

Quantitative Determination of Metformin Hydrochloride Tablet of Different Brands Available in Malaysia by UV Spectrophotometry and Fourier Transform Infrared Spectroscopy (FTIR)

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ABSTRACT

The present study aims to compare the quantitative determination of metformin hydrochloride brands available in Malaysia by spectroscopic method and Fourier transform infrared spectroscopy. Several different commercially available leading brands of metformin, within their shelf-life were purchase from various pharmacy outlets in Sungai Petani, Malaysia. Each brand of metformin hydrochloride tablets was labelled to contain 500 mg of metformin hydrochloride. The metformin tablets were blindly named as Brand A, Brand B, Brand C, Brand D, Brand E, Brand F and G by spectroscopic method and Fourier transform infrared spectroscopy in the present study. The average percentage purity of sample A, B, C, D, E, F and G was 99.15%, 99.03%, 99%, 99.66%, 98.31%, 99.42% and 99.38%. The brand A, B, C, D, F, and G passed the test whereas brand E failed the test according to the United States Pharmacopeia (USP). Several brands of Metformin Hydrochloride being assessed in this research, it showed that most of the brands were within the pharmacopoeia limits for various quality control parameters except one. Through the FTIR spectral measurements, a satisfactory vibrational band assignment of metformin hydrochloride has been carried out.

From the outcome of the various tests, it indicated that the process validation data provides a high degree of assurance of the manufacturing of these tablets' products meet its predetermined specification and quality attributes.

Keywords: U.V. Spectrophotometer; FTIR; Netformin; U.S. Pharmacopeia; Analysis

Introduction

Metformin is a class of drugs named biguanides prescribed for type 2 diabetes in the result of debilitated insulin discharge [1]. Treatment decision contains, oral antihyperglycemic and diet / insulin [1]. Metformin is an oral biguanide, enhances hyperglycemia to improve peripheral sensitivity toward insulin and reduces

gastrointestinal glucose formation [1]. As sulfonylureas, which doesn't stimulate secretion of insulin, aggravate hyperinsulinemia or because hypoglycemia or gain of weight (weight balances or reduces). It has also useful effects on serum lipid profiles. Metformin is sheltered and successful equally as monotherapy

and mix through antihyperglycemic specialists to sort diabetic 2 patients' needful extra glycemic control might be advantage when control of weight is desirable [1]. Metformin is currently a first-line treatment for Type 2 diabetes [2-5]. It is not insulin but is considered as insulin sensitizer [6,7]. It is in the biguanide family, isolated from the flower *Galega officinalis*, commonly known galega or goat's-rue and was used as treatment for diabetes in the medieval period because it relieved the intense urination [8]. The guanidine structure of metformin can be thought of as a nitrogenous analog of carbonic acid and includes two methyl groups, whereas phenformin and buformin have apparently toxic aromatic rings or alkyl chains.

In Figure 1, the differences between phenformin and metformin can be observed. The botanical *Galega officinalis*, previously mentioned, was given to diabetic patients because it relieved the excessive urination symptom. *G. officinalis*, also known as either

with the name of French lilac or Italian fitch was also given as a medicine during the plague epidemics to perspiration [8]. In the French lilac, there is an active ingredient that can lower the blood glucose level and the ingredients was shown to be galegine or isoamylene guanidine (Hakeem-Habeeb, B.,2011). Metformin's official chemical name is N, N-dimethylimidodicarbonimidic diamide (also called 1, 1-dimethylbiguanide) or $C_4H_{11}N_5$ [9]. In 1922, metformin was described firstly by Werner and Bell, and it involved a precipitation reaction of dimethylamine hydrochloride and 2-cyanoguanidine over heat. This one step synthesis can be seen in Figure 2 [10]. Metformin hydrochloride, the salt version of the drug, is synthesized via the reaction of equimolar quantities of 2-cyanoguanidine with dimethylamine and thus made into a solution using toluene with the process of cooling, with equimolar amounts of hydrogen chloride slowly added.

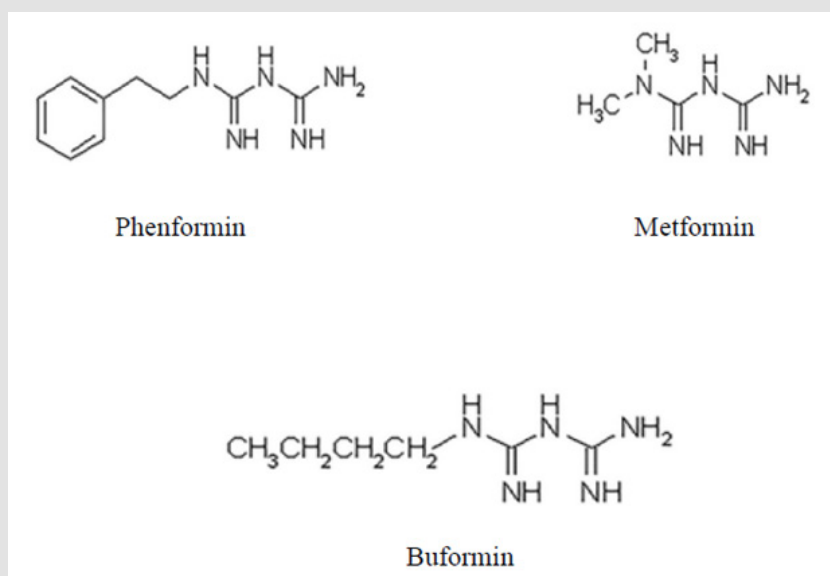


Figure 1: Differences in structures between phenformin, metformin and buformin.

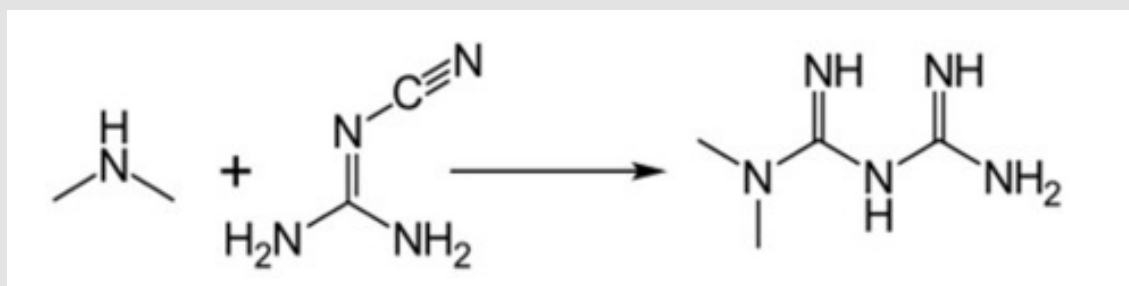


Figure 2: One step Synthesis of metformin.

The mixture boils and then, after cooling, metformin hydrochloride is yielded in a precipitate with 96% yield [7-11]. The dosage of metformin is usually 1-3 pills per day at different sizes of 500 mg or 850 mg or 1000 mg, taken with meals in order to reduce adverse gastrointestinal side effects [7]. The dosage is usually changed when taken with the combination with other drugs. Metformin can be used concomitantly with other drugs such as glipizide, glyburide, rosiglitazone, pioglitazone, repaglinide, sitagliptin, and insulin to create beneficial glucose lowering effects [12]. According to IUPAC, spectroscopy can be defined as “the study of physical systems by the electromagnetic radiation with which they interact or that they produce. Spectrometry is the measurement of such radiation as a means of obtaining information about the systems and their components [13]. The interpretation of the resulting spectrum can be used to analyze elements and chemical compounds, examine molecular structures, and determine the composition of a material [13]. UV spectrophotometry is another analytical technique which is being widely used in the chemical research for quantitative and qualitative analysis or organic and inorganic compounds.

Generally, UV spectra can be used with confirmation by comparing the spectra of the suspected compound [14]. For determining the overall structure, data from other instruments, such as FTIR, NMR and other supporting data are usually used

[15]. This paper can be used as a guide for researchers and novice students in understanding and interpreting UV spectrum data. Fourier Transform Infrared or FTIR is a valuable analytical technique for researchers. This type of analysis is mainly used for characterizing unknown compounds in the forms of solutions, liquids, powders, fibres, pastes, films and gases. It is also possible in analysing the material which is on the surfaces of substrate [16]. Compared to other types of characterization analysis, FTIR is quite popular. This analysis which helps in characterization is good in accuracy, sensitive and also rapid. [17]. During the FTIR analysis, samples will get subjected to contact with IR radiation. The interaction of infrared radiation with molecules in the sample with cause the impact of atomic vibrations of a molecule and then further resulting the specific absorption or transmission of energy. This makes FTIR is quite useful for determining and analyzing the specific molecular vibration in the sample [18].

Hypothesis

The concentration determination of each brand of metformin hydrochloride tablets available in Malaysia is comparative with official monograph. According to United State Pharmacopeia (USP), metformin hydrochloride must contain not less than 98.5 percent and not more than 101.0 percent of $C_4H_{11}N_5 \cdot HCl$, calculated on the dried basis (Figure 3).

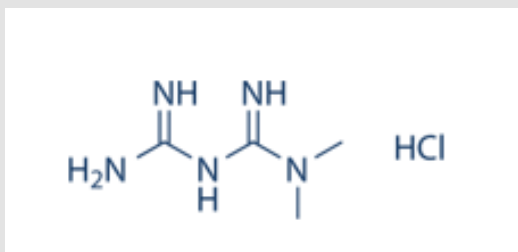


Figure 3: Chemical structure of Metformin HCl.

Materials and Method

Equipment

Spectroscopic analysis including absorbance measurements was carried out using UV-VIS spectrophotometer Shimadzu UV-1800 with 1cm path length quartz cells as shown in Figures 4 and 5. It is connected to the analyzed computer for interpretation and determination of absorbance. For weighing, Fisher brand™ analytical balance comprising of large draft shield with sliding top and side doors is used.

Chemicals and Reagents

Metformin hydrochloride BP powder as standard solution,

several brands of metformin (branded and generic version) as sample solution and distilled water as blank solution. They are all of analytical grade.

Collection of Metformin Hydrochloride Tablets

Several commercially available leading brands of metformin, within their shelf-life were purchased from various pharmacy outlets in Sungai Petani, Malaysia. Each brand of metformin hydrochloride tablet was labelled to contain 500 mg of metformin hydrochloride. The metformin tablets were blindly named as Brand A, Brand B, Brand C, Brand D, Brand E Brand F and Brand G in the present study. The descriptions about the different brands are shown in Table 1.

Table 1: The descriptions about the different brands.

Medication	Description
Brand A	• Chemical name: Metformin
	• 1 strip contain 10 film-coated tablets
	• Oval in shaped
	• White in colour
Brand B	• Chemical name: Metformin hydrochloride – gibenclamide
	• 1 strip contain 20 film-coated tablets
	• It is in caplet shaped
	• It is pale orange in colour
Brand C	• Chemical name: Metformin hydrochloride
	• 1 strip contain 20 film-coated tablets
	• It is oval in shaped
	• It is white in colour
Brand D	• Chemical name: Metformin hydrochloride
	• 1 strip contain 10 film-coated tablets
	• It is oval in shaped
	• It is white in colour
Brand E	• Chemical name: Metformin HCl
	• 1 strip contain 10-film coated tablets
	• It is oval in shaped
	• It is white in colour
Brand F	• Chemical Name: Metformin hydrochloride- gibenclamide
	• 1 strip contain 10 film-coated tablets
	• It is in caplet shaped
	• It is yellow in colour
Brand G	• Chemical name: Metformin hydrochloride
	• 1 strip contain 10 film-coated tablets
	• It is in oval in shaped
	• It is white in colour

Assay of the tablets

Twenty tablets of metformin hydrochloride were weighed and then powdered using mortar and pestle. A quantity of the powder equivalent to 0.1 g of metformin hydrochloride was made into solution by adding distilled water, the resulting solution was then diluted with distilled water and the absorbance of the resulting solution was measured using UV- visible spectrophotometer at a wavelength 233 nm. The content of metformin hydrochloride was then calculated using calibration curve. The experimental procedure was then repeated for the other brands.

UV Spectrophotometric Analysis

The Lambert-Beer law allows for the determination of the sample concentration from the measured absorbance value. If

the extinction coefficient ϵ and the path length d are known, then concentration c can be calculated from absorbance A as given below:

- c = concentration (in mol/L or g/mL)
- d = path length (in cm)
- ϵ (epsilon) = special constant describing how much the sample is absorbing at a given wavelength (in L/(cm*mol) or mL/(cm*g))

When the path length is 1 cm and the concentration is 1% w/v, the extinction coefficient is called specific absorbance

Concentration Determination

Based on the Lambert-Beer Law, the concentration of a

compound in a solution can be determined quantitatively by UV/VIS spectroscopy. To perform that, a calibration line is first determined by measuring the absorption of several standard solutions of known concentration. In this way, the concentration of samples such as, DNA, RNA, proteins, carbohydrates, organic compounds can be determined.

Calibration Curve

A calibration curve of standard metformin hydrochloride tablet was drawn. The relationship between concentration and absorbance was plotted and the equation and correlation values of the curve were generated from the scatter plot.

Comparison of Concentration of Metformin Hydrochloride

USP is used as one of the standards for the evaluation study. The concentration of several metformin HCl samples were compared with standard to determine whether the sample comply with the official limits. The samples were also compared with each other to determine whether which one is better than others [19].

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Table 2A: CCM METFORMIN.

	Size (mm)	Weight (mg)
	11.03	530
	11.02	530
	11.01	540
	11.01	530
	11.02	520
	11.02	520
	11.03	520
	11.01	520
	11.03	520
	11.02	520
	11.02	520
	11.03	530
	11.02	530
	11.02	530
	11.03	530
	11.02	520
	11.03	530
	11.02	520
	11.02	520
	11.03	530
Average	11.02	525.5

Table 2B: GLUMET DC.

	Size (mm)	Weight (mg)
	11.06	531.2
	11.05	530
	11.07	537.7
	11.05	534.8
	11.07	535.5
	11.07	530
	11.06	532.9
	11.05	534.2
	11.06	535.1
	11.07	534.4
	11.05	534.4
	11.07	534.8
	11.05	535.2
	11.08	533.8
	11.05	534.2
	11.06	534
	11.07	533.8
	11.07	534.5
	11.08	532.7
	11.05	532.1
Average	11.06	533.8

Table 2C: GLUCOXIT DUOPHARMA.

	Size (mm)	Weight (mg)
	11.05	529.8
	11.06	552.6
	11.04	530.8
	11.07	553.9
	11.05	550.9
	11.06	533.5
	11.07	541.5
	11.05	530.8
	11.07	554
	11.06	526.7
	11.07	542
	11.05	523
	11.07	539.5
	11.06	552.1
	11.06	544.8
	11.04	539.3
	11.07	536.6

	11.05	550.3
	11.05	543.2
	11.06	537.1
Average	11.06	540.6

Table 2D: METFORMIN HCL SUNWARD.

	Size (mm)	Weight (mg)
	12.06	616.7
	12.07	601.5
	12.05	606.9
	12.04	597.4
	12.05	596.1
	12.04	596
	12.05	598.5
	12.06	597.5
	12.05	605.6
	12.05	598.3
	12.06	595.5
	12.05	594.8
	12.06	595
	12.05	603.8
	12.04	595
	12.05	613.8
	12.05	597.3
	12.04	600
	12.05	616.2
	12.04	605.5
Average	12.05	601.6

Table 2E: GLUCOPHAGE FILM COATED.

	Size (mm)	Weight (mg)
	12.05	530
	12.03	530
	12.03	530
	12.02	530
	12.02	530
	12.03	530
	12.04	530
	12.03	530
	12.03	530
	12.03	530
	12.03	530
	12.03	530
	12.04	530
	12.05	540

	12.04	530
	12.04	530
	12.03	530
	12.03	530
	12.03	530
	12.04	530
	12.03	530
Average	12.03	530.5

Table 2F: GLUCOVANCE MERK SANTE.

	Length (mm)	Width (mm)	Weight (mg)
	36.4	28.39	630
	36.36	28.38	640
	36.43	28.43	620
	36.46	28.45	630
	36.41	28.38	630
	36.4	28.4	630
	36.39	28.4	630
	36.45	28.44	630
	36.41	28.45	620
	36.42	28.41	620
	36.38	28.41	630
	36.39	28.4	630
	36.42	28.42	640
	36.42	28.4	630
	36.4	28.43	640
	36.4	28.46	630
	36.39	28.4	620
	36.43	28.42	630
	36.42	28.43	630
	36.42	28.44	630
Average	36.41	28.42	629.5

Tables 2A-2G: Weight uniformity of different brands of Metformin hydrochloride.

Table 2G: DIAMIDE FILM COATED.

	Length (mm)	Width (mm)	Weight (mg)
	34.66	28.45	610
	34.66	28.46	620
	34.67	28.45	620
	34.67	28.45	620
	34.67	28.46	610
	34.67	28.45	610
	34.66	28.45	610

	34.65	28.44	610
	34.66	28.45	620
	34.66	28.44	620
	34.68	28.45	620
	34.68	28.46	630
	34.66	28.44	620
	34.65	28.46	620
	34.68	28.44	620
	34.68	28.44	620
	34.65	28.44	610
	34.66	28.45	620
	34.67	28.46	620
	34.65	28.46	610
Average	34.66	28.45	617

At different intensities and locations of the IR spectrum, there are different functional groups which can produce bond absorptions. When we get to recognize the absorptions generated by the common functional groups occur, this will help in interpreting IR spectra. The Table 2 below shows the list of the intensities and the locations of the absorption being produced by typical functional groups [20].

Result and Discussion

Metformin Hydrochloride was white crystalline powder, odorless and has bitter taste. For the solubility test, metformin hydrochloride is freely soluble in water, and it is slightly soluble in alcohol, and it is practically insoluble in acetone and methylene chloride. Also, the pH value of 1% aqueous solution of metformin hydrochloride is 6.68.

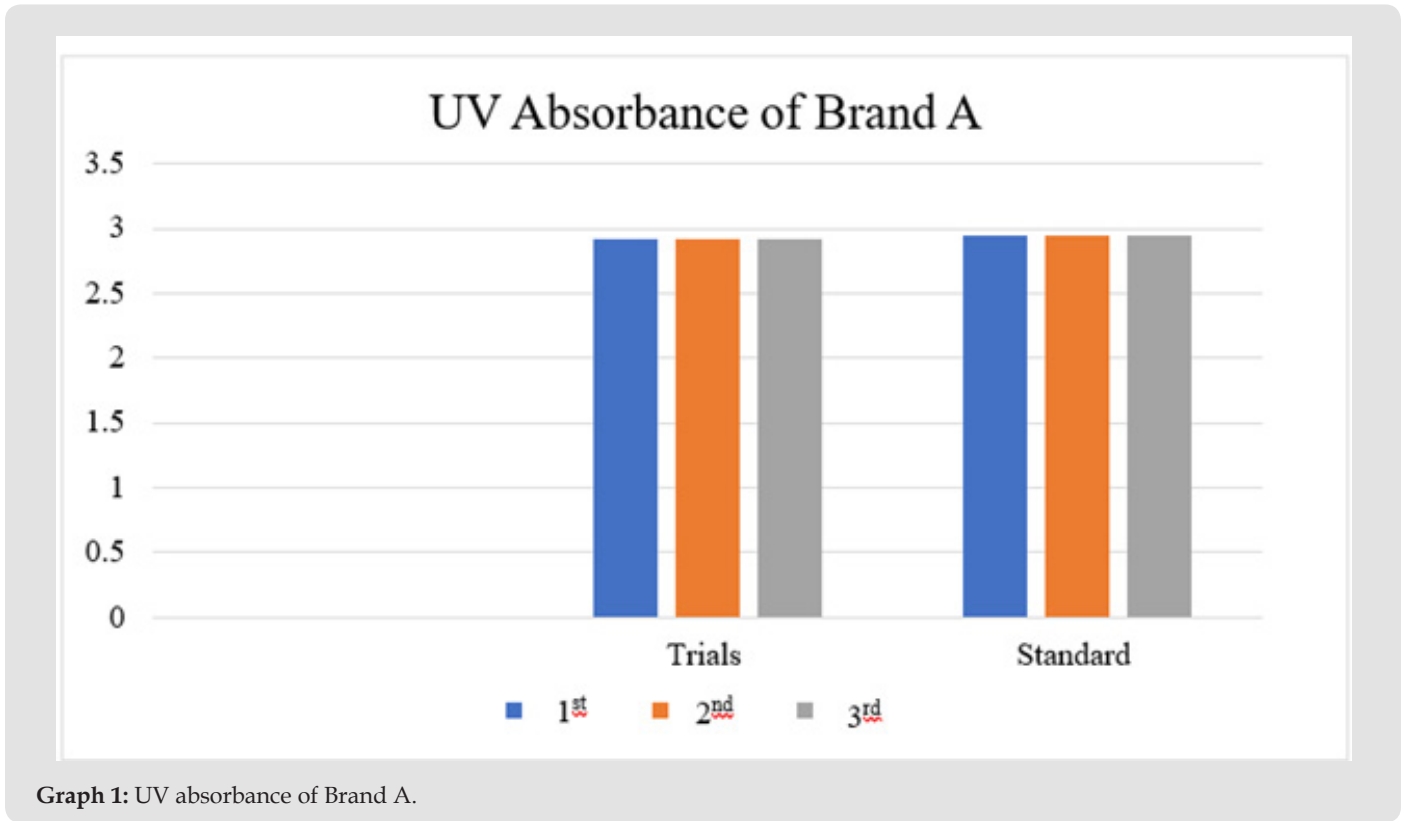
Weight Uniformity of Different Brands of Metformin Hydrochloride

The average weight of different brands of metformin hydrochloride tables are shown in Tables 2a-2g. These tables shows that the Glucovance had the highest average weight 629.5 per tablet while Metformin had the lowest average weight 525.5 per tablet. The average weight for Glumet DC were 533.8 mg, Glucoxit Duopharma 540.6 mg, Metformin HCl Sunward 601.6 g, Glucophage 530.5 mg per tablet and Diamide Film Tablet 617 mg

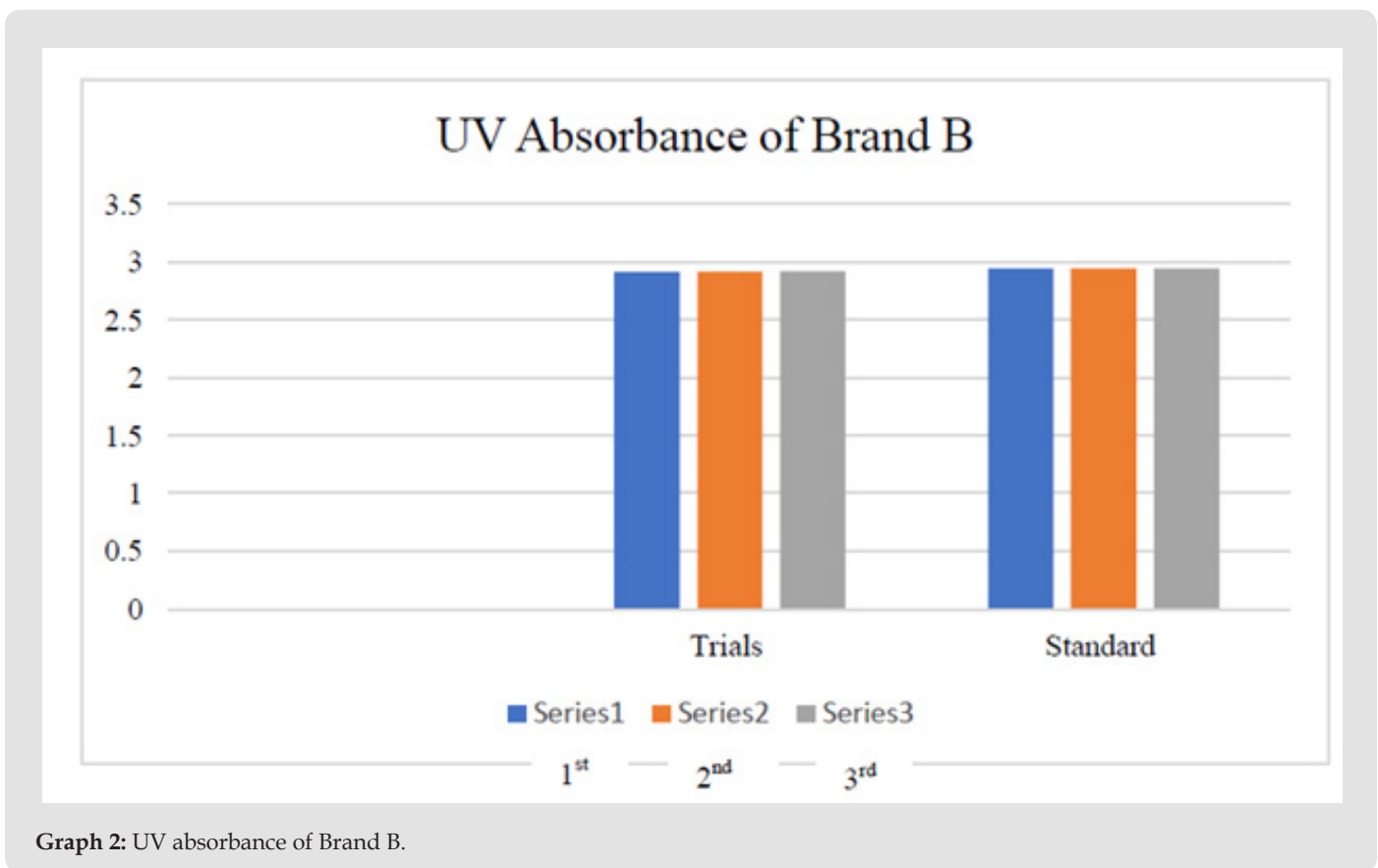
per tablet respectively. The purpose of this test is to verify the uniformity of each batch that reflect the drug content uniformity in all the formulation batches. This test was performed as the official procedure. Furthermore, the different weights of the tablet may differ in the quality parameters. This includes the content of the active pharmaceutical ingredient. The average weight of the tablets of the seven brand ranges from 520 to 620 mg.

UV Analysis

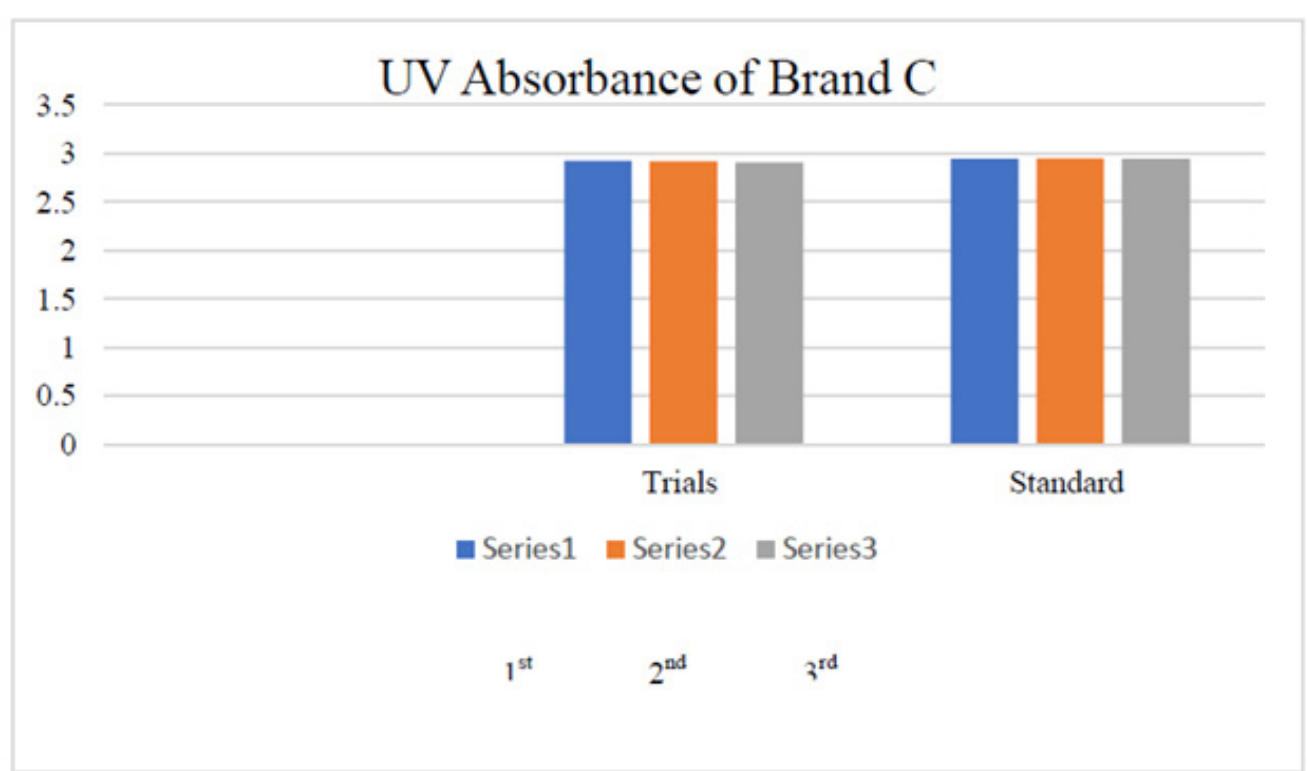
Absorbance of different brands of Metformin Hydrochloride using distilled water as solvent is laid down in Table 3 and Graphs 1-7 shows the UV absorbance of different brands. The UV absorbance of different brands of Metformin Hydrochloride is shown in Table 1. The first, second and third readings of standard Metformin Hydrochloride were 2.948, 2.947 and 2.946. In the trial, the first, second and third readings were 2.923, 2.921 and 2.922. The average for trial is 2.922 and the average for standard is 2.947. The first, second and third readings of standard metformin hydrochloride were 2.947, 2.946 and 2.945 and the average for the standard was 2.946. There was trial conducted for assay of brand B and the trial has 3 readings. The first, second and third readings were 2.915, 2.918 and 2.919. The average for the trial was 2.917. The first, second and third readings of standard metformin hydrochloride were 2.945, 2.947 and 2.946. The average standard reading was 2.946. There was trial conducted for assay of brand C and the trial had 3 readings. The first, second and third readings were 2.921, 2.919 and 2.91. The average for the trials was 2.917. The first, second and third readings of standard metformin hydrochloride were 2.94, 2.944 and 2.946. The average for the standards was 2.943. There was trial conducted for assay of brand D and the trial had 3 readings. The first, second and third readings were 2.931, 2.934 and 2.935. The average for the trials was 2.933. The first, second and third readings of standard metformin hydrochloride were 2.946, 2.947 and 2.943. The average reading for the standards was 2.945. There was trial being conducted for the assay of brand E and the trial had 3 readings. In the trial, the first, second and third readings were 2.83, 2.934 and 2.923. The average reading for the trials was 2.896. The first, second and third readings of standard metformin hydrochloride were 2.941, 2.940 and 2.938. The average reading for the standards was 2.940.



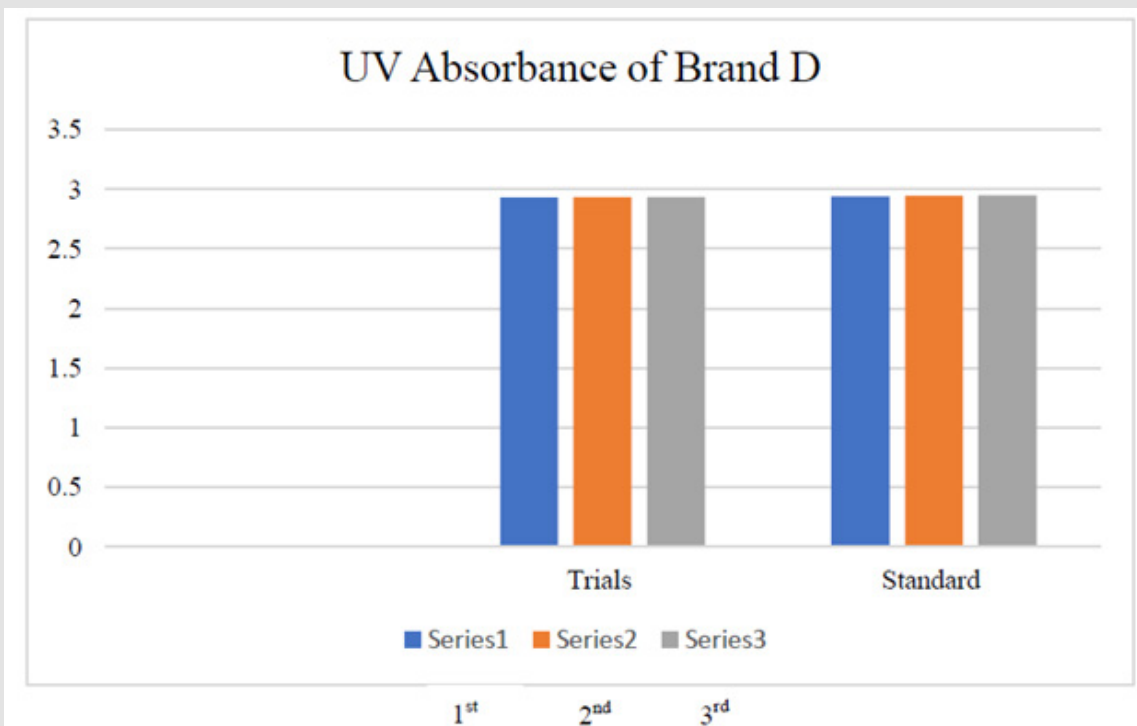
Graph 1: UV absorbance of Brand A.



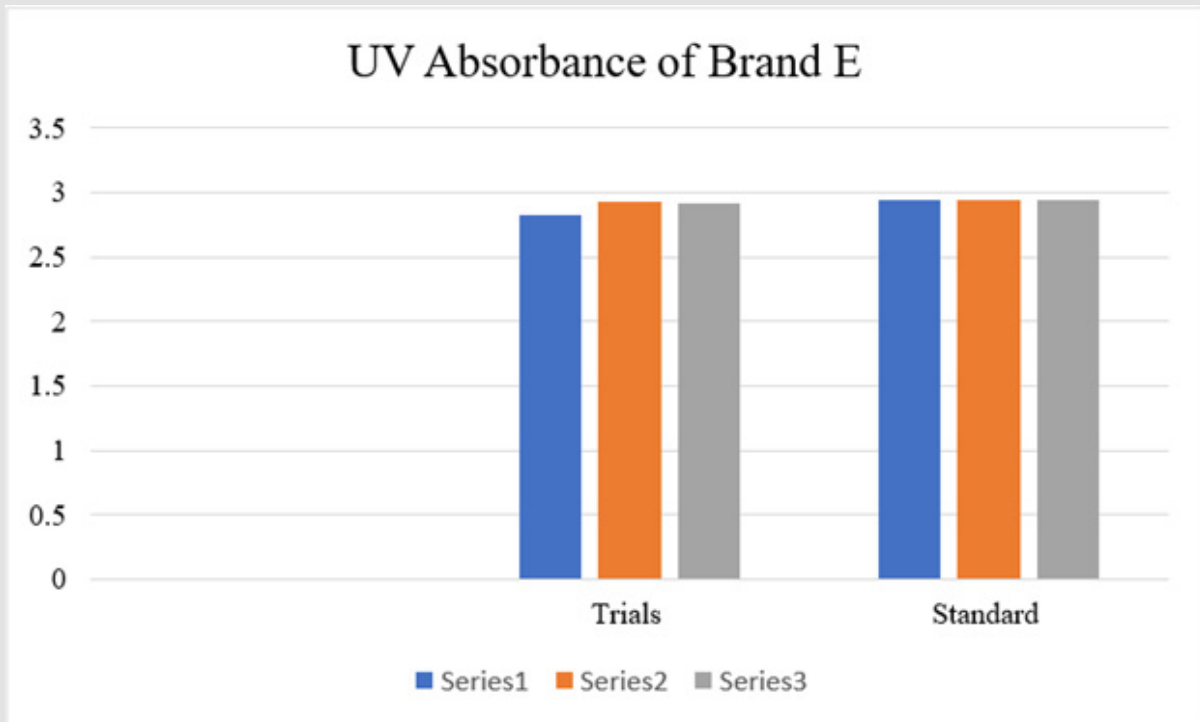
Graph 2: UV absorbance of Brand B.



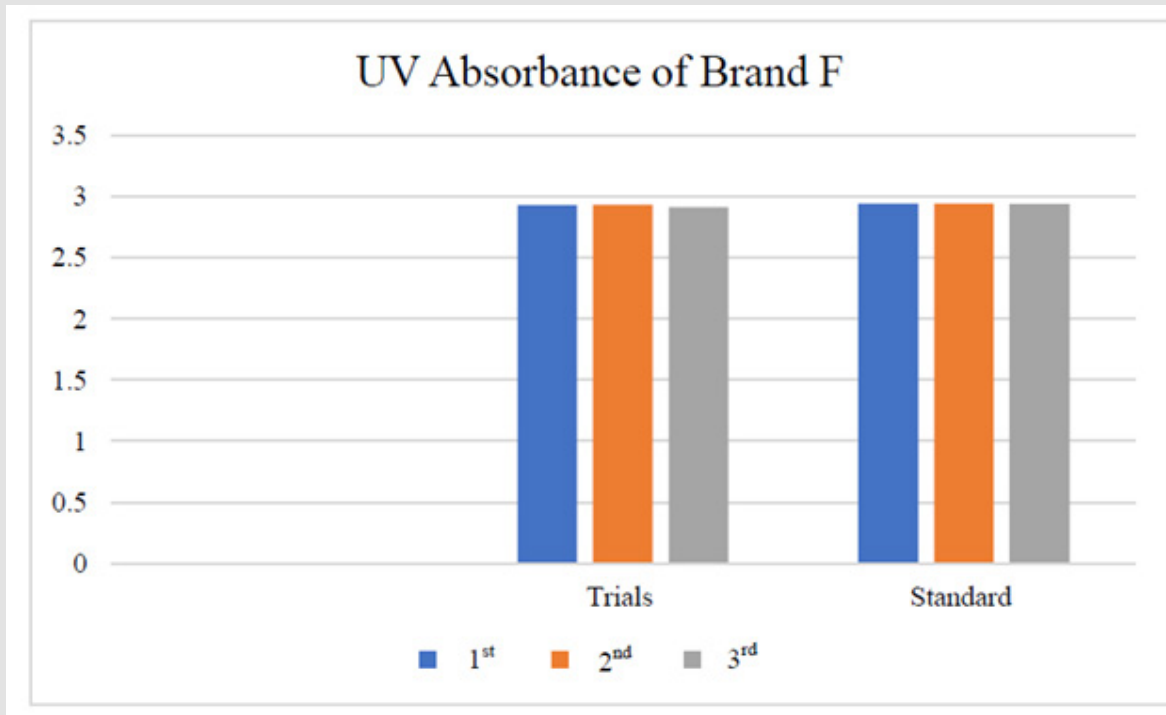
Graph 3: UV absorbance of Brand C.



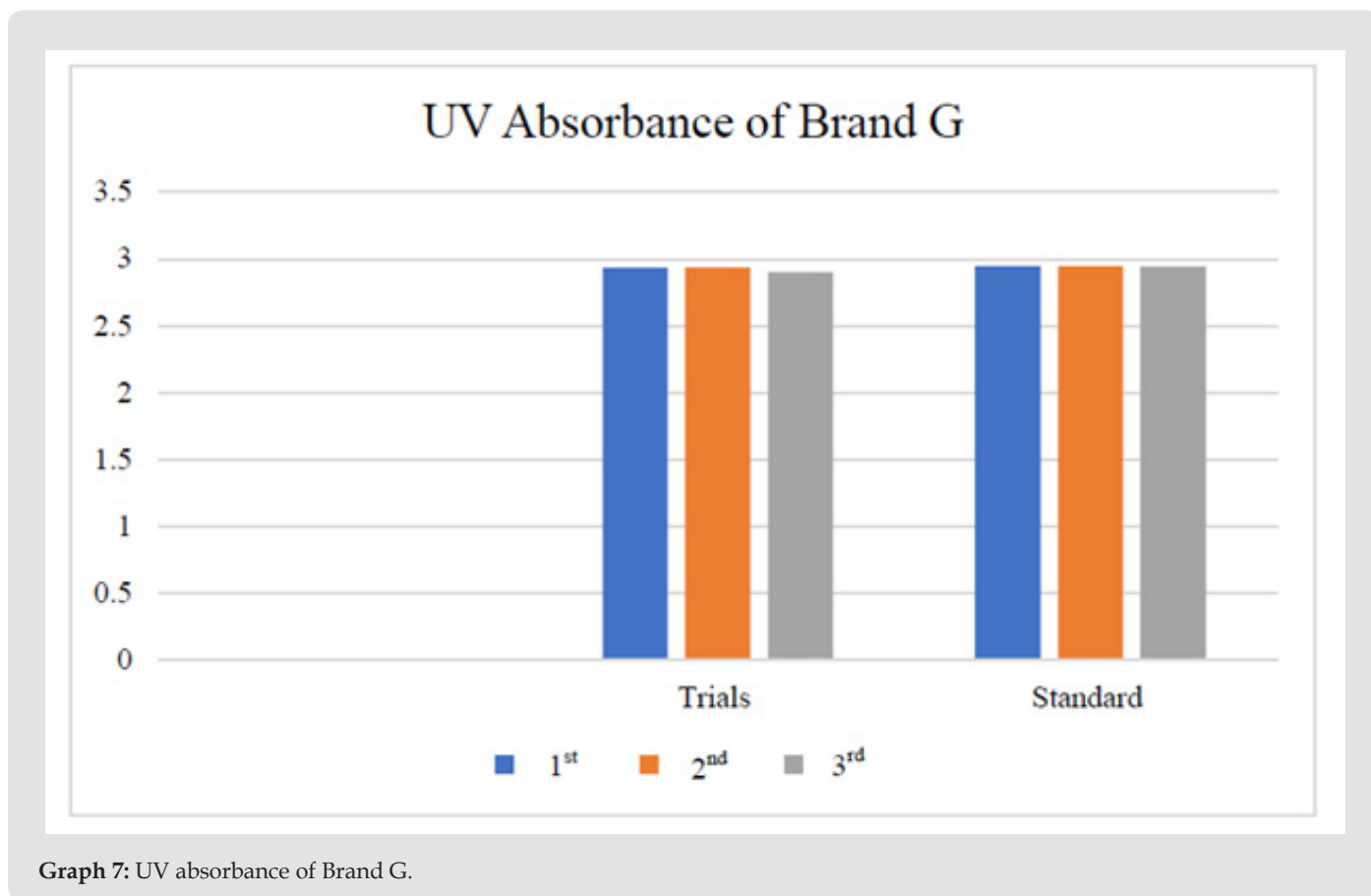
Graph 4: UV absorbance of Brand D.



Graph 5: UV absorbance of Brand E.



Graph 6: UV absorbance of Brand F.



Graph 7: UV absorbance of Brand G.

There was trial being conducted for the assay of brand F and the trial had 3 readings. In the trial, the first, second and third readings were 2.928, 2.930 and 2.910. The average reading for the trials was 2.923. The Percentage purity of different brands of Metformin Hydrochloride is shown in Table 4 by UV spectrophotometric method. The first, second and third readings of standard metformin hydrochloride were 2.943, 2.942 and 2.940. The average reading for the standards was 2.942. There was trial being conducted for the assay of brand G and the trial had 3 readings. In the trial, the first, second and third readings were 2.934, 2.936 and 2.90. The average reading for the trials was 2.923. The average percentage purity of sample A, B, C, D, E, F and G was 99.15%, 99.03%, 99%, 99.66%, 98.31%, 99.42% and 99.38%. The brand A, B, C, D, F, and G passed the test whereas brand E failed the test. According to the United States Pharmacopeia (USP), the percentage purity of the Metformin Hydrochloride should be in the range of 98.5% to 101.0%. The comparison of percentage purity of sample with the standard (%) is given in Graph 8. According to the United States Pharmacopoeia (USP), the percentage purity of the Metformin Hydrochloride should be in the range of 98.5% to 101.0%. All of the brands passed when using the distilled water as solvent. To conclude, six of the

seven samples which were brand A, B, C, D, , F, and G complied with U.S.P official limits with percentage purity of 99.15%, 99.03%, 99%, 99.66%, 99.42% and 99.38% respectively. For brand E, it failed to comply with the limits and had the percentage purity of 98.31%.

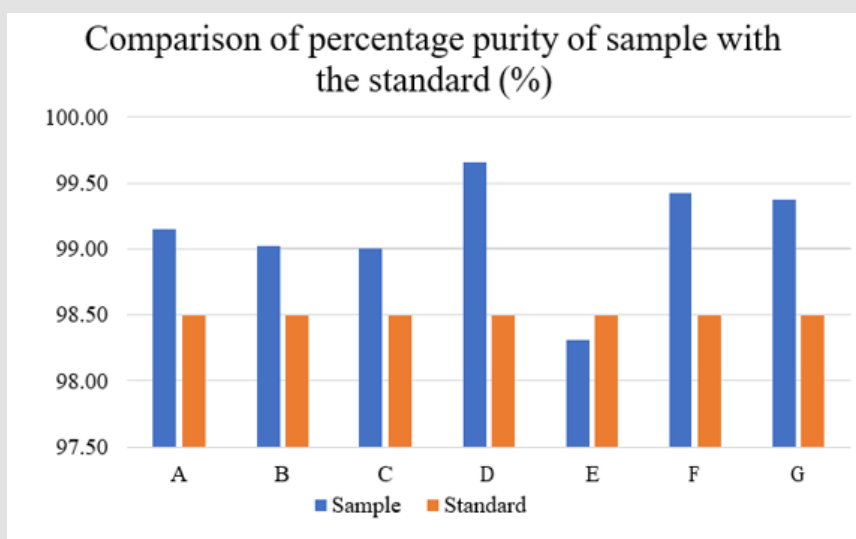
This is most probably due to the hygroscopicity of the metformin hydrochloride. Metformin hydrochloride is high water soluble. It can absorb the atmospheric moisture so rapidly that the concentration of the prepared solution is usually changes over time. This concern is reflecting in the absorbance readings. To eliminate this problem, when preparing the solution for the UV spectrophotometry, it has to be standardized to know the exact concentration and only prepared when needed to keep it as fresh as possible. Once the drug content has been exposed to the atmosphere and become extremely sensitive to the temperature, light and humidity. Although the samples had been stored well in a plastic zipper bag and placed in a desiccator, the result showed the stability of the samples might be slightly affected in the factor of consideration of the tablet sample solution. Factors such as light, temperature and humidity may cause significant drug degradation and thereby affect its label content and purity [21].

Table 3: Absorbance of different brands of Metformin Hydrochloride using distilled water.

Brand	Blank	Readings			
			1st	2nd	3rd
A	0	Trials	2.923	2.921	2.922
		Standard	2.948	2.947	2.946
B	0	Trials	2.915	2.918	2.919
		Standard	2.947	2.946	2.945
C	0	Trials	2.921	2.919	2.91
		Standard	2.945	2.947	2.946
D	0	Trials	2.931	2.934	2.935
		Standard	2.948	2.947	2.946
E	0	Trials	2.83	2.934	2.923
		Standard	2.946	2.947	2.943
F	0	Trials	2.928	2.93	2.91
		Standard	2.943	2.942	2.940
G	0	Trials	2.934	2.936	2.9
		Standard	2.943	2.942	2.940

Table 4: Percentage purity of different brands of metformin hydrochloride UV spectrophotometric method.

Brand	Equivalent weight (mg)	Percentage purity (%)				Standard (%)
		1st	2nd	3rd	Average	
A	500	99.15	99.12	99.19	99.15	98.5
B	500	98.91	99.05	99.12	99.03	98.5
C	500	99.19	99.05	98.78	99.00	98.5
D	500	99.69	99.66	99.63	99.66	98.5
E	500	96.06	99.56	99.32	98.31	98.5
F	500	99.56	99.66	99.05	99.42	98.5
G	500	99.69	99.80	98.64	99.38	98.5



Graph 8: Comparison of the percentage purity of sample with the standard (%).

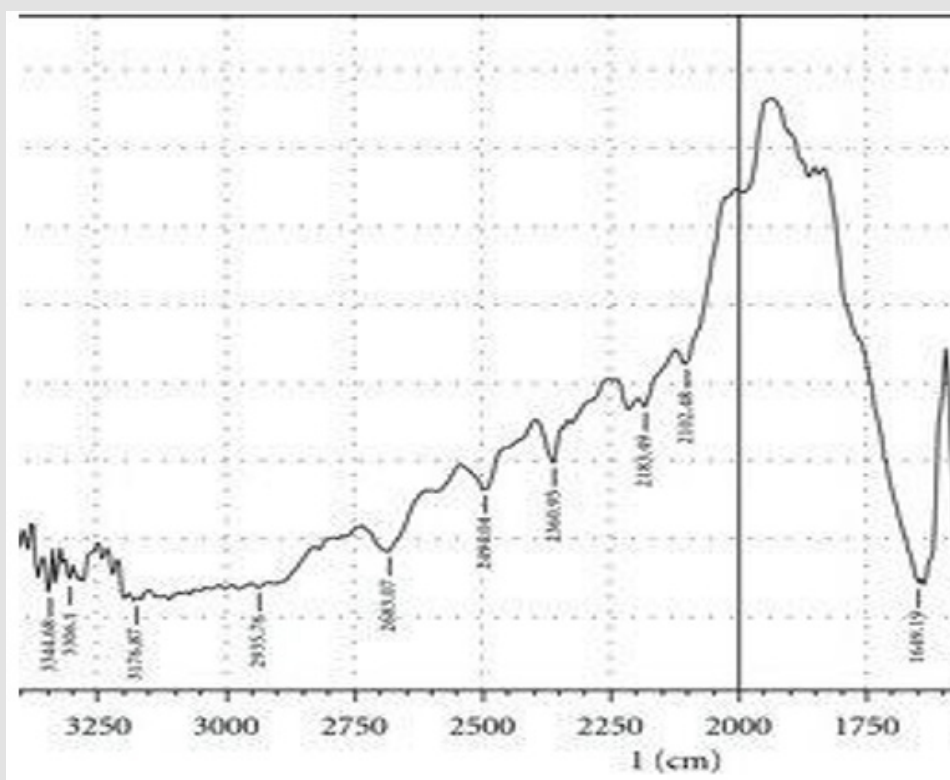
FTIR Spectroscopy

Infrared spectrum gives information about the functional groups present in the compound. The FTIR spectrum for the metformin hydrochloride is recorded and given in Graphs 9-10. The wave number values for each peak have been explained in Table 5. The FTIR spectrum was found to be in compliance with the various peaks for metformin HCl. All the tests of the identification studies were found to be compliance with the data available in the literature. Also, it is confirmed from these results that the procured drug Metformin HCl was free from the impurities. Other than that, background spectrum contains information about the species of gases and solvent molecules, which may be subtracted away from our sample spectrum in order to gain information about just the sample. The reference standard for the metformin hydrochloride

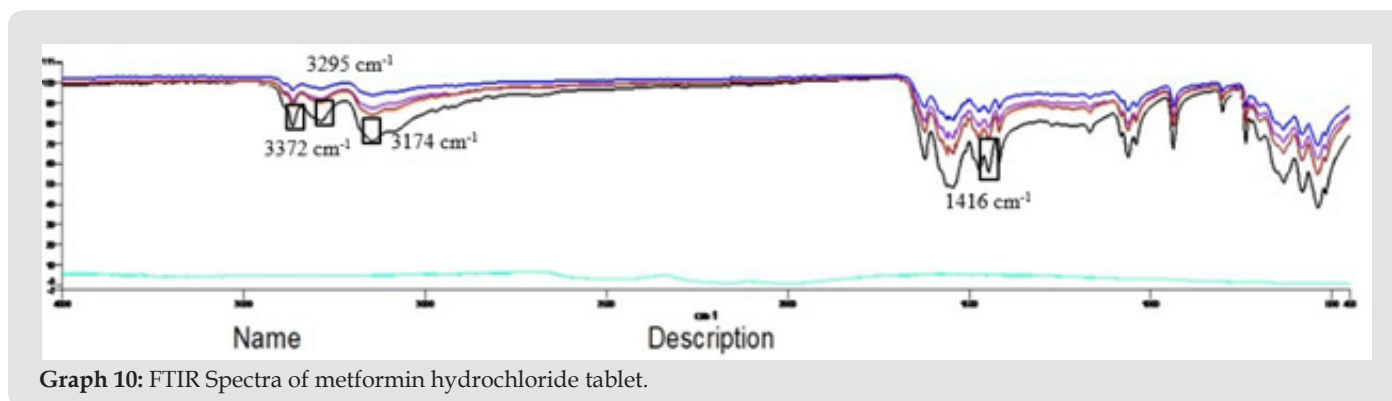
during the infra-red (IR) scan is characterized by the wave numbers of 740, 935, 1075, 1063, 1580 and 1620 cm⁻¹. And for the N-H wagging vibrations it occurs at 740 and 935 cm⁻¹: C-N stretch vibrations for 1063 and 1075 cm⁻¹ and C=N stretch vibrations accounting for absorption bands at 1580 and 1620 cm⁻¹. The identification test results indicated that the generics of metformin hydrochloride tablets being used for this research as Active Pharmaceutical Ingredient (API). The N-H stretching vibrations are found in the region 3300- 3500 cm⁻¹. In the IR spectrum, there is a peak appearing at this region as it is assigned to the N-H stretching vibration. There is C-H stretching vibration generally occur in the region 3500- 3300 cm⁻¹ and in the region of 3200-3000 cm⁻¹. There is C-N stretching vibration generally occur in the region of 1500-1000 cm⁻¹ with the peak at 1416 cm⁻¹.

Table 5: Wave number values for peak in the FTIR spectrum.

Observe number (cm-1)	Characteristic wave number ran
3372	3500-3300 cm-1
3295	3600-3200 cm-1
3174	3200-3000 cm-1
1416	1500-1000 cm-1



Graph 9: Reference FTIR spectra of Pure Metformin Hydrochloride (20-21).



Graph 10: FTIR Spectra of metformin hydrochloride tablet.

Conclusion

From the several brands of Metformin Hydrochloride being assessed in this research, it showed that all of them were within the pharmacopoeial limits by looking at the various quality control parameters. Through the FTIR spectral measurements, a satisfactory vibrational band assignment of metformin hydrochloride has been carried out. From the outcome of the various test, it indicated that the process validation data provides a high degree of assurance of the manufacturing of these tablets' products meet its predetermined specification and quality attributes. Metformin hydrochloride solution of about 100 ppm was accurately prepared for determination of wavelength of maximum absorption. The absorbance of this solution was scanned and measured as a function of wavelength in the 200-400 nm UV regions. The maximum absorption wavelength (λ_{max}) was observed at 234nm, and this wavelength was adopted for absorbance measurement. All spectrophotometer measurements were at room temperature laboratory standard.

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Conflict of Interest

Authors declare that there is no conflict of interests.

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