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Method Development, Validation, and Concentration Determination of Metformin Hydrochloride and Atorvastatin Calcium Using UV-Visible Spectrophotometry

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ABSTRACT

It is very important to have simple but fast analytical procedures to determine the active ingredient concentrations in drug substances as the significant deviation from the labelled amount might negatively affect the patients. The goal of this study is to develop a simple and accurate method to determine the active ingredient concentrations of metformin hydrochloride (type II diabetes) and atorvastatin calcium (hypercholesterolemia) using a simple ultraviolet photo spectroscopy (UV). The method has been developed according to the International Conference on Harmonization (ICH) guidelines and validated using the acceptance criteria of linearity, range, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), and stability. From the UV-Vis spectra, the wavelength of maximum absorbance (λ_{max}) values of metformin hydrochloride and atorvastatin calcium tablets were measured and used for the rest of the study. A five-point calibration curve was obtained within a concentration range from 2-10 ppm with a correlation coefficient (R^2) of 0.999 for metformin hydrochloride and 5-15 ppm concentration range with an R^2 value of 0.998 for atorvastatin calcium. The accuracy was tested with the spike recovery method which showed the mean recoveries occur from 92.14% to 95.04% for metformin hydrochloride and 90.10% to 102.90% for atorvastatin calcium, respectively. Test sample analysis was carried out by using different brands purchased from various places in Sri Lanka, manufactured in different countries. The developed method resulted that the actual concentration of the active ingredients of metformin hydrochloride ranged from 382.70 to 454.19 mg per 500 mg tablet while 9.00 to 9.88 mg per 10 mg tablet for atorvastatin calcium. Since the calibration plot can be prepared using premade standard solutions stored at room temperature or refrigerated conditions, it can be concluded that the developed analytical procedure can be used for quantitative analysis of the active compound for the selected drugs. Since standard samples have proven stability of seven days, no need of repeat preparation of calibration plots for each analysis.

Keywords: Method development; Spectroscopy; Metformin hydrochloride; Atorvastatin calcium

Introduction

Metformin hydrochloride (1,1-dimethylbiguanide hydrochloride) and atorvastatin calcium (calcium(3R,5R)-7-[2-(4-flurophenyl)- 3-phenyl-4-(phenylcarbomyl)-5-propan-2ylpyrrol-1-yl]-3,5-dihydroxyheptanoate) are commonly administered drugs for type II diabetes and for hypercholesterolemia, respectively[1,2]. Metformin hydrochloride (MH) tablets are available in two types as immediate release tablets and extended-release tablets [3]. Immediate release tablets deliver the components to the body mostly within 8 hours while extended-release tablets deliver the active components over the time (Usually extended-release tablets are used only once a day) [3]. Atorvastatin calcium (AC) tablets are available as immediate release tablets with 7 hours of biological half-life [4]. The assay of immediate release tablets of MH is usually carried out by UV spectrophotometry as per British Pharmacopoeia (BP) and United States Pharmacopoeia (USP) while HPLC method has been developed for extended-release tablets in the USP monograph. The assay of AC is determined by using a liquid chromatography method as per USP [4].

The MH is used along with a prescribed diet plan and with exercise schedule to control the blood sugar levels of patients who are suffering from type II diabetes (non-insulin dependent diabetes). This control will reduce the amount of glucose made by the metabolism of liver, lowers the amount of glucose absorb to the body and increases the effect of insulin inside the body [5]. The AC is indicated for primary hypercholesterolemia in patients who have not responded adequately to diet and other appropriate measures. This lowers plasma cholesterol and lipoprotein levels by acting as a selective, competitive inhibitor of HMG-CoA reductase and lowers the cholesterol synthesis in liver [6].

In this study, the applicability of UV-visible method has been developed to determine the active pharmaceutical ingredient (API) concentration of each tablet as API is responsible for the pharmacological activity of the drug [7-9]. Determination of drug concentration is important as a specific dose is required to be administered to each patient. This dose is different from person to person since the effective dose (ED50) for one person could be deadly for another person. Therefore, it is important to ensure the drug has correct amount of API prior to administer it to a patient, as overdosing or under dosing may cause critical consequences [10]. The spectroscopic approach is a simple, accurate, specific and a sensitive

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method that has been employed for most of similar studies [10-15]. Both MH and AC are white crystalline powders which do not possess any odor. The molecular weights of MH and AC are 129.163 g mol^{-1} and 1155.363 g mol^{-1}, respectively.

Methodology

Description of materials

Standard samples of MH and AC were obtained from the State Pharmaceutical Manufacturing Corporation (SPMC), Rathmalana, Sri Lanka. De ionized water was obtained from the in house supply while the methanol was obtained from SRL chemicals, India. Test samples manufactured in different countries for both drugs were purchased from the local pharmacies in different areas in Colombo district. The MH samples were purchased in 500 mg tablets while the AC was of 150 mg tablets (Figure 1).

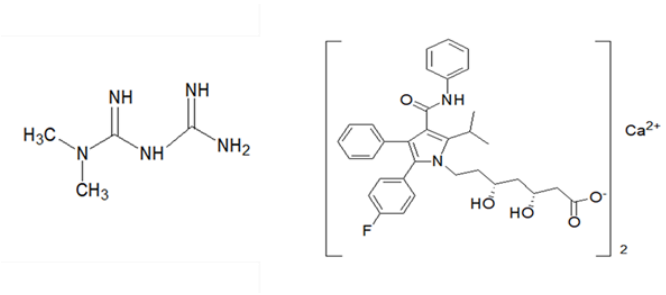

Figure 1: Chemical structures of MH and AC.

Apparatus and instruments

Double beam UV spectrophotometer (HITACHI-U2910) located in Institute of Chemistry Ceylon (IChemC) laboratory was used to measure absorbance of all prepared samples.

Standard preparation

Metformin hydrochloride (100 ppm): A 10 mg sample of MH standard was weighed accurately and transferred into a 100 mL volumetric flask carefully. Then, about half of the volume of de-ionized water was added and the solution was sonicated 2 minutes for better solubility. The resulting solution was topped up to the mark with deionized water. Then, the absorbance of MH standard was measured for the wavelength range from 200 nm to 400 nm using the UV vis spectrophotometer to determine the maximum absorbance for the drug. This procedure was triplicated to ensure the reproducibility of results. The standard calibration curve was constructed by diluting the standard solutions for the range of 2.00 ppm to 10.00 ppm and measuring the absorbance values at 233 nm which was determined in the wavelength scan [16,17].

Atorvastatin calcium (100 ppm): A 10 mg of pure drug powder was weighed and transferred carefully into a 100 mL volumetric flask and dissolved with the selected solvent mixture of methanol: water 50:50. To enhance the solubility, the sample was sonicated about 3 minutes prior to top up with the solvent. Absorbance of AC standard was measured for the wavelength range from 200 nm to 400 nm using the UV vis spectrophotometer. This procedure was followed in triplicate to ensure the reproducibility of results. The standard stock solution was used to prepare a calibration plot ranging from 5 ppm to 15 ppm measuring the absorbance at 245 nm [17,18].

Sample preparation

Metformin hydrochloride (6 ppm): Pre-weighed five tablets from each test sample were crushed and powdered to make a homogenous mixture. A 10 mg sample from this mixture was weighed accurately and transferred into a 100 mL volumetric flask. The contents were dissolved using deionized water and resulting solution was sonicated to enhance the solubility for 2 minutes prior to adjust the volume to 100 mL. A 1.25 mL sample from this solution was withdrawn and transferred to a 25 mL volumetric flask to prepare MH 6 ppm test sample solution. The absorbance of each test sample was measured at 233 nm.

Atorvastatin calcium (5 ppm): Three tablets from each test sample were weighed and average weight was calculated. They were powdered and 10 mg of equivalent active ingredient was measured. The sample was transferred to a 100 mL volumetric flask and dissolved in methanol: water (50:50) solvent mixture. After 3 minutes of sonication the volumetric flask was topped up with the same solvent mixture. The test sample solutions were filtered to remove the undissolved particles present in the tablets. A 2.50 mL sample from this solution was withdrawn and transferred to a 50 mL volumetric flask to prepare AC 5 ppm test sample solution. The absorbance of each test sample prepared was measured at 245 nm.

Statistical analysis

All statistical analyses were done by using Microsoft Office Excel (Microsoft apps for enterprise), Origin 8.1 (2018) packages and structures were drawn using ChemDraw Ultra 7.0 software package.

Results and Discussion

The wavelength scans for standard MH and AC show that their maximum absorbances occurred at 233 nm and 245 nm, respectively as shown in Figure 2.

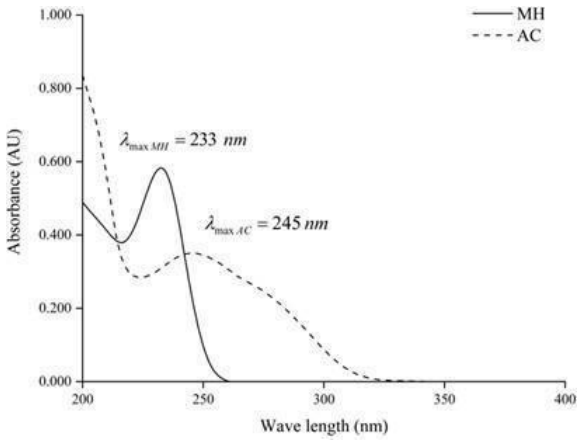

Figure 2: Absorption spectra of MH and AC.

Method validation

The method validation was conducted according to the International Conference on Harmonization (ICH) guidelines issued by Organization for Economic Co-operation and Development (OECD).[19,20] A full method validation was done using standard

samples with parameters of specificity, linearity, precision, accuracy, stability, range, limit of detection (LOD) and limit of quantification (LOQ).[21-27].

Specificity

Specificity of the standard samples was measured by comparing the absorption of solvent (vehicle) with and without the sample. The de-ionized water was used as the solvent for MH while methanol: water (50:50) solvent mixture was used for AC. It could be concluded that the solvent system adopted for each drug component was suitable for the study.

Linearity

Five points calibration curve was prepared for the concentration range from 2 ppm to 10 ppm for MH and from 5 ppm to 15 ppm for AC. The responses of both drugs were found to be linear in the selected concentration range and are shown in Figure 3. For each drug, the linear regression equation, the correlation coefficients were determined.

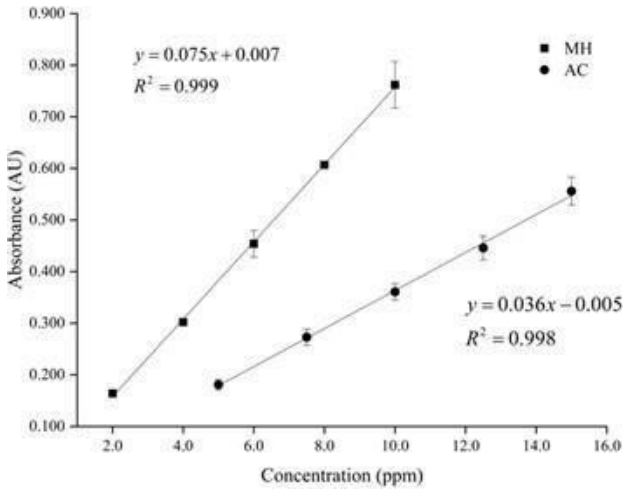

Figure 3: The calibration plots for MH and AC.

Range

According to Figure 3, lower concentration limits were 2 ppm and 5 ppm for MH and AC, respectively while 10 ppm to 15 ppm were the upper concentration limits, respectively. Therefore, the range used to validate the method is acceptable.

LOD and LOQ

Through the calibration curve, the LOD and LOQ values corresponding to MH and AC were determined using the equations (1), and Figure 3. The results are shown in the Table 1.

$$LOD=3.3\frac{\sigma}{b} \qquad LOQ=10\frac{\sigma}{b} \qquad (1)$$

Name	σ	b	LOD (ppm)	LOQ (ppm)
MH	0.001	0.007	0.609	1.845
AC	-0.001	-0.005	0.909	2.754

Table 1: LOD and LOQ values for MH and AC.

Precision

Precision data were analyzed for repeatability and inter-day precision.

Repeatability

Repeatability of both samples was studied by analyzing a predetermined concentration (10 ppm) from the standard sample for six times. The MH and AC repeatability samples were prepared by withdrawing 10.00 mL from the standard solution of 100 ppm and making it up to 100 mL using the relevant vehicle for each drug sample. Table 2 shows the absorbance values for the six samples at 10 ppm.

Sample Number	MH	AC
1	0.546	0.365
2	0.546	0.365
3	0.525	0.362
4	0.527	0.364
5	0.531	0.364
6	0.522	0.362
Average	0.533	0.364
SD	0.011	0.001
%RSD	1.989	0.376

Table 2: Repeatability data MH and AC.

The standard deviation (SD) and percent relative standard deviation (%RSD) were calculated using equations (2) and (3), respectively. From the Table 2, it can be concluded that the %RSD values for both MH and AC were below 2% and satisfied the ICH guidelines.

$$SD=\sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i-\bar{x})^2} \qquad (2)$$

S – Standard deviation

N – Number of values in the data set

x – Each value in the data set

$\bar{x}$ – Mean of all values in the data set.

$$\%RSD=\frac{SD}{Average}\times 100 \qquad (3)$$

Inter-day precision

Inter-day precision was confirmed by repeating the analysis for each drug in different concentrations in three consecutive days. For MH, 2 ppm, 6 ppm, and 10 ppm concentrations were analyzed while 5 ppm, 10 ppm, and 15 ppm concentrations were analyzed for AC. Three replicates from each concentration were prepared. The same procedure was carried out in 3 different days and the results are shown in Table 3. According to Table 3, % Relative Standard Deviation (%RSD) for inter-day precision values was <2% and satisfied the ICH guidelines.

Concentration (ppm)	%RSD of MH	Concentration (ppm)	%RSD of AC
2	1.275	5	1.995
6	0.714	10	1.204
10	1.367	15	0.64

Table 3: Inter-day precision for MH and AC.

Tablet type	Added tablet Concentration (ppm)	Recovery Level %	Percentage recovered (%)	Mean recovery %	Standard Deviation	RSD %
Immediate release tablet	10	50	93.74	92.14	1.39	1.51
	10	100	91.22			
	10	150	91.45			
Extended-release tablet	10	50	90.09	91.70	1.41	1.54
	10	100	92.33			
	10	150	92.69			

Table 4a: Accuracy data for MH.

Tablet brand	Added tablet Concentration (ppm)	% Recovery Level	Percentage Recovered %	Mean Recovery %	Standard Deviation	%RSD
A	5	50	100.8	101.00	1.81	1.79
	5	100	102.9			
	5	150	99.3			
C	5	50	90.1	91.03	1.62	1.78
	5	100	90.1			
	5	150	92.9			
F	5	50	92.4	91.53	1.03	1.13
	5	100	91.8			
	5	150	90.4			

Table 4b: Accuracy data for AC.

The developed method for MH and for AC analysis was found to be precise as the %RSD values.

Accuracy

Accuracy was confirmed by standard addition method at three recovery levels (50%, 100%, and 150%) of concentrations as shown in Tables 4a and 4b. The standard solutions (100 ppm) were spiked with the test sample solutions (100 ppm) and corresponding absorbance values were measured at of 233 nm and 245 nm for MH and AC, respectively. The recovery percentage was evaluated using the data obtained as shown in column 4 of Tables 4a and 4b. For MH, two test samples were used while for AC, three test samples were used.

The recovery was calculated using the equation (4). Tables 4a and 4b shows that both the drugs have a recovery percentage in between 90% and 110% which satisfied the ICH guidelines.

$$\%\ \text{Recovery} = \frac{\text{Amount found (amount recovered)}}{\text{Amount added (total amount present)}} \times 100 \quad (4)$$

Stability

Tables 5a and 5b shows the results of stability studies for each drug at different time intervals at room temperature (25°C) and refrigerator conditions (5°C), respectively. It can be concluded that the preparations were stable up to 7 days at room temperature and refrigerator conditions for both MH and AC. The values were compared with the 10% acceptance criteria as per the ICH guidelines.

Drug	Concentration/ ppm	Initial Absorbance	Percent stability (%)			
			24 hours	72 hours	7 Days	14 Days
MH	2	0.164	95.73	95.12	90.24	41.46
	6	0.454	99.11	98.46	94.27	70.26
	10	0.762	99.34	98.43	95.53	85.43
AC	5	0.181	101.66	102.21	93.37	98.34
	10	0.361	101.11	101.66	97.23	101.39
	15	0.556	100.18	100.90	97.84	99.10

Table 5a: Stability data at room temperature for MH and AC.

Drug	Concentration / ppm	Initial Absorbance	Percent stability (%)			
			24 hours	72 hours	7 Days	14 Days
MH	2	0.164	98.17	96.34	92.07	38.41
	6	0.454	99.34	98.24	95.37	79.74
	10	0.762	99.61	99.08	95.41	85.96
AC	5	0.181	99.45	98.34	100.55	117.68
	10	0.361	103.32	103.05	103.32	114.04
	15	0.556	101.26	101.44	102.34	112.23

Table 5b: Stability data at refrigerator condition for MH and AC.

Statistical analysis

From the data on Tables 5a and 5b, it can be concluded that both drugs were stable at room temperature condition and refrigerator condition up to 7 days since they met the acceptance criteria. Also, it is evident that the refrigerator condition shows a higher stability than the room temperature condition. However, none of the drugs were stable up to 14 days in the media it was prepared.

Test sample analysis

Eight different brands of MH and seven different brands of AC were purchased from different pharmacies in Colombo district of Sri Lanka. These tablets were analyzed according to the method develop in previous sections, and the percentages of active ingredient were calculated. The Tables 6a and 6b show the results for MH and AC, respectively.

Brand	Average powder weight (mg)	Label claim (mg)	Amount active ingredient found (mg)	% Active ingredient
A	572.3 ± 1.59	500	411.3 ± 0.26	82.2
B	618.3 ± 1.80	500	440.4 ± 0.75	88.3
D	646.2 ± 0.67	500	424.1 ± 0.30	84.8
E	618.8 ± 0.95	500	454.1 ± 1.03	90.8
F	841.5 ± 1.93	500	399.1 ± 1.03	79.8
G	522.8 ± 1.47	500	419.5 ± 0.24	83.8

Table 6a: Test sample analysis for 500 mg MH samples.

Brand	Average powder weight (mg)	Label claim (mg)	Amount active ingredient found (mg)	% Active ingredient
A	155.0 ± 0.90	10	9.88 ± 0.11	98.8
B	154.8 ± 1.91	10	9.01 ± 0.04	90.1
C	152.2 ± 0.86	10	9.10 ± 0.41	91
D	147.8 ± 0.68	10	9.00 ± 0.21	90
E	179.6 ± 1.30	10	9.22 ± 0.43	92.2
F	155.8 ± 0.19	10	9.55 ± 0.36	95.5

Table 6b: Test sample analysis for 150 mg AC samples.

According to the second column of Table 6a, the average powered weight of MH tablet varies from (522.8 ± 1.47) mg to (841.5 ± 1.93) mg even though the label claim was 500 mg for each as shown in column 3. The fourth column of Table 6a shows the calculated amount of active ingredient according to developed method and the amount ranges from (399.1 ± 1.03) mg to (440.4 ± 0.75) mg. The fifth column of Table 6a shows the percentage of active ingredient determined by the developed method. It is very clear that brand E contains the highest amount of active ingredient which is 90.8% while the brand F, which has the highest amount of inactive ingredient, contains the lowest percentage of active ingredient. Therefore, it can be concluded that the brand F could have more side effect compared to all other brands tested.

For AC, the manufacture formulates the tablets in such a way that each tablet contains 10 mg of active ingredients. The remaining weight of the tablet would be the excipient. As per the second column of Table 6b, the average powered weight of AC tablet varies from (147.8 ± 0.68) mg to (179.6 ± 1.30) mg. The fourth column of Table 6b shows the calculated amount of active ingredient according to developed method and the amount ranges from (9.00 ± 0.21) mg to (9.88 ± 0.11) mg. The fifth column of Table 6a shows the percentage of active ingredient determined by the developed method and all the drug components have over 90% of active ingredients. Therefore, it can be concluded that all brands tested met the acceptance criteria and hence suitable to administer.

Conclusion

An analytical method was developed to determine metformin hydrochloride (MH) and atorvastatin calcium (AC) concentrations in commercially available tablets as per the ICH guidelines. The results demonstrated that the method was satisfactory, as each of the measured parameter has met the acceptance criteria recommended by the ICH. The method was validated and found to be simple, sensitive, rapid, linear, accurate and precise as per ICH guidelines. Further, it has been determined that the standards were stable at the room temperature and the test sample analysis can be done even in remote areas with less facilities using a portable UV-Vis spectrophotometer. Conclusively, the developed analytical method can be used for quantitative analysis of the active compound of MH and AC.

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Conflicts of Interest Statement

The author declares that he has no competing financial interests.

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