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Assessment of Miligram(Mg) Content of some Notable Analgesics used by Residents of Maiduguri Metropolis: Evidence from High Performance Liquid Chromatographic Technique

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Abstract

Diclofenac sodium and paracetamol are Over the counter (OTC) medications used as analgesic and antipyretic agents to relieve pains, fever and headaches. The aim of this work was determine the mg content of paracetamol and diclofenac sodium in selected samples across the Maiduguri metropolis using High Performance liquid chromatography (HPLC) method. For paracetamol samples an intersil ODS-3V column (150 mm × 4.6 mm; 5 µm pore size) was used at the temperature of 35^{o}C the mobile phase was methanol: water (20:80) using isocratic elution at the flow rate of 2 mL/min, injection volume of 10 µL and a diode array detector. For diclofenac sodium samples, the chromatographic conditions were the same except the mobile phase which was methanol: water (63:37), flow rate of 0.8 mL min, injection volume of 20 µL and column temperature was set at 30 ^{o}C. A total of 45 samples were analysed; which comprised of paracetamol tablet, paracetamol syrup and diclofenac sodium tablet which were randomly selected from three different sources; hospital pharmacy, community pharmacy and drug patent stores. The percentage mg content for paracetamol tablets ranged from 48 to 83 % while that of the paracetamol syrups ranged from 72 to 120 % and finally diclofenac sodium tablets ranged from 73 to 174 %. Adopting the United State Pharmacopoeia (USP) as reference for assessment tool, it was observed that all the paracetamol tablet samples failed, 4 out of 15 paracetamol syrup samples failed, and 11 out of 15 diclofenac sodium tablet samples failed. There was statistical significance ($p< 0.05$) between class of drug and percentage mg content but there was no significance ($p< 0.05$) between source of drug and percentage constituent. HPLC technique was used successfully for quantitative determination of paracetamol and diclofenac sodium dosage forms.

Keywords: Paracetamol, diclofenac sodium, High-performance liquid chromatography, Pharmacy, Maiduguri

INTRODUCTION

Pharmaceutical drug analysis has over the years advanced from classical methods to more sophisticated and highly sensitive techniques, it is part and parcel of drug quality control without which the quality and safety of products cannot be ascertained (Siddiqui *et al*., 2013).

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Paracetamol is widely used for management of pain and fever. It is part of the class of drugs known as aniline analgesics, it is the only such drug still in use today. It is the active metabolite of phenacetin, once popular as an analgesic and antipyretic in its own right, but unlike phenacetin and its combinations, paracetamol is not considered to be carcinogenic at therapeutic doses. Paracetamol is often classified as a non-steroidal anti-inflammatory drug, but it has few anti-inflammatory effects in many tissues (Franeta et al., 2002). However, aspirin, paracetamol and other NSAIDs all act by the same mechanism (inhibition of prostaglandin synthesis) and all show varying levels of analgesic, anti-inflammatory and antipyretic actions (Bertolini *et al*., 2006).

A wide range of assay methods for paracetamol have been developed over the years (Espinosa *et al*., 2006) but most commonly used ones are those described by the BP and USP (USP, 2017 and BP, 2018).

Diclofenac sodium is a NSAID belonging to the phenyl acetic acid derivative sub-class. Its structure was specially designed based on information gained about structure activity relationships of other NSAIDs. The chemical name to diclofenac sodium is: 2- [(2,6-dichlorophenyl) amino] benzene acetic acid mono sodium salt. It is white to off white in color, odorless, crystalline and slightly hygroscopic in nature (BP, 2018).

In the United States, diclofenac is approved for long-term symptomatic treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. The normal daily dosage for those indications is 100 to 200 mg, given in several divided doses. It is also used for the short-term treatment of acute musculoskeletal pain, postoperative pain, and dysmenorrhea. Diclofenac is also available in combination with misoprostol, a PGE1 analog (Arthrotec®). This combination, which retains the efficacy of diclofenac while reducing the frequency of gastrointestinal ulcers and erosions, is cost-effective relative to the selective COX-2 inhibitors despite the cost of the added misoprostol (Morant *et al*., 2002).

Apart from methods described in USP and BP for the assay of diclofenac in bulk and also in dosage forms, several other methods have been developed over the years ranging from titrimetric, UV as well as modified HPLC techniques (Ebeshi *et al*., 2013; Naveed and Qamar 2014; Naveed *et al*., 2015, USP, 2017 and BP, 2018).

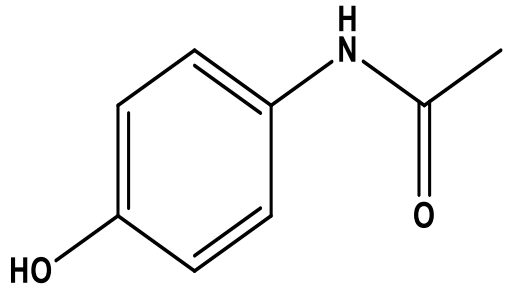

Figure 1: Paracetamol

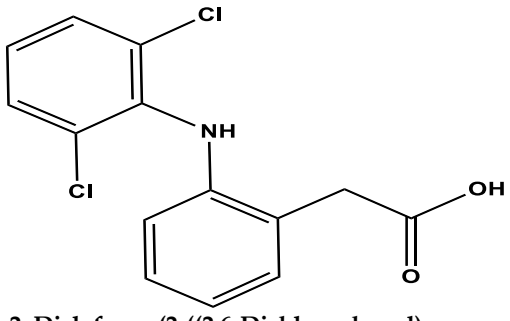

Fig. 2: Diclofenac (2-((2,6-Dichlorophenyl) amino benzene acetic Acid)

MATERIALS AND METHOD

Instruments and Reagents

The Instruments obtained for the study are follows; Hitachi HPLC machine (pump model L-2130), Intersil ODS-3V column (150mm × 4.6mm; 5µm pore size) and mechanical shaker. While the standard drugs and reagents include: paracetamol and diclofenac sodium (Sigma Aldrich). Solvents: methanol (Sigma Aldrich HPLC grade) and distilled water

Sampling and Sample Size

Using random selection, samples were selected from the different pharmacy premises as well as the patent stores. Dosage forms of tablets and syrup were used. In the same manner, pharmacies and the patent stores of each area were selected randomly. Three Hundred (300) tablets and Fifteen (15) syrups of paracetamol produced by five (5) different companies were randomly selected. Three hundred (300) tablets of diclofenac sodium produced by five (5) different companies were randomly selected. Different doses and types were randomly selected.

Data Analysis

Data analysis was carried out by using statistical package of social science (SPSS) program version 21 (SPSS, 2016). Data coding and entry, statistical examination such as frequency Crosstabs, chi square test, distribution, ANOVA and Post Hoc tests were carried out and the level of significance was performed at $p \leq 0.05$

Quantitative Determination of Paracetamol

Solutions of paracetamol were prepared using the standard powder and calibration curve was generated using various concentrations. 25 mg of the standard was dissolved in 2 mL of methanol and made up to 10 mL using distilled water. 1.5 mL of the resulting solution was diluted to 25 mL with distilled water. From the second solution 1, 0.8, 0.6, 0.4 and 0.2 mL were taken and diluted to 25 mL with distilled water to give 6, 4.8, 3.6, 2.4 and 1.2 µg/mL respectively. The graph plot of peak area against concentration was used to obtain the calibration curve. To quantify paracetamol, samples analyzed were prepared to obtain 150 µg/mL concentration. For tablets, quantity of powdered sample containing 25 mg equivalent of paracetamol was weighed and dissolved in 2 mL methanol and made up to 10 mL with distilled water. After filtration of the resulting solution, 1.5 mL was taken and diluted to 25 mL with distilled water to yield 150 µg/mL concentration. For syrups, 1 mL as measured and dissolved in 2 mL methanol and made up to 10 mL with distilled water. After filtration of the resulting solution, 1.5 mL was taken and diluted to 25 mL with distilled water to yield 150 µg/mL concentration.

The chromatographic conditions are: Column: Intersil ODS-3V, Column dimension: 150 mm × 4.6 mm; 5 µm pore size, Mobile phase: methanol: water (20 : 80), Flow rate: 2 ml/min, Injection volume: 10 µL Mode of elution: isocratic, Column temperature: 35^{O}C Detector: diode array detector (DAD L-2455) (Crevar *et al*., 2008).

Quantitative Determination of Diclofenac Sodium

Using the standard powder, solutions of diclofenac sodium was prepared. Various concentrations were used to obtain the calibration curve. 20 mg of each standard was dissolved in the mobile phase (methanol: water; 63:37) and made up to 25 mL using the same mobile phase as the diluent. 5 mL of the resulting solution was diluted to 10 mL with mobile phase. From the second solution 1mL was taken and diluted to 10 mL with mobile phase to

give 0.04 mg/mL. Further dilutions were made to get concentrations of 5, 10, 15, 20 and 25 µg/mL concentrations. Peak area was plotted against concentration to obtain the calibration curve. To quantify diclofenac sodium, samples to be analyzed was prepared to obtain 0.04 mg/mL concentration. For diclofenac sodium tablets, quantity of powdered sample containing 20 mg equivalent of diclofenac sodium was weighed and dissolved in mobile phase and made up to 25 mL with mobile phase. The sample was then filtered. After filtration of the resulting solution, 5 mL was taken and diluted to 10 mLwith mobile phase, 1 mL of the second solution was then diluted with mobile phase up to 10 mL to yield 0.04 mg/mL concentration.

The chromatogram condition are as follows;
Column: Intersil ODS-3V, Column dimension: 150 mm × 4.6 mm; 5 µm pore size, Mobile phase: methanl: water (63 : 37), Flow rate: 0.8 ml/min, Injection volume: 20 µl, Mode of elution: isocratic, Column temperature: 30 0C, Detector: diode array detector (DAD L-2455) (Korodi *et al*., 2012)

Abbreviations: USP: United States Pharmacopoeia; ANOVA: Analysis of Variance; BP: British Pharmacopoeia; HPLC: High Performance Liquid Chromatography; LSD: Least Significant Difference; OTC: Over-The-Counter; 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.

RESULTS

Quantitative Determination of Paracetamol

In quantitative analysis standard drugs are often used to obtain a background peak to which other peaks obtained will be compared. In fig 1 below. Standard paracetamol was run using HPLC, which gave a prominent peak with peak height at 2minutes.

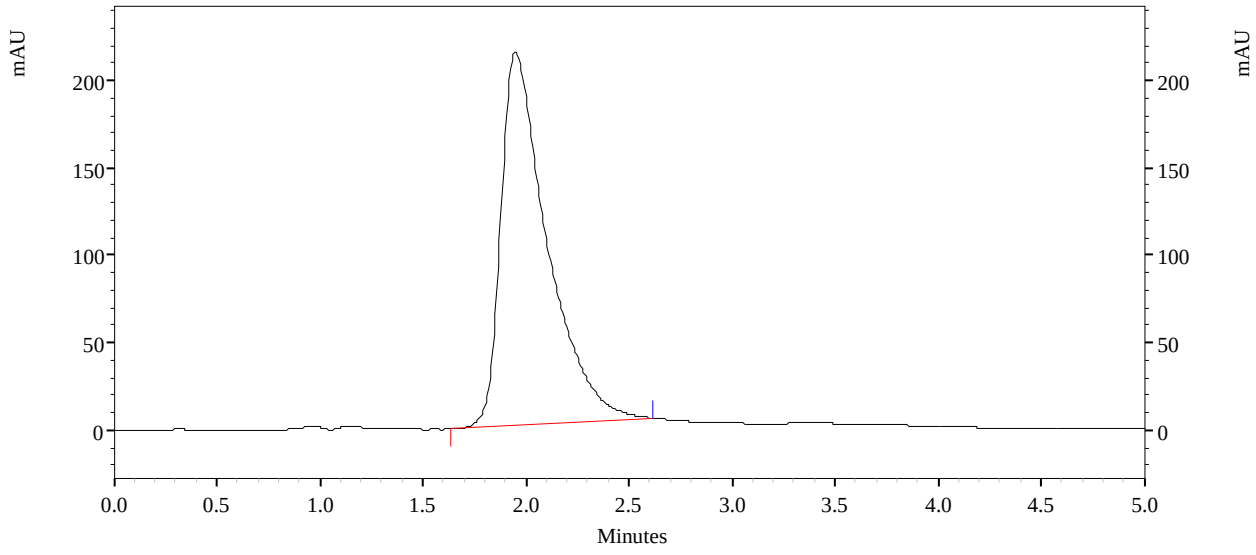

Figure. 3: HPLC Chromatogram of Paracetamol Standard at 150 µg/ml

Paracetamol Standard Calibration Curve

The absorbance of standard paracetamol solution was taken at 244 nm while regression analysis was carried out using microsoft excel 2016 program. It has the equation y = 87170x + 41505 and a correlation factor of R2 = 0.9587. The data was plotted as shown in figure 4

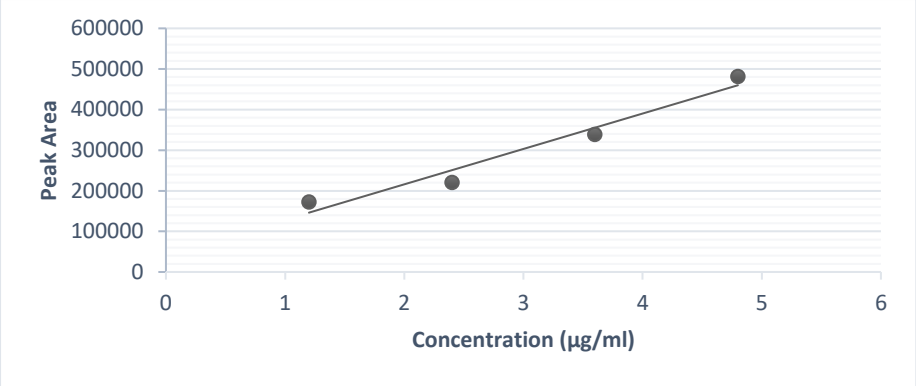

Fig. 4: Calibration Curve of Paracetamol Standard using HPLC

A list of description of the acronyms used in capturing all drug samples are shown in Table 1 below.

Table 1: Description of Acronyms of all Drug Samples

Sample ID	Description
PTH	Paracetamol Tablet Hospital
PSH	Paracetamol Syrup Hospital
DSTH	Diclofenac Sodium Tablet Hospital
PTCP	Paracetamol Tablet Community Pharmacy
PSCP	Paracetamol Syrup Community Pharmacy
DSTCP	Diclofenac Sodium Tablet Community Pharmacy
PTPS	Paracetamol Tablet Patent Store
PSPS	Paracetamol Syrup Patent Store
DSTPS	Diclofenac Sodium Tablet Patent Store

Paracetamol Constituents in assayed Tablet

Quantitative analysis of paracetamol tablets from different manufacturers was conducted. The percentage weight of paracetamol lies between 48 and 83%The results are expressed as percentage (w/w) and are summarized in Table 2.

Table 2: Paracetamol content in tablets

Sample ID	Percentage Content (w/w)	Milligram content (mg)
PTH-1	51	255
PTH-2	70	350
PTH-3	48	240
PTH-4	69	345
PTH-5	81	405
PTCP-1	72	360
PTCP-2	72	360
PTCP-3	81	405
PTCP-4	74	370
PTCP-5	83	415
PTPS-1	75	375
PTPS-2	74	370
PTPS-3	75	375
PTPS-4	72	360
PTPS-5	76	380

Paracetamol Content in Syrup

Quantitative analysis of paracetamol syrups from different manufacturers was conducted. The percentage weight of paracetamol lies between 72 and 120%. The results are expressed as percentage (w/w) and are summarized in Table 3.

Table 3: Paracetamol content in syrups

Sample ID	Percentage Content (w/w)	Mg content (mg/5ml)
PSH-1	88	110
PSH-2	92	115
PSH-3	95	118.75
PSH-4	102	127.5
PSH-5	92	115
PSCP-1	92	115
PSCP-2	90	112.5
PSCP-3	102	127.5
PSCP-4	90	112.5
PSCP-5	94	117.5
PSPS-1	98	122.5
PSPS-2	116	145
PSPS-3	72	90
PSPS-4	120	150
PSPS-5	110	137.5

Quantitative Determination of Diclofenac Sodium

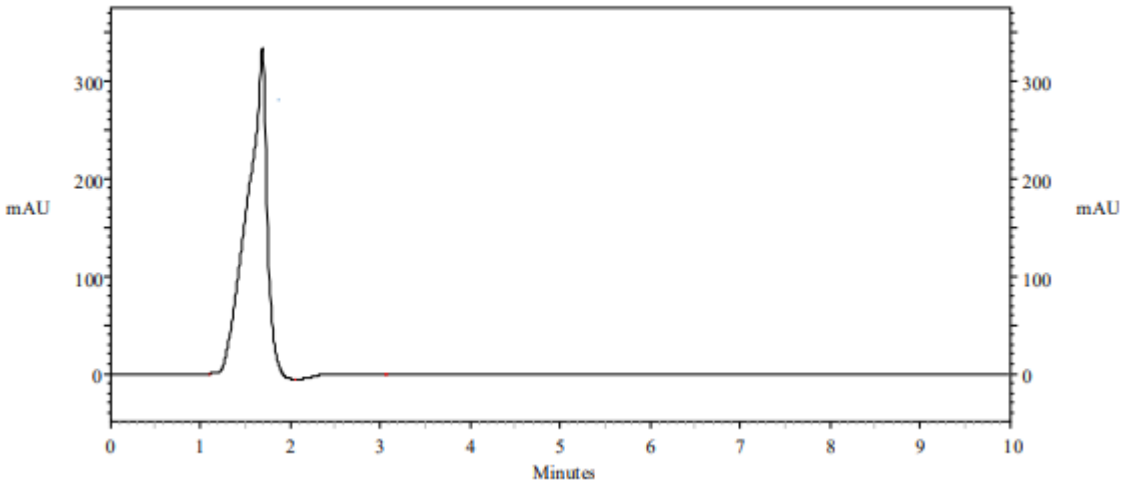

Figure 5: HPLC Chromatogram of Diclofenac Sodium Standard

Calibration curve of Diclofenac sodium standard

The absorption of diclofenac sodium standard solutions was measured at 283nm while regression analysis was carried out using microsoft Excel 2016 program. It gave the equation y = 5×10-9x – 0.0103 and a correlation factor of R2 = 0.9993. The data were plotted and shown in figure 6.

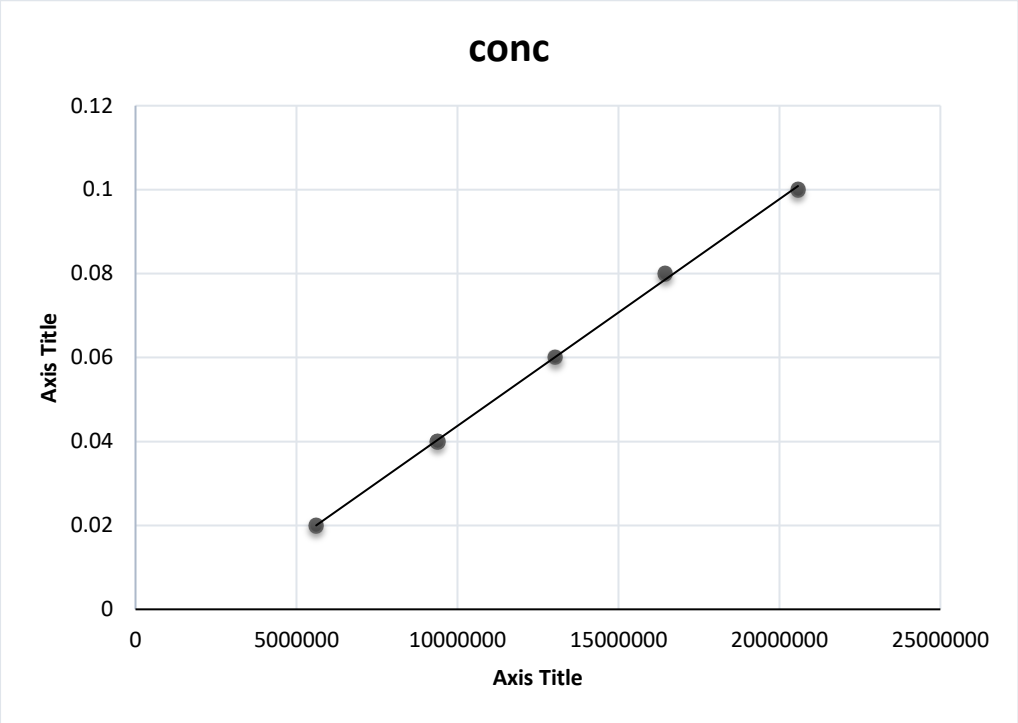

Fig. 6: Calibration curve of Diclofenac sodium standard using HPLC

Quantitative analysis of Diclofenac sodium tablets from different manufacturers was conducted. The percentage weight of diclofenac sodium lies between 73 and 174%. The results are expressed as percentage (w/w) and are summarized in Table 4.

Table 4: Diclofenac Content in Tablets

Sample ID	Percentage Content (w/w)	Milligram content (mg)
DSTH-1	139	69.5
DSTH -2	116	116
DSTH -3	80	40
DSTH -4	86	43
DSTH -5	88	44
DSTCP-1	94	47
DSTCP -2	174	174
DSTCP -3	85	85
DSTCP -4	117	58.5
DSTCP -5	85	42.5
DSTPS -1	73	36.5
DSTPS -2	95	47.5
DSTPS -3	108	54
DSTPS -4	92	92
DSTPS -5	81	40.5

The Correlation Between Analyzed and Reference Content of the Drug Samples

To test if the difference between the drug samples and sources of the drugs in relation to the percentage mg content is significant, ANOVA tests were applied. A p-value of 0.000079 (Table 5) for drug sample versus percentage mg content, and 0.669 (Table 6) for sources of drug versus percentage mg content, 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 5: Statistical Analysis of the drugs versus percentage milligram (mg) content

	N	Mean	Std. Deviation	Std. Error
PCM Tabs	15	71.5333	9.82611	2.53709
PCM Syrup	15	96.8667	11.95746	3.08740
Diclofenac Na Tabs	15	100.8667	26.72577	6.90056
Total	45	89.7556	21.81759	3.25237

Source of Variation	Sum of Squares	Degree of freedom	Mean Square	F	Sig.
Between Groups	7591.111	2	3795.556	11.938	0.000079
Within Groups	13353.200	42	317.933		
Total	20944.311	44			

Table 6: Statistical Analysis of the sources versus percentage milligram (mg) content

	N	Mean	Std. Deviation	Std. Error
Hospital Pharmacy	15	86.4667	22.98716	5.93526
Community Pharmacy	15	93.6667	25.13866	6.49077
Patent Dug Store	15	89.1333	17.52902	4.52597
Total	45	89.7556	21.81759	3.25237

Source of Variation	Sum of Squares	Degree of freedom	Mean Square	F	Sig.
Between Groups	397.511	2	198.756	0.406	0.669
Within Groups	20546.800	42	489.210		
Total	20944.311	44			

The Relationship Between Drug Samples and Percentage Milligram(mg) Content

There was statistical significance between PCM tabs and PCM syrup, Diclofenac Na tabs and PCM tabs as p-value < 0.001 after conducting Post Hoc Test and performing LSD. The results are listed in Table 7.

Table 7: Drugs versus percentage mg content one-way ANOVA post hoc LSD

Drug	Drug	Mean Difference
PCM Tabs	PCM Syrup	-25.33333*
	Diclofenac Na Tabs	-29.33333*
PCM Syrup	PCM Tabs	25.33333*
	Diclofenac Na Tabs	-4.00000
Diclofenac Na Tabs	PCM Tabs	29.33333*
	PCM Syrup	4.00000

* The mean difference is significant at the 0.05 level.

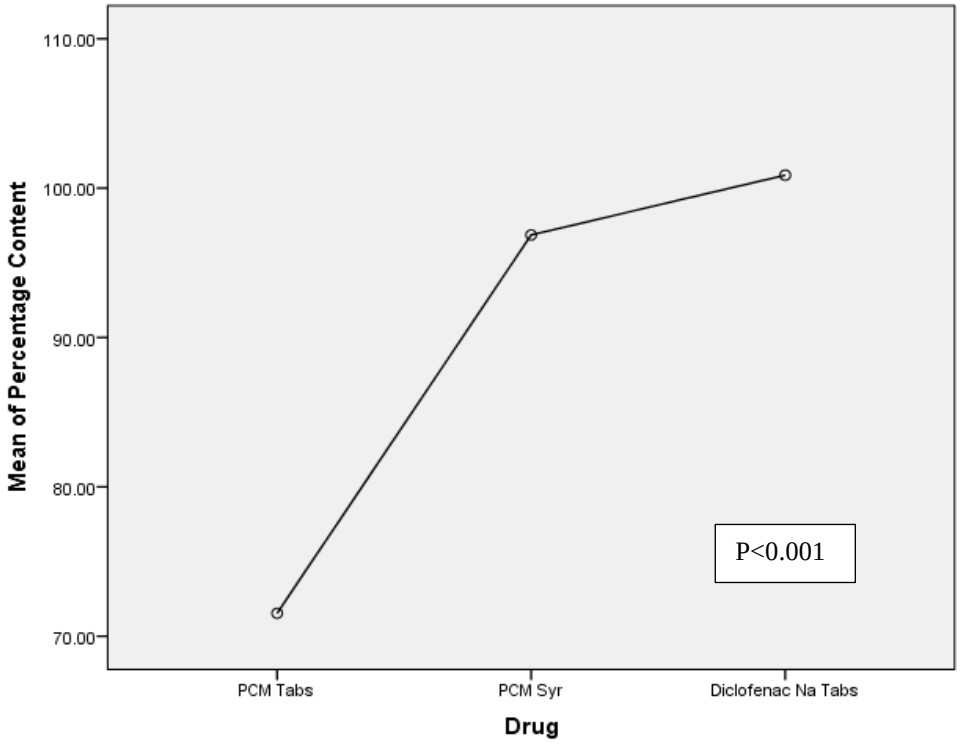

Fig. 7: Plot of the means of the drugs versus percentage mg content using HPLC

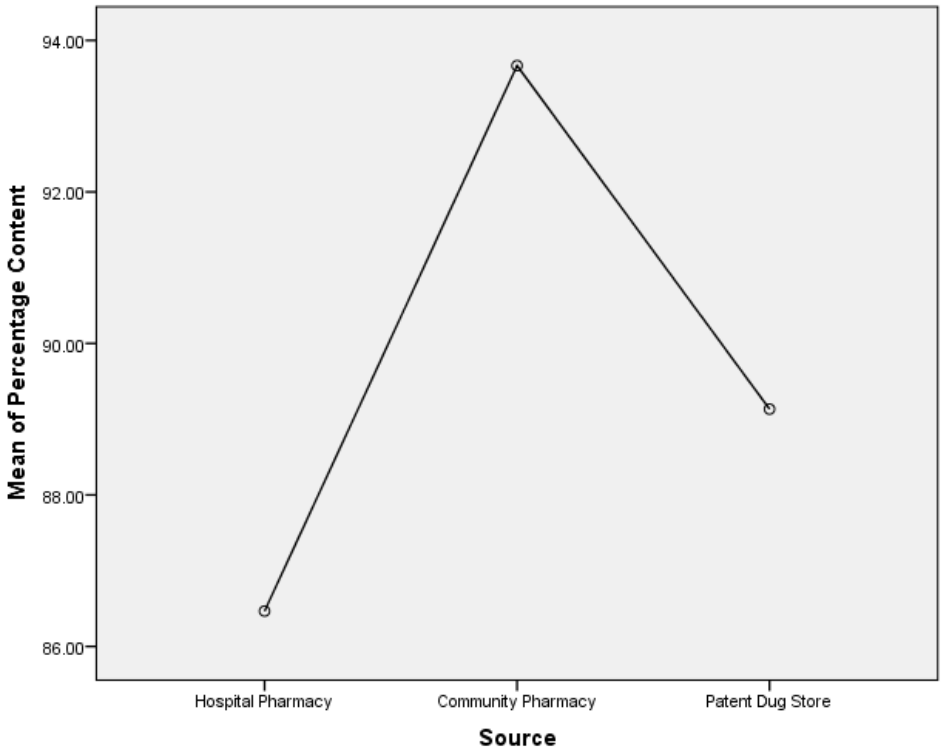

Fig. 8: Plot of the means of the sources versus percentage mg content using HPLC

The Relationship Between Diclofenac Sodium Content and Expired Date

Crosstabs Chi square test was applied to test the statistical relationship between the two variables; expired date and diclofenac sodium content among tablets. It was found that 100%

of the samples with shelf life 6 months or less were rejected and 0% were accepted, while 71.4% of the samples with shelf life more than 6 months were rejected and 28.6% accepted. The aspirin contents were categorized into two groups accepted and rejected according to the criteria of the USP, which allowed diclofenac sodium content of 95-105%. There was no statistical significance (P>0.05) between the expired date and the rejection of the samples . The results are summarized in Table 8.

Tablet 8: Crosstabs Chi square test for Diclofenac sodium content in tablets using HPLC

Shelf Life * Score Cross-tabulation

			Score		Total	Sig.
			Passed	Failed		
Shelf Life	> or = 6 months	Count	4	10	14	
		% within Shelf Life	28.6%	71.4%	100.0%	
		% within Score	100.0%	90.9%	93.3%	
		% of Total	26.7%	66.7%	93.3%	
	< 6 months	Count	0	1	1	1.000
		% within Shelf Life	0.0%	100.0%	100.0%	
		% within Score	0.0%	9.1%	6.7%	
		% of Total	0.0%	6.7%	6.7%	
Total		Count	4	11	15	
		% within Shelf Life	26.7%	73.3%	100.0%	
		% within Score	100.0%	100.0%	100.0%	
		% of Total	26.7%	73.3%	100.0%	

Correlation Between Analyzed and Reference Content of the Drug Samples

To test if the difference between the drug samples and sources of the drugs in relation to the percentage mg content is significant, two-way ANOVA tests were applied. The p-value was 0.000107 as illustrated in Table 9 for drug sample versus percentage mg content, 0.541 for sources of drug versus percentage mg content and 0.340 for the drug*source interaction versus percentage mg content. This means that there is significant difference between drug samples and percentage mg content but not between sources of drug and drug*source interaction and percentage mg content.

Table 9: Statistical Analysis of drugs, source and drug source Interaction versus percent milligram (mg) content

Drug	Source	Mean	Std. Deviation	N
PCM Tabs	Hospital Pharmacy	63.8000	13.91761	5
	Community Pharmacy	76.4000	5.22494	5
	Patent Drug Store	74.4000	1.51658	5
	Total	71.5333	9.82611	15
PCM Syrup	Hospital Pharmacy	93.8000	5.21536	5
	Community Pharmacy	93.6000	4.97996	5
	Patent Drug Store	103.2000	19.31839	5
	Total	96.8667	11.95746	15
Diclofenac Na Tabs	Hospital Pharmacy	101.8000	25.00400	5
	Community Pharmacy	111.0000	37.56993	5
	Patent Drug Store	89.8000	13.44247	5
	Total	100.8667	26.72577	15
Total	Hospital Pharmacy	86.4667	22.98716	15
	Community Pharmacy	93.6667	25.13866	15
	Patent Drug Store	89.1333	17.52902	15
	Total	89.7556	21.81759	45

Source of Variation	Sum of Squares	Degree of Freedom	Mean Square	F	Sig.
Drug	7591.111	2	3795.556	11.919	0.000107
Source	397.511	2	198.756	.624	0.541
Drug * Source	1492.089	4	373.022	1.171	0.340
Error	11463.600	36	318.433		
Total	20944.311	44			

* The mean difference is significant at the 0.05 level

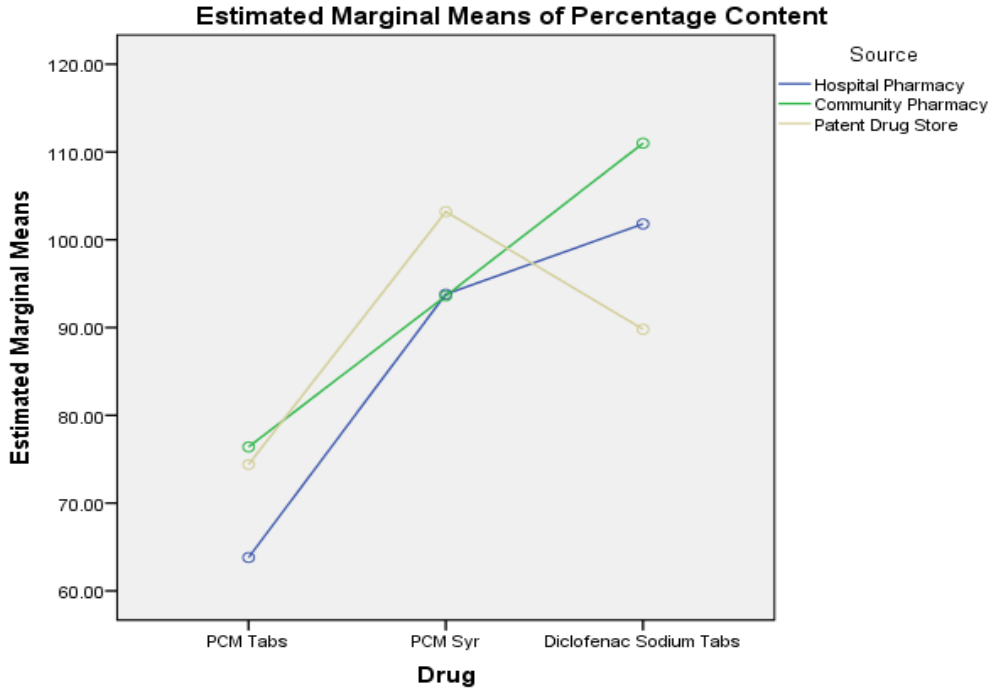

Fig. 9: Plot of the means of the drugs and sources versus percentage mg content using HPLC

DISCUSSION

Using the USP acceptance criteria, which described 90-110% of paracetamol content for tablets to be accepted, all paracetamol tablet samples were rejected as the percentage mg content was between 48 and 83%. In contrast not all paracetamol syrup and diclofenac sodium tablet samples were rejected (USP acceptance range 90-110%) as the percentage mg content were between 72 -120% and 73-174% respectively (USP 2017).

There was statistically significant difference between the class of drugs used and milligram (mg) content of the drugs $p < 0.001$ ($p = 0.000079$). Upon conducting post-hoc analysis (LSD), it was found that there was statistically significant difference between paracetamol tablet samples and the syrup samples, and as well between paracetamol tablet samples and the diclofenac sodium tablet samples as shown from the mean percentage content for paracetamol tablet samples (71.5%). The value is small when compared to that of paracetamol syrup and diclofenac sodium tablet samples at 96.9% and 100.9% respectively. The paracetamol syrup and diclofenac sodium tablet samples are comparable. However, there was no statistically significant difference between sources of the drugs and milligram (mg) content of the drugs, $p > 0.05$ ($p = 0.669$).

Upon conducting two-way ANOVA test, statistically significant difference was also observed between class of drugs and mg content of the drugs in question as p-value was <0.001 ($p = 0.000107$), but not between sources of the drugs and mg content of the drugs, $p > 0.05$ ($p =$

0.541) nor drug*source interaction (p = 0.340). However, the paracetamol tablet samples had low mean percentage mg content irrespective of the source.

All paracetamol tablet samples failed while 4 out of 15 paracetamol syrup samples also failed. For diclofenac sodium tablet samples, 4 out of 15 passed. This was in agreement with a similar study conducted by of Sani et al. (2015). His work reported an agreement with respect to statistical analysis results obtained (class of drug vs percentage mg content, class of drug vs source of drug and class of drug vs percentage mg content vs source of drug). Also, Osama et al. (2017) conducted a comparative study of Physical and chemical parameters of paracetamol tablet (10 brands) sold in Pharma Market in Libya. The findings showed that Seven (7) passed the USP requirement (within the range of 90-110 %) excluding three brands (Panadol (Tunisia), Parol (Turkey) and paracetamol tablet (UK)) that were considered inappropriate to be used in medical practice. Adeyemi et al. (2017) conducted a study using HPLC on 15 brands of Diclofenac tablets sold in Pharmacy stores within the mainland area of Lagos state. The Percentage content of the different Diclofenac tablets analyzed were within 4.58 % to 182.04 %, where only two samples (13.33%) passed the USP specified range of 90 – 110 %.

Only results of diclofenac sodium tablet samples were subjected to the chi-square test, paracetamol samples were not because all still had shelf-life greater than 6 months. There was no statistically significant difference between diclofenac sodium tablet samples with shelf-life greater or equal to 6 months and samples with shelf-life less than 6 months as the p = 1.00. This implies that, shelf-life left had no effect on the percentage mg content obtained.

CONCLUSION

High Performance Liquid chromatography (HPLC) technique was used successfully for quantitative determination of paracetamol and diclofenac sodium dosage forms sold within Maiduguri Metropolis. The percentage mg content for paracetamol ranged between 48 and 120 % while diclofenac sodium was between 73 and 174 %. All paracetamol tablet samples were rejected, 73% of paracetamol syrup samples were accepted and 27% of diclofenac sodium tablet samples were accepted. Statistically significance difference was observed between class of drug and percentage mg content but not between source of drug and percentage mg content.

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