg content of the drugs in question as p-value was <0.001 ($p = 0.000008$), but there was no significant difference between sources of the drugs and mg content of the drugs as the p-value was >0.05 ($p = 0.658$) like that of drug*source interaction ($p = 0.537$). Bonferroni post-hoc analysis was thus conducted and it revealed that there was statistically significant difference between paracetamol tablet samples and the syrup samples, as well between paracetamol tablet samples and the diclofenac sodium tablet samples. The meaning of this is there is no effect of source on the percentage content of the drug samples. The observed effect is due to paracetamol tablet samples because it is low all across irrespective of the source it is being obtained. This can be clearly seen from Fig. 6 which shows the mean plot of percentage milligram content of drug samples in relation to the classes of drug included in the study and sources of such drug. For paracetamol tablet, non of the means is up to 65% in contrast to community pharmacy with mean of 99.8 %. And declofenac sodium tablet from hospital pharmacy with mean of 91.20%

4.Conclusion

UV spectrophotometric analysis of paracetamol and diclofenac sodium was successfully applied to assay paracetamol and diclofenac sodium. Paracetamol range was from 18-104% while diclofenac sodium range was 77-95%. All paracetamol tablet samples were rejected, 80% of paracetamol syrup samples were accepted and 53% of diclofenac sodium tablet samples were accepted. There was statistical significance between class of drug and percentage mg content but no significance between source of drug and percentage mg content.

Abbreviations: USP: United States Pharmacopoeia; ANOVA: Analysis of Variance; BP: British Pharmacopoeia; UV: Ultra Violet, LSD: Least Significant Difference; PTH: Paracetamol Tablet Hospital sample; PSH: Paracetamol Syrup Hospital sample; DSTH: Diclofenac Sodium Tablet Hospital sample; PTCP: Paracetamol Tablet Community Pharmacy sample; PSCP: Paracetamol Syrup Community Pharmacy sample; DSTCP: Diclofenac Sodium Tablet Community Pharmacy sample; PTPS:Paracetamol Tablet Patent Store sample; PSPS: Paracetamol Syrup Patent Store sample; DSTPS: Diclofenac Sodium Tablet Patent Store sample; Tabs: Tablets and Na: sodium.

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