COMPARATIVE PHYSICAL, CHEMICAL AND MICROBIOLOGICAL STUDY OF COMMERCIALLY AVAILABLE PARACETAMOLTABLETS IN LOCAL MARKET: A CASE SUDY

*Azeem Arif Aziz[1] and Kashif Ahmed[1]
[1] Department of Chemistry, N.E.D. University of Engineering & Technology, Karachi, Pakistan
*azeem.arif.aziz@hotmail.com, kashif25473@yahoo.com

ABSTRACT: *The present work is directed toward the comparative study of physical, chemical and microbiological properties of different Multinational and local brands of paracetamol 500 mg in solid dosage form available in Karachi pharmacies. Pharmaceutically and chemically equivalency of all selected brands have been evaluated including thickness, diameter, hardness, friability, disintegration, % content by Pharmacopeial method. Additionally the microbial limit test, content uniformity by weight variation and dissolution also been performed. The results revealed that appearance & mostly physical parameters as well as microbial test are within the acceptance criteria of all brands while the assay, Disintegration and content uniformity by weight variation of local brands fails to comply the acceptance criteria. When drug release profile is compared against reference brand (brand-7 Multinational brand) it is revealed that less than 85% drug release in brand 2, 5 and 6 within 60 minutes and not meet with the acceptance criteria of drug release profile. On the basis of this analysis it can concluded that all these brands are not equivalent and the use of such type of substandard medicine will not be efficient for the patient hence it is the responsibility of the manufacturer to provide the safe and efficient medicine which is intended for use at the same time the local regulatory must ensure the availability of quality medicine in the local market.*

1. INTRODUCTION:

Paracetamol is the most frequently use OTC (Over the counter) medicine that is use to manage headache, flu, fever, cough and mild aches [1, 2, 3]. Paracetamol with different actives such as caffeine, pseudoephedrine, dextromethorphan, trimethoprim etc are also available in market [4]. Paracetamol is available as solid as well as in liquid dosage form [5]. The overdose of paracetamol can cause serious harm to liver and kidney, its use is limited in case of liver disease and history of alcoholism [6, 7, and 8].

The current study is mainly based on most significant issue, to evaluate the quality of a pharmaceutical products available in the local market because drug products must be chemically, biologically and pharmaceutically equivalent and identical in strength, quality, purity, active ingredient release profile and dosage form.. For this reason Paracetamol 500 mg tablet is selected for the evaluation of their quality. Brands were chosen on the basis of availability and purchased from the local market of Karachi city and its quality is checked by using different physical, chemical parameter and biological parameters.

Wide usage of this drug is one of the reasons for its selection and numerous producers including multinational and local companies manufacture this with variable price ranges. Numerous pharmaceutical companies are busy to manufacture thousands of drug medicine in Pakistan; these generic Medicines are easily obtainable in the market without any proper bioequivalence assessment [9, 10, 11]. Accessibility of right and quality medicine is also a big concern in Pakistan due to the use of counterfeit drugs [12]. In Pakistan various pharmacies are running without accredited pharmacist [13, 14]. Medicines are distributed without the supervision of pharmacist and many times without any prescription. Sometimes local operating pharmaceutical companies do not fully comply cGMP (current good manufacturing process) practices that results in production of medicine that can categorized as substandard [15, 16, 17]. This comparative study capture all this issues related to quality of drug product [18, 19, 20, 21, 22].

2. MATERIAL AND METHOD

Chemical and Reagents

Reagents that were used in this study were methylene chloride (analytical grade), Methanol (HPLC grade) from Merck Germany, USP Paracetamol reference standard from sigma Aldrich, Mono Basic potassium phosphate (analytical grade) and Sodium hydroxide (analytical grade) from Fischer scientific.

Apparatus

Make of glassware was of Class A Pyrex included Beaker, Jerry Can 10 Liter, HPLC vials, Volumetric Flasks of different volume, 2 Liter Mobile phase Bottle, Plastic Cups, Stainless steel spatula.

Preparation of pH 5.8 buffer Dissolution Medium

Dissolved around 68 gm of potassium dihydrogen phosphate and 36 ml of 1N NaOH and make up with water to produce 10 liter of solution (adjust pH to 5.8 if necessary).

Preparation of 0.1 N NaOH for Dissolution

Used 4.0 gm of sodium hydroxide in 1 liter flask volumetric flask, added 500 ml of water, shake to dissolve and allowed to cool at room temperature and made up the volume up to 1000 ml.

Preparation of Mobile Phase for Content determination

Methanol (2000 ml) and Water (6000 ml) were added in 10 Liter jerry can. Filtered through 45 µm and degassed through filtration and sonication.

Preparation of Standard Solution for Content determination

Paracetamol (10 mg) USP standard in 100 ml volumetric flask dissolved and dilute with mobile phase (stock standard). Prepared 100 ml by diluting 10 ml from stock to 100 ml with mobile phase (working Standard).

Preparation of Test Solution for Content determination

Around 110 mg of each brands powder obtained by crushing 20 tablets of each brands was taken into 200 ml volumetric flask added 100 ml of mobile phase sonicate, dissolved and dilute up to 200 ml with mobile phase (stock Test solution).

Prepared 250 ml by dilute 5 ml from stock test solution into 250 ml with mobile phase.

Instrumentation and Parameter

For Assay content, HPLC system (LC solution Software), LC -20 AT, SPD-20A, SIL10A, CBM-20A, LC-20 AHCT (Auto sampler) with parameter L1 packing column at LC mode ,10 µL injection volume elute at 1.5 ml\min at 243nm.Dissolution apparatus DIS-6000 COOPLY work at rate of 75 rpm speed in 45 minutes at body temperature 37.5 degree Celsius. Tablet disintegrator automatic (Logan instrument DST-3), Tablet hardness tester (pharmetest), Ultrasonic Cleaner (UC-10P JEIO TECH), UV spectrometer 1700, Friblator, mobile phase recycler chromatographic technology and Analytical Balance JP105DUG Mettler Toledo.

Quality Assurance

All tests have been performed according to the approved British and United State pharmacopeia methods [20, 21]. A Latest 2016 publication of pharmacopeias is being use for this study to maintain reliability of overall test results for comparison purpose.

Sample Collection and Study Design

7 Brands have been selected for comparison purpose. For this 100 tablets of each brand have been purchased in different pharmacies located in Karachi.

These seven brands are belongs to local and multinational Company. Breakups of these brands are as follow. Brands 1, 3, 7, are of multinational company and 2, 4, 5, 6 are from local company.

Quality of Paracetamol 500 mg tablets of each brands have been evaluated by performing Physical, Chemical and Microbiological test by in-vitro Mean. These same tests are performed by individual pharmaceutical company to analyzed quality of their products. Physical, chemical and microbiological tests of different brands of paracetamol tablet are performed are mentioned below.

Weight Variation: weight of 10 random tablets from each individual brands was carried out through analytical balance and calculate the average and weight variation.

Limit: No more than 2 tablet deviate by 5 %.

Hardness: Hardness of ten individual tablets was determined through Tablet hardness tester (pharmetest).

Limit: Hardness is not more than 15 kilo Pascal is the acceptance requirement.

Diameter and Thickness: Diameter and Thickness of 10 units was determined through Tablet hardness tester (pharmetest) having integrated with diameter, length and thickness measurement.

The proportionality tolerance is about ±3% to ±5% for both diameter and thickness.

Disintegration: Disintegrator automatic (Logan instrument DST-3) was used to find out the Disintegration time of six individual tablets from each 7 brands operated at 37±2ºC for 45 minutes.

Friability: Friblator is operated at 25 RPM for about 4 minutes and calculated the loss by the given formula. % friability = (Initial wt – final wt)/ (initial wt) x 100

Limit: .Maximum loss NMT 1 %.

Content Uniformity: It is uncover by using USP Method. Content uniformity by weight variation is calculated by taking 10 individual tablet weights , assay and average weight of 10 tablets of individual brands.

Limit: NMT 7.5 percent variation.

Assay: Twenty tablets of each brands is crushed into finely divided powder using motel and parcel. Compression weight is calculated by taking 10 tablets average weight by using Analytical Balance JP105DUG Mettler Toledo and use as multiplication factor for content determination. Reference Standard use for content analysis is 99.8 % pure. Dilution factor is use to calculate concentration constant by using formula included standard weight, dilution and purity.

Limit: The content must be lie within 90% to 110%.

Drug release profile: Dissolution apparatus DIS-6000 COOPLY work at rate of 75 rpm speed in body temperature 37.5 degree Celsius is used for evaluating dissolution study of different brands.5. 8 pH dissolution buffer is use to release drug content in 60 minutes .Absorbance of Solution is measured at 243 nm in UV spectrometer 1700.

Limit: NLT 80 %(Q) of content dissolved.

Microbiological Test: Total aerobic Microbial count, total yeast and mould count, pseudomonas aregonisa and gram negative test has been performed for each local and multinational paracetamol brand .

3. RESULTS AND DISCUSSION

Table 1: Area of standards (Paracetamol)

AREA	Reference Standard-01	280799	% RSD
	Reference Standard-02	280986	1-5 Ref Standard
	Reference Standard-03	280708	0.06%
	Reference Standard-04	280932	Response Factor=RF
	Reference Standard-05	280510	(Areax100) / (Standard wt x Purity)
	Check Standard-01	281966	
	Check Standard-02	281642	0.536 %

In the present comparison study seven brands of paracetamol tablets with different trade name were tested by Pharmacopeial test methods.

Appearance of each tablet is found satisfactory with respect to shape, cleanness, color, damage and dirt or any foreign contamination.

Table-2 shows the results of physical parameter e.g. thickness, hardness, weight variation and diameter including their standard deviation and relative standard deviation. Results show that all the brands comply with the acceptance limit. Hardness and Diameter was graphically represented in Fig 1 & 2 respectively.

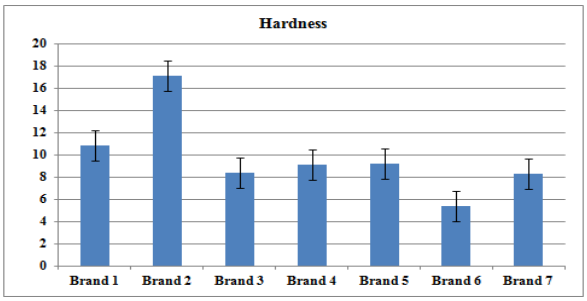

Figure 1: Hardness of different brands with Standard deviation plot

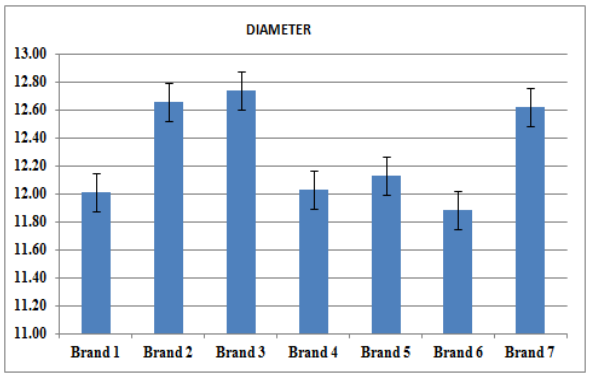

Figure 2: Diameter of different brands with Standard deviation plot

Table-3 shows the results of Disintegration, assay, Friability, uniformity of dose by weight variations and Microbial limit test. Results shows that friability and Microbial limit test are within the acceptance limit.

Assay performed by using HPLC, successfully met system suitability criteria with 0.068% RSD, 1.6 tailing factor, and 2160 theoretical plates. Assay results all the brands fulfill their label claim except brand-6 that is 7.6%.

Chromatograms of each brands is attached for reference in Fig 3, 4, 5, 6, 7, 8 and 9 respectively. Brand 6 contains an extra peak in line with a paracetamol peak. In brand 6 peak area of paracetamol is low and show low response in term of mV.

Standard precision is used to check the system response upon change in weights. Its limit for assay determination is about 1.5% and for impurity determination limit should be within 5.0 %. Therefore two standards are run throughout the batch table one for calculation and system suitability and other for standard precision.**(Table 1)**

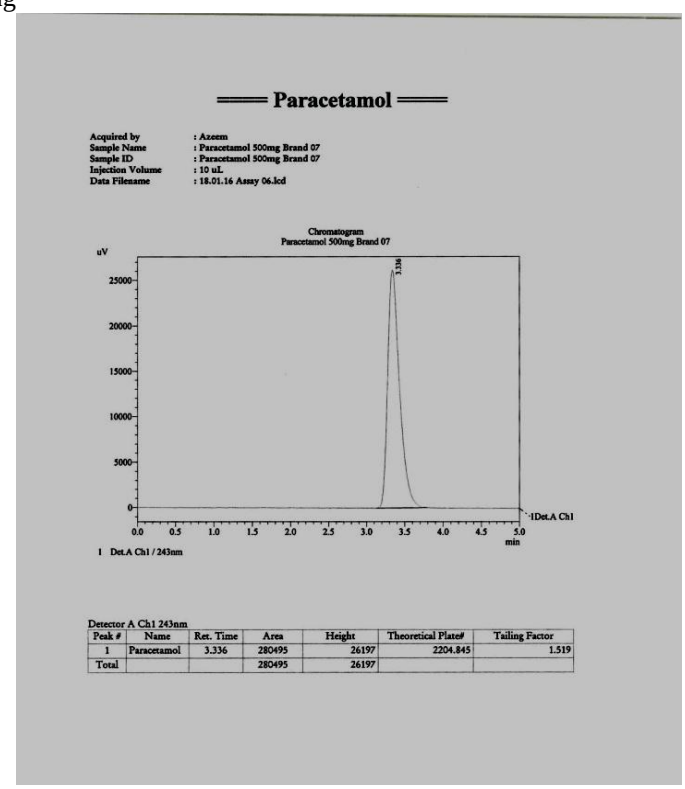

Figure 3: HPLC Assay Chromatograms of Brand -7 (Reference/Multinational Brand)

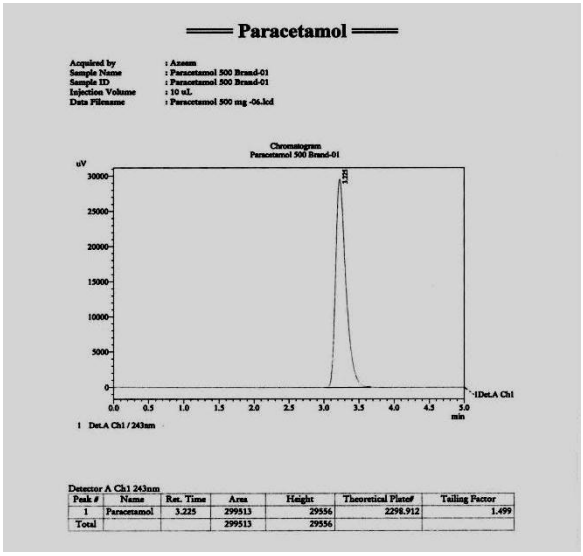

Figure 4: HPLC Assay Chromatogram of Brand-1

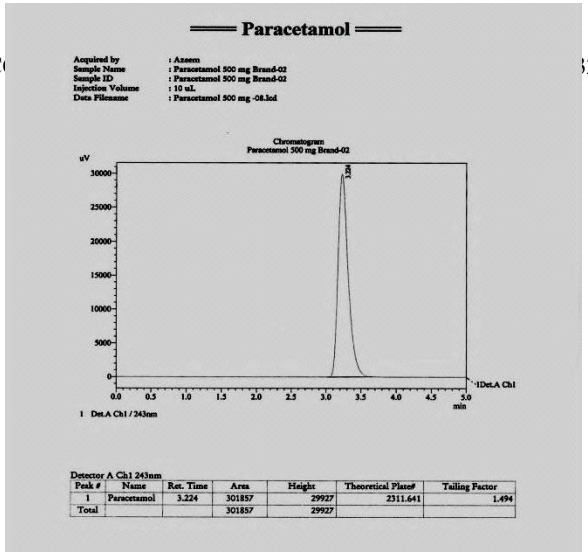

Figure 5: HPLC Assay Chromatogram of Brand-2

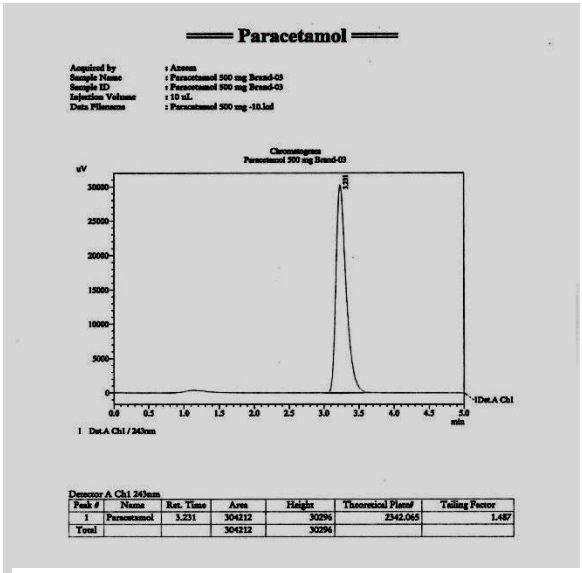

Figure 6: HPLC Assay Chromatogram of Brand-3

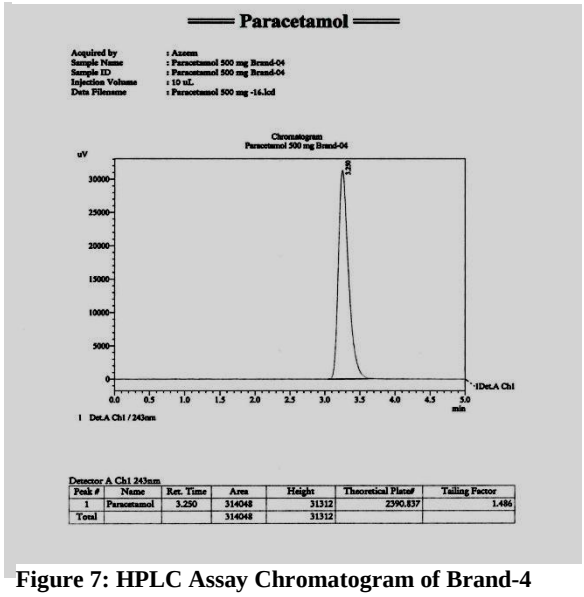

Figure 7: HPLC Assay Chromatogram of Brand-4

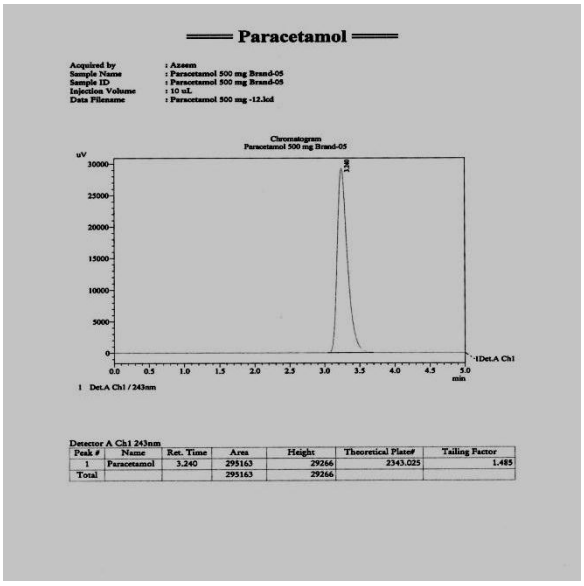

Figure 8: HPLC Assay Chromatogram of Brand-5

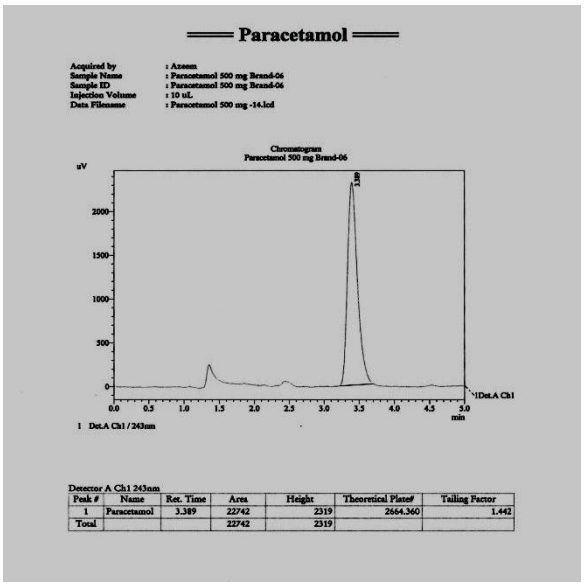

Figure 9: HPLC Assay Chromatogram of Brand-6

Table 2:

Physical parameter of different Brands

Physical Parameter	Thickness			Hardness			Weight Variation			Diameter		
	Mean	S.D	RSD %	Mean	S.D	RSD %	Mean	S.D	RSD %	Mean	S.D	RSD %
Brand-1	4.48	0.05	1.2%	10.9	0.90	8.3%	0.5907	0.01	1.4%	12.01	0.013	0.1%
Brand-2	4.75	0.03	0.5%	17.13	0.80	4.7%	0.6463	0.01	1.6%	12.66	0.026	0.2%
Brand-3	4.06	0.04	0.9%	8.41	0.30	3.6%	0.5816	0.14	2.5%	12.74	0.023	0.2%
Brand-4	4.58	0.13	2.9%	9.13	0.58	6.3%	0.5565	0.01	1.6%	12.03	0.019	0.2%
Brand-5	4.29	0.06	1.4%	9.26	0.74	8.0%	0.5628	0.01	1.3%	12.12	0.01	0.1%
Brand-6	4.32	0.10	2.3%	5.4	0.71	13.1%	0.5102	0.01	1.2%	11.89	0.01	0.1%
Brand-7	4.42	0.01	0.3%	8.3	0.43	5.2%	0.6092	0	0.6%	12.61	0.01	0.2%

Table 3 Assay, Disintegration, Friability, uniformity of dosage by weight variation and Microbial test

Parameter	Assay		Disintegration	Friability	Uniformity of Dosage by Weight Variation	TAMC	TYMC	Pathogens
Results	**mg/tab**	**% L.S**	**Min**	**%**	**Acceptance Value %**	**cfu/ml**	**cfu/ml**	**Status**
Brand-1	503	100.3%	2.3	0.1 %	1.66%	< 0	< 0	Absent
Brand-2	508.2	101.6%	45	0.2%	2.12%	< 0	< 0	Absent
Brand-3	502.6	100.5%	0.5	0.15 %	3.18%	< 0	< 0	Absent
Brand-4	521.2	104.2%	1.25	0.24%	3.3%	< 0	< 0	Absent
Brand-5	501.2	100.2%	35	0.1%	5.87%	< 0	< 0	Absent
Brand-6	37.2	7.4%	0.53	0.5%	90.02%	< 0	< 0	Absent
Brand-7	501	100.1%	1.0	0.13%	0.17%	< 0	< 0	Absent

Table 4: Drug release profile

DRUG RELEASE PROFILE (%)							
Time (min)	**Brand-1**	**Brand-2**	**Brand-3**	**Brand-4**	**Brand-5**	**Brand-6**	**Brand-7**
5	39.4%	3.7%	42%	29.4%	8.6%	2.3%	44.8%
10	68.2%	19.8%	77.4%	68.4%	23.3%	6.4%	89.1%
15	87.1%	32.6%	85.9%	91.9%	37.4%	11.2%	99.4%
30	98%	41.4%	99.8%	96.4%	38.5%	15.4%	100.3%
45	99.2%	48.9%	99.9%	98.3%	41.8%	27.6%	100.3%
60	100.2%	61.3%	99.9%	98.8%	49.5%	39.6%	100.8%

Brand 2 and 5 failed to comply the disintegration criteria.

Uniformity of dosage of weight variation of brand 6 is found to be 90.02% that is too high as compare to the Pharmacopeial requirement NMT 7.5%.

% drug release is displayed in Table-4. Results show that more than 85% of drug is released within 30 minutes in brand 1, 3, 4 and 7. Brand 2, 5 and 6 failed to meet the USP criteria as less than 85% of drug released within 60 minutes. Release Graph is shown on fig 10.

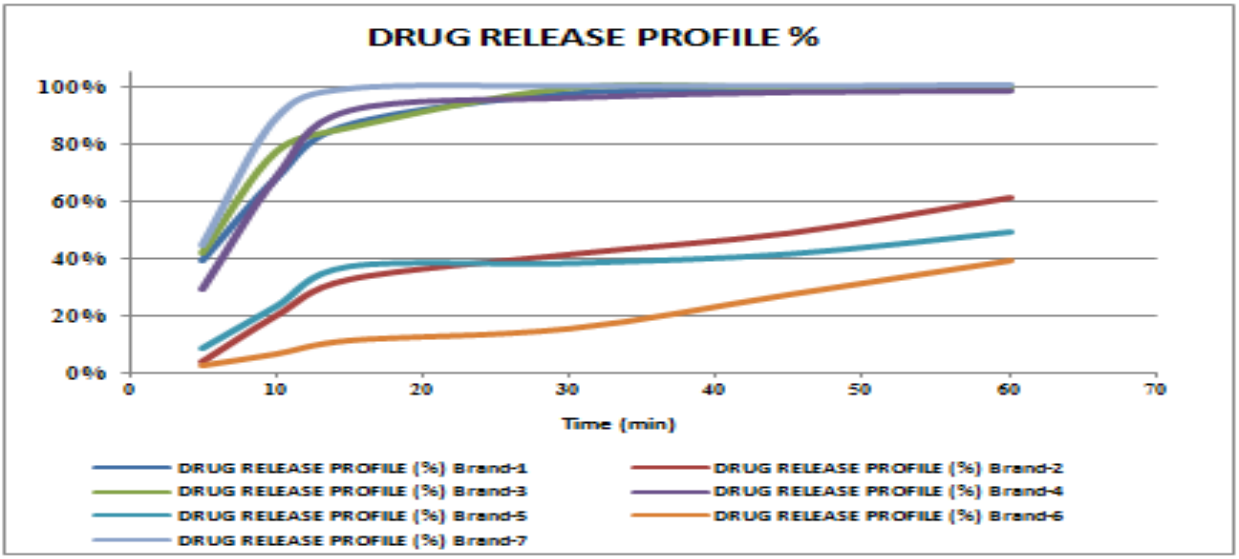

Figure 10: Drug Release Profile of Different Brands

4. CONCLUSION

On the basis of above observations following conclusion can be made

- It has been observed that appearance and the physical test such as weight variation, hardness, thickness and diameter were within the acceptance limits of all the brands.
- The assay results, disintegration time and weight variation of some of the local brands fails to meet the acceptance criteria.
- % drug release of some of the local brands was not within the defined limits.

Pakistan is one of those developing country in where health facilities is not easily accessible, lack of pharmacist in hospitals and retail pharmacies to direct the consumers and patients, resources are less for the population. Due to existence of counterfeit and substandard medicine in local market it is very difficult to distinguish them from the original one. It's all due to some local manufacturer doesn't care of cGMP and during manufacturing of drugs. It is the responsibility of health care regulatory and health care authorization to ensure the quality, safety with bio equivalency and right efficacy of the drugs reach to the consumer and patients that is fit for use and safe as well.

REFERENCES:

[1] Joydeb, N. et al, "market research and recommendation on packaging and labeling of Various OTC (over the counter) drugs available in Bangladesh," *International journal of science and technology*, The Experiment, **10**(3):651-662(2013).

[2] Saha, S, Dey, B. K. Chakraborty, S. Choudhury, T., "Development of reverse phase high performance liquid chromatographic method and method validation of paracetamol by using economical single mobile phase," *Journal of applied pharmaceutical research*, **5**(6):56-75(2013).

[3] Afifi S.A. Al Gohary, O.M.N. Al-Shalabi, R. Eltahir, E.K.D. and Darwish, H.W., "Comparative Evaluation of the Pharmaceutical and Chemical Equivalence of Some Commercial Brands of Acetaminophen Tablets*," Life Science Journal*, **10(**3):2013.

[4] Aziz, A.A. Ahmed, K., " Simultaneous determination of paracetamol with different Active pharmaceutical ingredient (API) and Excipient in Various dosage forms," *sci.int.(Lahore)*, **27**(6),6173-6176(2015).

[5] Mathur, N. Kumar, R. Tiwari, K. Singh, S. Fatima, N., "Evaluation of quality control parameters on various brands of paracetamol," *world journal of pharmacy and pharmaceutical sciences*, **4**(7):976-984(2015).

[6] Dixit, R.B. and Patel, J.A., "Spectrophotometric determination of paracetamol drug using 8-hydroxyquinoline," *International journal of Pharmaceutical sciences and research*, **5**(6):2393-2397(2014).

[7] Obuekwe, C.O. Obuekwe, I.F. Ogbimi, A.O., "Changes in the disintegration properties of some brands of paracetamol tablets inoculated with four bacterial species," *Polish pharmaceutical society*, **58**(1):17-20(2001).

[8] Al-Shalabi, R. Al-Gohary, O. Afify, S. Eltahir, E., "Comparative Evaluation of the Biopharmaceutical and Chemical Equivalence of the Some Commercial Brands of Paracetamol Tablets," *International Journal of Medical, Health, Biomedical, Bioengineering and Pharmaceutical Engineering*, **6**(9): (2012).

[9] Nagabandi, V. Kumar, M.S. Someshwari, G.P.K. Varaprasad, A., "Comparative Dissolution Studies of Marketed Preparations and Treatment of Data by Using ANOVA," *International Journal of Advances in Pharmaceutical Science*, ISSN: 0976-**1055**(1):142-146(2010).

[10] Jadge, D.R. deshpande, A.N. gadugul, Y.A. patil, O.P., "Comparitive in-vitro evaluation of different brands of paracetamol tablets in maharstra state," *International Journal of Pharmaceutical Science and Health Care*, **4**(4):40-47 (2014)

[11] Igbinaduwa P.O. Aghayere, GE., "Determination of lead in paracetamol oral liquid dosage formulations in Nigeria," *African journal of pharmaceutical research & development*, **7**(1):37-44(2015).

[12] Chandrasekaran, A.R. Han, Y.C. Chung, A.C.Y. Cheang, L.W. Ping, S.L., "Post market in-vitro equivalency evaluation of paracetamol tablets in Kedah, Malaysia," *International journal of pharmaceutical and nanotechnology*, **4(**2):1403-1407(2011).

[13] Hundie, K. Debella, A. Hymete, A.,"Short Communication: Comparative Quality Evaluation of Some Brands of Paracetamol Tablets, Suppositories and Syrups," *Ethiopian Pharmaceutical Journal*, **26**(1):59-64(2008).

[14] Mamatha, T. Raheem, MD. Pasha, S. Zubair, MD., "Comparative In-Vitro Evaluation of Marketed Paracetamol Tablets," *Research Journal of Pharmaceutical Dosage Forms and Technology***,** **1**(2):100-102(2009).

[15] Audu, S.A. . Taiwo, A.A.E. Okuolape, F.I.W. Sani, A.S. Bukola, A.R Mohammed, I., "Comparative Evaluation Study On Different Brands Of Lisinopril Tablet Using Hplc And UV Spectrophotometer," *Journal of general science results***, 2**(7):(2012)

[16] Deisingh, A.K., "Pharmaceutical counterfeit," Analytical Abstracts, 130:271-279(2005).

[17] Shakoor, O. Taylor, R.B. Behrens, R.H., "assessment of incidence of substandard drugs in developing country*," Tropical medicine and international health*, **2**(9):845-851(1997).

[18] Rani, T.N. , Kumari, C.T. L, Reddy ,B.M. Reddy, B.D.R., "comparative in vitro evaluation of different commercially available brands of pantoprazole tablets," *International journal of Pharmaceutical sciences and research*, **3**(4):1108-1111(2012).

[19] Jakaria, Md. Hasanat, A. Adnan, Md. Nazimuddin, Md. Mousa, A.Y., "Comparative degradation study of different brands of Rabeprazole tablet using UV-spectrophotometer," *American journal of chemistry and application*, **2(**2):32-35(2015).

[20] Howtan, K. Bergen, H. Simkin, S. Arensman, E. Corcoran, P. Cooper, J. Waters, K. Gunnell, D. Kapur, N., "Impact of different pack size of paracetamol in the united kingdom and Ireland on international overdoses: a Comparitive study," *Biomed Central public health*, **11**:460(2011).

[21] Martino, R. Malet-Martino, M. Balayssac, S. Gilard, V., "counterfeit drugs: analytical techniques for their identification," *Springer* **398**(1):77-92(2010).

[22] Rodomonte, A.L. Gaudiano, M.C. Muleri, N., "Counterfeit drugs detection by measurement of tablet and secondary packaging color," Science direct**,** **53**(2):215-220, (2010).

[23] Bate, R. Jensen, P. Hess, K. Mooney, L. Milligan, J., "substandard and falsified anti-tuberculosis drugs: a primarily field analysis," *Social science research network, **17(**3):308-311(2013).*

[24] USP-31, NF 26 (2), Page 18.1 May, 2014

[25] British Pharmacopeia, paracetamol monograph 2016.