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Fast and Simple Method for Assay Determination of Metformin and Glyburide from Combination Tablet Dosage form by UV Spectrophotometer

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ABSTRACT

Fast, simple, economic and accurate methods have been developed for simultaneous determination of assay of metformin and glyburide from combination tablet dosage form. UV-Visible spectrophotometric method is developed with measurement of absorption at maximum wavelength of 233 nm and 301 nm for metformin and glyburide respectively. The developed method was validated and proved to be precise and robust. UV responses were found to be linear in the concentration range of 8 to 12 ppm for metformin and 80-120 ppm for glyburide. The correlation coefficient was found to be 0.9994 for metformin and 0.9998 for glyburide. The mean percentage recovery was found to be 99.8% for metformin and 99.9% for glyburide. This method will be useful for the determination of assay in combination tablet dosage form.

Keywords: Metformin, Glyburide, UV-Visible spectrophotometer

INTRODUCTION

Metformin hydrochloride (1,1-Dimethylbiguanide monohydrochloride) has molecular formula $C_4H_{11}N_5$.HCl and molecular weight 165.62 g/mol (Figure 1) [1]. It is an anti-hyperglycemic drug from biguanide class used in management of Type 2 diabetes. Metformin hydrochloride is a white to off-white crystalline compound and is freely soluble in water, slightly soluble in alcohol, practically insoluble in acetone and in methylene chloride. Glyburide has chemical name 1-[[p-[2-(5-chloro-o-anisamido)ethyl]phenyl]sulfonyl]-3-cyclo-hexylurea, with molecular formula $C_{23}H_{28}ClN_3O_5S$ and molecular weight 494.0 (Figure 2) [1]. Glyburide is an antihyperglycemic drug of the sulfonylurea class. Glyburide is a white to off-white crystalline which is insoluble in water.

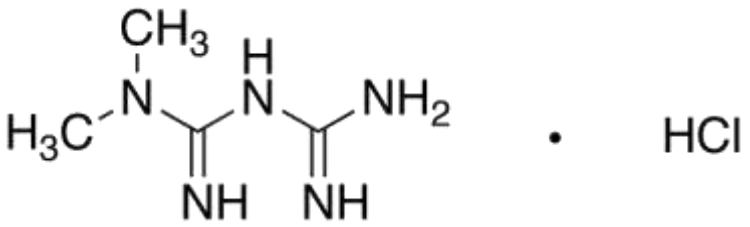

Figure 1: Metformin hydrochloride

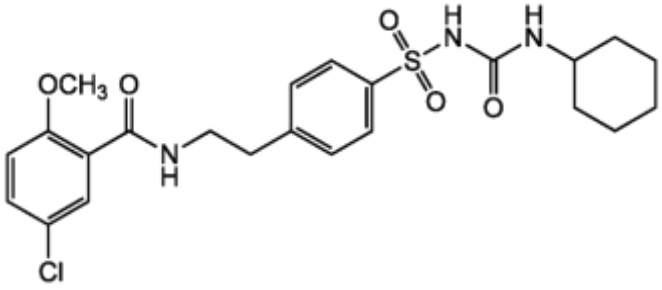

Figure 2: Glyburide

Metformin hydrochloride and glyburide tablets contain two oral antihyperglycemic drugs used in the management of type 2 diabetes, i.e., Metformin hydrochloride and glyburide. This combines' two anti-hyperglycemic agents with complementary mechanisms of action, to improve glycemic control in patients with type-2 diabetes. Metformin hydrochloride is an antihyperglycemic agent that improves glucose tolerance in patients with type-2 diabetes, lowering both basal and postprandial plasma glucose. Metformin hydrochloride decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Glyburide appears to lower blood glucose acutely by stimulating the release of insulin from the pancreas, an effect dependent upon functioning beta cells in the pancreatic islets. With chronic administration in patients with type-2 diabetes, the blood glucose lowering effect persists despite a gradual decline in the insulin secretory response to the drug. Extra-pancreatic effects may be involved in the mechanism of action of oral sulfonylurea hypoglycemic drugs [2].

Literature survey reveals few chromatographic methods and also methods using UV-Visible spectrophotometric techniques for estimation of combination drugs from various formulation dosage forms [3-10]. Official monograph in the United States Pharmacopoeia (USP) has a monograph for glyburide and metformin hydrochloride tablets consisting of separate High Performance Liquid Chromatography (HPLC) methods for assay and impurities of glyburide and metformin respectively [1]. The present study is aimed to develop and validate a fast, economic and reliable method of analysis using UV-Visible spectrophotometer for determination of Assay of metformin and glyburide. Special emphasis is given on development of a rapid and cost effective method of analysis; thereby to save upon precious analytical time, avoid costly instrumentation like HPLC and expensive chemicals. Easily available chemicals are been used for this developmental work. Development of a UV-Visible spectrophotometric method for assay of metformin and glyburide from combination tablets becomes a tricky task as both the drug have absorbance in the UV region with maxima of each drug in close proximity of each other. Hence use of acquired technical knowledge and expertise in method development becomes a vital tool to develop, execute and validate method thereby proving its effectiveness for routine use.

MATERIALS AND METHODS

Chemicals and reagents

Metformin API was received as a gift sample from M/S USV Ltd. Glyburide API was received as a gift sample from M/S Cadila Pharmaceuticals Ltd. These API were used as working standard. Combination tablets used for this activity of metformin HCl 500 mg and glyburide 5 mg were purchased from local medical stores. Methanol and acetonitrile used were of AR grade solvents of Rankem Ltd. Purified water used were from Millipore water purification system.

Instruments and equipment

UV-Visible spectrophotometer of the make-Jasco, Model No. V-630 was used in the experiment. Ultrasound Sonicator of local make was used during sample preparation.

Method of analysis for metformin

Preparation of diluent

Purified water was used as diluent.

Preparation of standard (10 ppm)

About 25 mg of metformin standard was accurately weighed and dissolved in sufficient water and diluted to 100 ml in a volumetric flask. Further, 2 ml of stock solution is diluted to 50 ml with diluent.

Estimation of metformin in tablet dosage form

Five intact tablets were crushed to fine powder. Transferred powder equivalent to 1 tablet into a dry 250 ml volumetric flask. About 120 ml of hot boiling water was added to this flask. Swirl to mix the contents and sonicated for 30 min with intermittent shaking. After sonication contents of flask was cooled to room temperature and diluted to volume upto the mark with diluent. From this solution, 1 ml of solution is diluted to 200 ml with diluent. Further, the solution is filtered with 0.45 µ filter porosity membrane filter before use.

Detection on UV-spectrophotometer

For the selection of analytical wavelength diluent, standard solution and sample solution were scanned in the spectrum mode from 400-200 nm separately. From the spectra of standard solution maximum wavelength 233 nm is selected for analysis, using water as blank and 1 cm quartz cuvettes.

Method of analysis for glyburide

Preparation of diluent

Acetonitrile and methanol was mixed in the proportion of (1:1) in a flat bottom flask and used as a diluent.

Preparation of standard (100 ppm)

About 20 mg of glyburide standard was accurately weighed and dissolved in sufficient diluent, diluted to 200 ml in a volumetric flask.

Estimation of glyburide in tablet dosage form

Five intact tablets were crushed to fine powder and transferred powder equivalent to 1 tablet into a dry 50 ml volumetric flask. About 25 ml of diluent was added to this flask. Swirl to mix the contents and sonicated for 30 min with intermittent shaking. After sonication the flasks was cooled to room temperature and dilute to volume upto the mark with diluent. Further, the solution was filtered with 0.45 µ filter porosity membrane filter before use.

Detection on UV-spectrophotometer

The diluents, standard and sample solutions were scanned in the spectrum mode from 200-400 nm and maximum wavelength 301 nm is selected with 1 cm quartz cuvette.

RESULTS AND DISCUSSION

Method development

The primary aim was to develop selective method for each drug using spectrophotometry. This was a challenge, as metformin and glyburide both exhibit absorbance in the range of 200-400 nm. Hence solutions of each drug were prepared separately and scanned. It was observed that Metformin exhibits maxima at about 233 nm, whereas glyburide exhibits maxima at about 230 nm and 301 nm (Figures 3 and 4). Since metformin does not have any maxima at about 301 nm, this wavelength was ideal for assay of glyburide as the interference of metformin at this wavelength would be negligible. However this was not the case vice-versa, as glyburide has a significant absorbance at 230 nm, considering the maxima of metformin at 233 nm. This causes a substantial interference for assay of metformin. Hence other characteristics of these drugs needed to be explored to develop a selective method. In this context, solubility characteristics of both drugs were studies. It was found that glyburide was insoluble in water whereas metformin was freely soluble in water. Selecting water as diluent for metformin in tablet sample would eliminate the interference of glyburide, as glyburide present in tablet matrix would not get solubilized in water, when used as diluent for assay of metformin. Hence method was developed with water as diluent for metformin with detection at 233 nm and Acetonitrile: Methanol (1:1) as diluent for glyburide with detection at 301 nm.

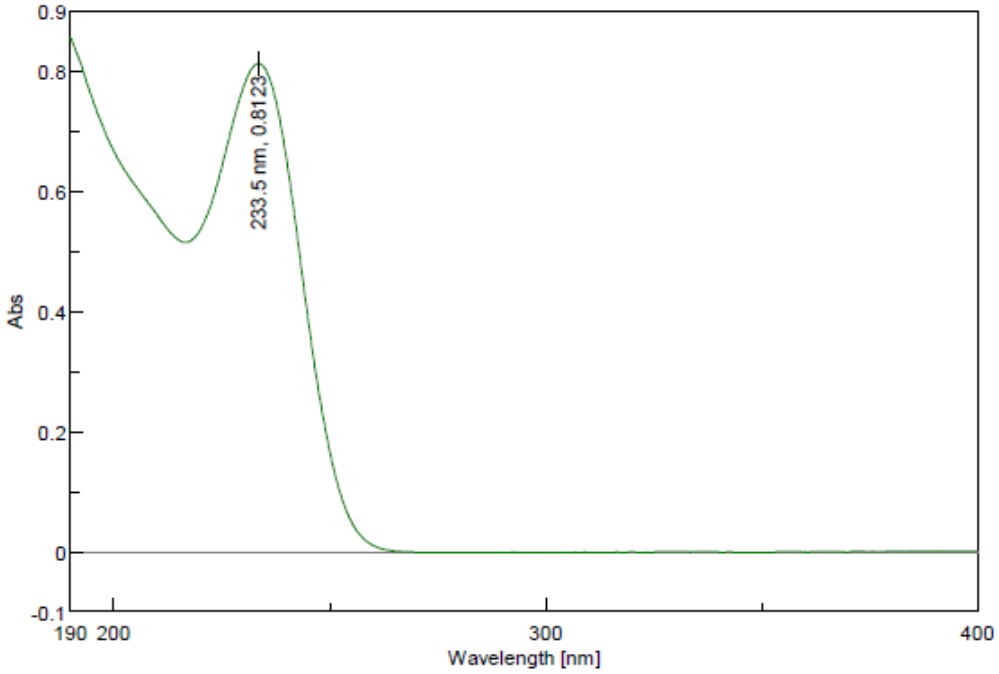

Figure 3: UV scan of metformin between 200-400 nm

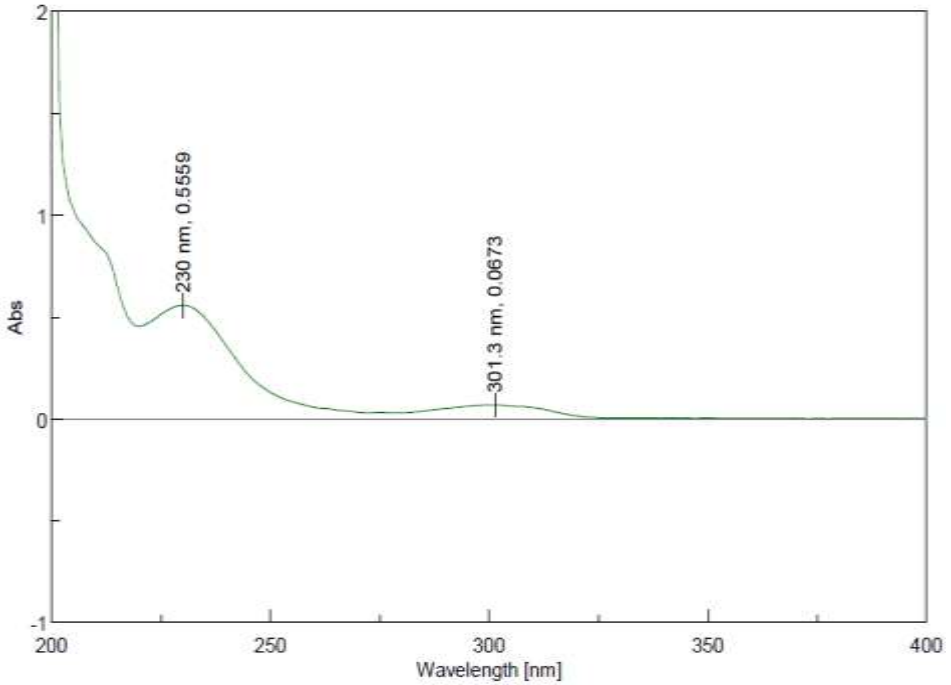

Figure 4: UV scan of glyburide between 200-400 nm

Method validation

The methods developed for each drug from combination tablet were validated for the assay of metformin and glyburide separately using validation parameters as mentioned in International Conference on Hormonization (ICH) guidelines [11].

Method validation for assay of metformin

Specificity

The specific and selective nature of method for metformin was proved by checking the interference of diluent (water) and glyburide at the 233 nm (Figure 5a). The scan of standard and sample were compared to check its conformance with respect to maxima observed in UV spectrum (Figure 5b and c). No interference for diluent (water) and glyburide was found at 233 nm. The spectrum of metformin standard shows maxima at 233.5 nm and that of tablet sample solution shows maxima at 233.4 nm. Hence the method is selective for assay of metformin at 233 nm.

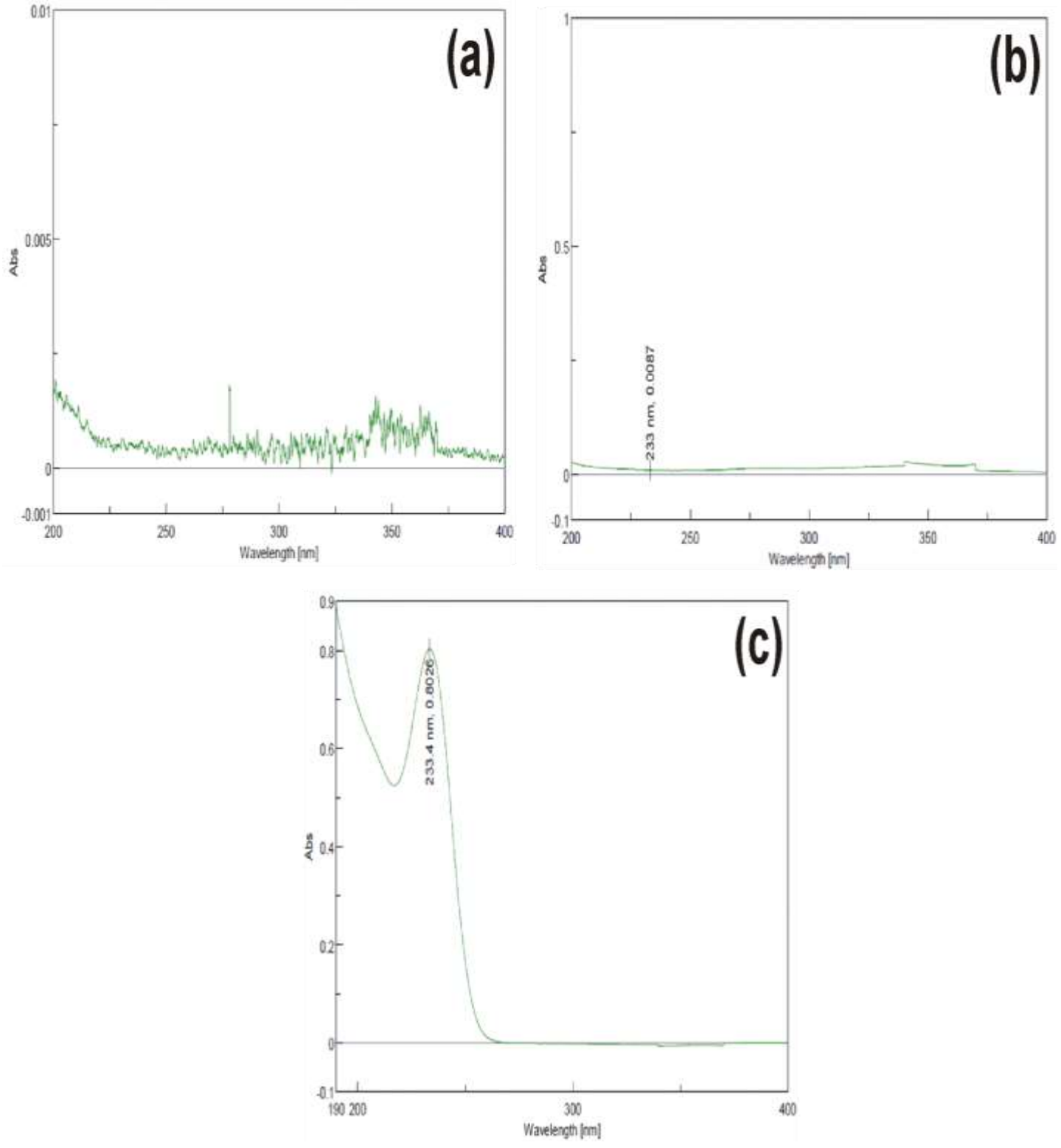

Figure 5: UV scan for specificity for (a) Diluent, (b) Glyburide in diluent and (c) Tablet sample for metformin content

Precision

Sample no.	Absorbance	Sample Wt. (mg)	mg/tablet	% assay of Label claim
Precision 1	0.8036	657.8	493.85	98.77
Precision 2	0.8095	658.1	497.25	99.45
Precision 3	0.8053	657.9	494.82	98.96
Precision 4	0.8067	656.1	497.04	99.41
Precision 5	0.8106	657.6	498.31	99.66
Precision 6	0.8089	658.1	496.88	99.38
Average	-			99.27
Std. deviation	-			0.33
% RSD	-			0.34

To prove the precise nature of method; system precision, method precision and intermediate precision was carried out for metformin content. System precision was carried out on five replicate measurements of metformin standard solution at 233 nm. For system precision, the %RSD for absorbance should be less than 2%. Method precision was carried out by preparing sample solution six times and recording its absorbance at selected wavelength. The assay value and the %RSD were calculated. For method precision, %RSD which should be less than 2%. Intermediate precision was carried out by carrying out the precision experiment on different day by different analyst. For intermediate precision, % cumulative RSD of 12 preparations of precision and intermediate precision should not be more than 2%. Following were the observation from precision study of metformin (Tables 1-3):

Table 1: System precision for metformin

Replicates	Standard absorbance
1	0.8036
2	0.8095
3	0.8053
4	0.8067
5	0.8106
Mean	0.8089
%RSD	0.36

Table 2: Method precision for metformin

Sample no.	Absorbance	Sample Wt. (mg)	mg/tablet	% assay of label claim
Precision 1	0.8036	657.8	493.85	98.77
Precision 2	0.8095	658.1	497.25	99.45
Precision 3	0.8053	657.9	494.82	98.96
Precision 4	0.8067	656.1	497.04	99.41
Precision 5	0.8106	657.6	498.31	99.66
Precision 6	0.8089	658.1	496.88	99.38
Average	-			99.27
Std. deviation	-			0.33
% RSD	-			0.34

Table 3: Intermediate precision for metformin

Sample no.	Absorbance	Sample Wt. (mg)	mg/tablet	% assay of label claim
Inter. Precision 1	0.8106	659.1	503.17	100.63
Inter. Precision 2	0.8109	661.4	501.6	100.32
Inter. Precision 3	0.8024	659.7	497.62	99.52
Inter. Precision 4	0.8134	662.1	502.62	100.52
Inter. Precision 5	0.8032	654.1	502.38	100.48
Inter. Precision 6	0.8012	649.7	504.53	100.91
Average			501.99	100.4
Std. deviation			2.35	0.47
% RSD			0.47	0.47
Average of 12 assays	-			99.83
Std dev. of 12 assays	-			0.7
% RSD of 12 assays	-			0.71

For system precision, it was found that %RSD for replicates absorbance of standard solution were 0.36% (Table 1). In method precision %RSD of six preparations was found to be 0.34% (Table 2). Intermediate precision was carried out by carrying out the precision experiment on different day by different analyst. For intermediate precision, % cumulative RSD of 12 preparations of precision and intermediate precision was found to be 0.71%. Hence it is concluded that the method was precise.

Linearity and range

For determination of linearity and range of the method, five solutions of different concentration from 80%-120% of the working level were prepared. Responses of these solutions were recorded between 200-400 nm (Figure 6). Correlation coefficient, slope and intercept were determined by statistical calculations. For a method to be linear within the workable range, the correlation coefficient should be more than 0.99.

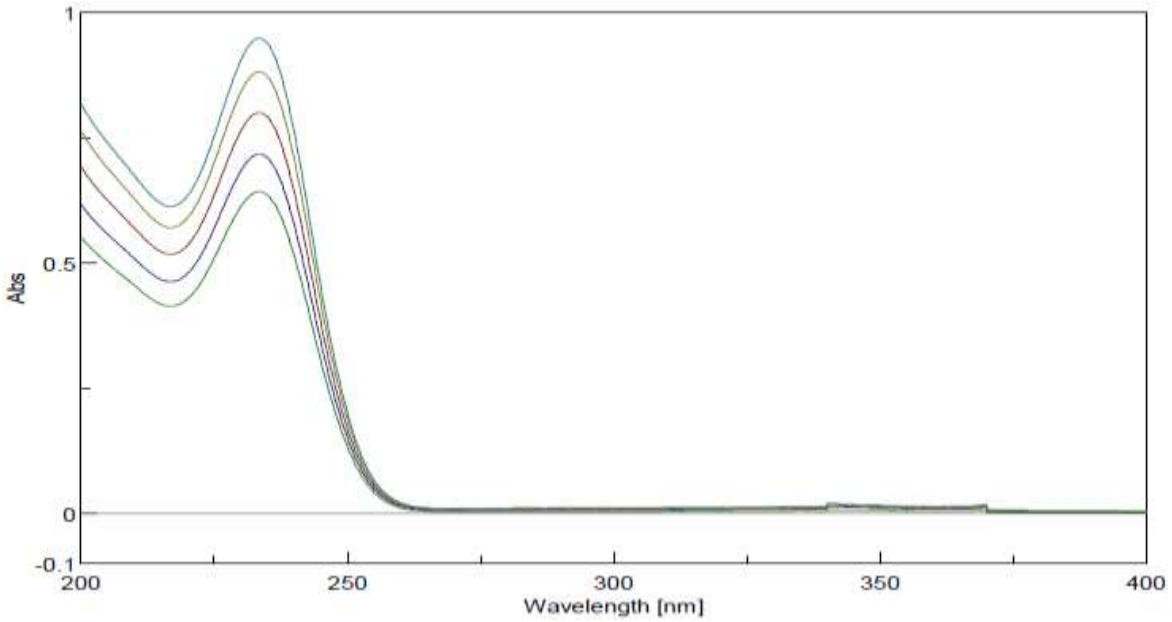

Figure 6: Overlay of spectrum for Metformin-Linearity levels from 80% to 120%

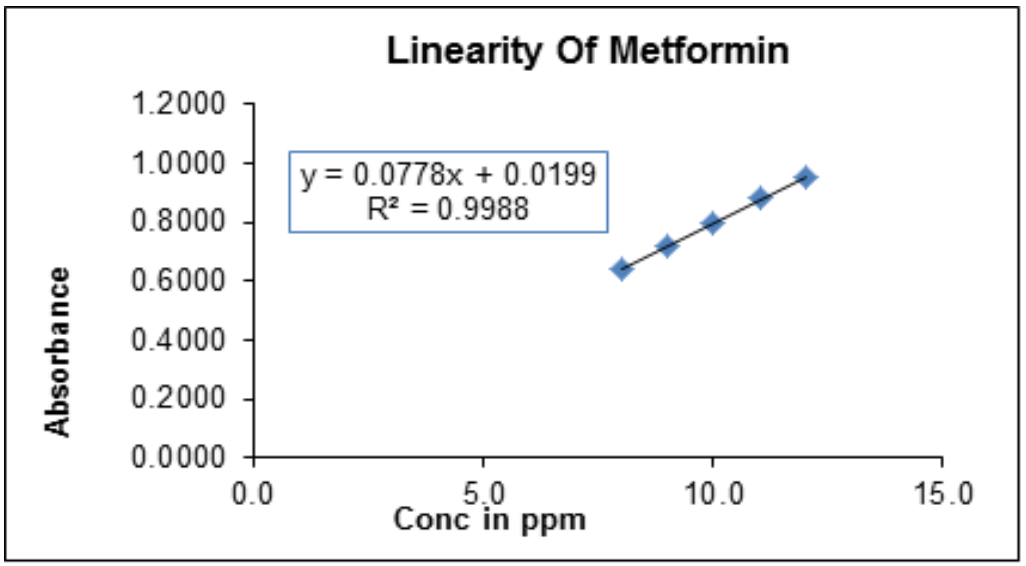

Figure 7: Linearity plot for Metformin-Absorbance vs. concentration

From the linearity data and the subsequent statistical analysis, it was found that the correlation coefficient (R) was 0.998, slope was found to be 0.077 and Y-Intercept was 0.019 (Figure 7). Hence it is concluded that the method is linear within the working range of 8 ppm to 12 ppm.

Accuracy

Accuracy was determined by recovery study of metformin at three levels in the range of 80%-120%. The percentage recovery was found to be 99.0%, 99.2% and 101.1% respectively at each level. The mean recovery of all the three levels was found to be 99.8%. Hence it is proved that the method is accurate for recovery of metformin.

Robustness

Robust nature of method needs to be proved by small deliberate change in the experimental condition. Being a spectrophotometric method, the wavelength of detection was changed by ± 1 nm and the assay was determined at these wavelengths. These results were compared with the results of as such condition. For a method to be robust, the absolute difference between as such condition and deliberately changed condition should not be more than 2%. It was found that %difference in assay value at 234 nm was 0.33% and at 232 nm was 0.75%. As results were within the acceptable criteria even after deliberate change to the experimental conditions, it is proved that the method remained unaffected by small variations of parameter.

Method validation for assay of glyburide

Specificity

The specific and selective nature of method for glyburide was proved by checking the interference of diluent (Acetonitrile: Methanol) and metformin at 301 nm (Figure 8a). The scan of standard and sample were compared to check its conformance with respect to maxima observed in UV spectrum. No interference for diluent (Acetonitrile: Methanol) and no interference of metformin was found at 301 nm (Figure 8b). The

spectrum of glyburide standard shows maxima at 300.6 nm and that of tablet sample solution shows maxima at 300.6 nm (Figure 8c and d). Hence the method is selective for assay of glyburide at 301 nm.

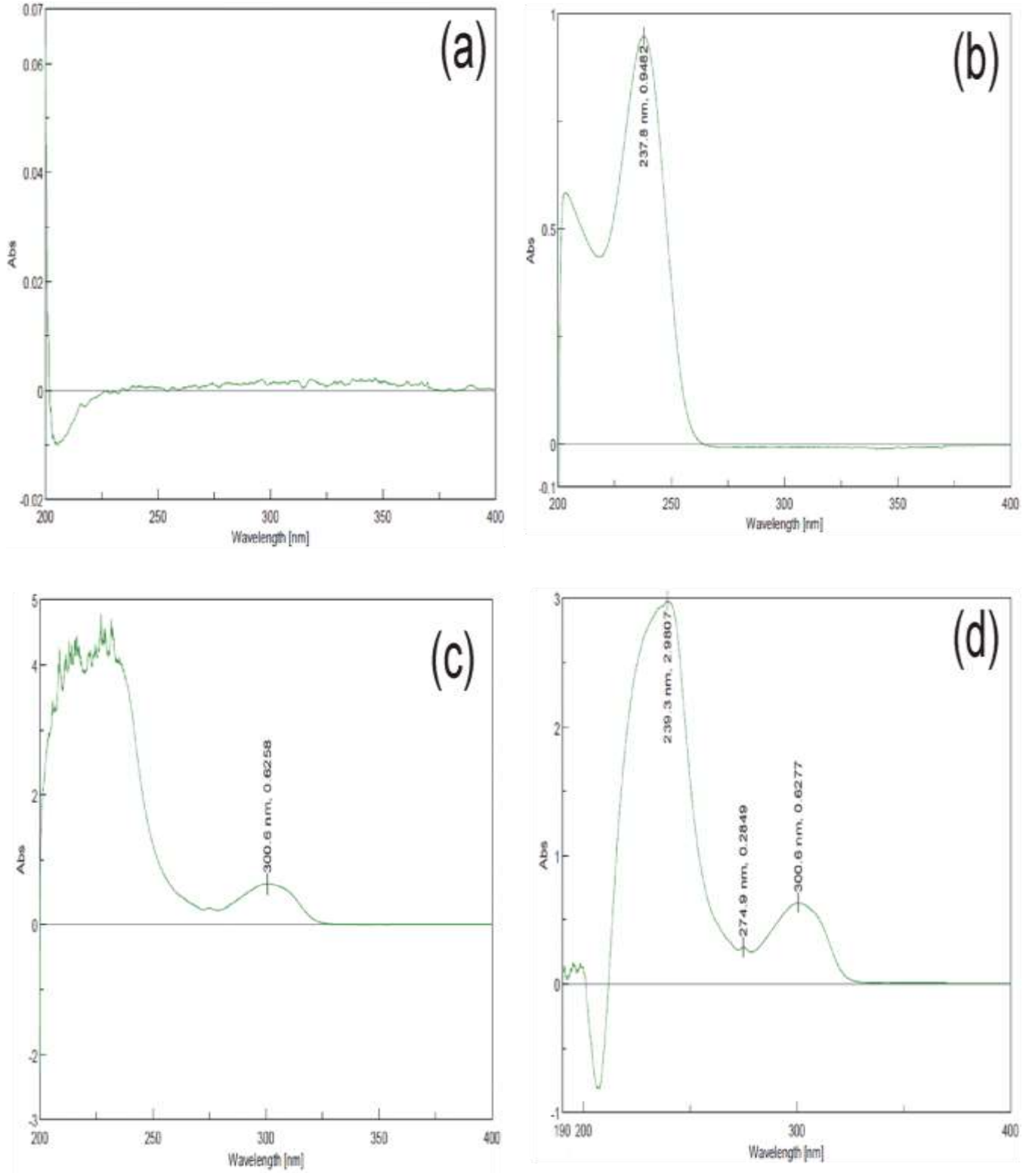

Figure 8: UV scan for Specificity (a) Diluent, (b) Metformin in diluents, (c) Glyburide standard and (d) Tablet sample for Glyburide content

Precision

To prove the precise nature of method; System precision, method precision and intermediate precision was carried out for glyburide. System precision was carried out on five replicate measurements of glyburide standard solution at 301 nm. Method precision was carried out by preparing sample solution six times and recording its absorbance at selected wavelength (Table 4). The assay value and the %RSD were calculated. Intermediate precision was carried out by carrying out the precision experiment on different day by different analyst. For intermediate precision, % cumulative RSD of 12 preparations of precision and intermediate precision was calculated. Following were the observation from precision study of glyburide:

Table 4: System precision for glyburide

Replicates	Standard Absorbance
1	0.6260
2	0.6251
3	0.6262
4	0.6249
5	0.6266
Mean	0.6258
%RSD	0.12

Table 5: Method precision for glyburide

Sample no.	Absorbance	Sample Wt. (mg)	mg/ tablet	% assay of Label claim
Precision 1	0.6277	648.9	5.05	101.1
Precision 2	0.6227	655.1	4.97	99.34
Precision 3	0.6201	648.9	4.99	99.87
Precision 4	0.6199	641.2	5.05	101.04
Precision 5	0.6287	658.4	4.99	99.8
Precision 6	0.6267	648.3	5.05	101.03
Average	-			100.36
Std. deviation	-			0.78
% RSD	-			0.78

Table 6: Intermediate precision for glyburide

Sample no.	Absorbance	Sample Wt. (mg)	mg/ tablet	% assay of Label claim
Inter. Precision 1	0.6197	654.1	5.00	100.09
Inter. Precision 2	0.6204	652.7	5.02	100.42
Inter. Precision 3	0.6229	653.6	5.03	100.69
Inter. Precision 4	0.6232	659.2	4.99	99.88
Inter. Precision 5	0.6217	649.7	5.05	101.10
Inter. Precision 6	0.6228	642.1	5.12	102.47
Average	-			100.78
Std. deviation	-			0.94
% RSD	-			0.93
Average of 12 assays	-			100.57
Std dev. of 12 assays	-			0.85
% RSD of 12 assays	-			0.84

For system precision, it was found that %RSD for replicates absorbance of standard solution were 0.12%. In method precision %RSD of six preparations by was found to be 0.78% (Table 5). Intermediate precision was carried out by carrying out the precision experiment on different day by different analyst. For intermediate precision, % cumulative RSD of 12 preparations of precision and intermediate precision was found to be 0.84% (Table 6). Hence it is concluded that the method was precise.

Linearity and range

For determination of linearity and range of the method, five solutions of different concentration from 80% to 120% of the working level of glyburide were prepared. Responses of these solutions were recorded between 200-400 nm (Figure 9). Correlation coefficient, slope and intercept were determined by statistical calculations. For a method to be linear within the workable range, the correlation coefficient should be more than 0.99

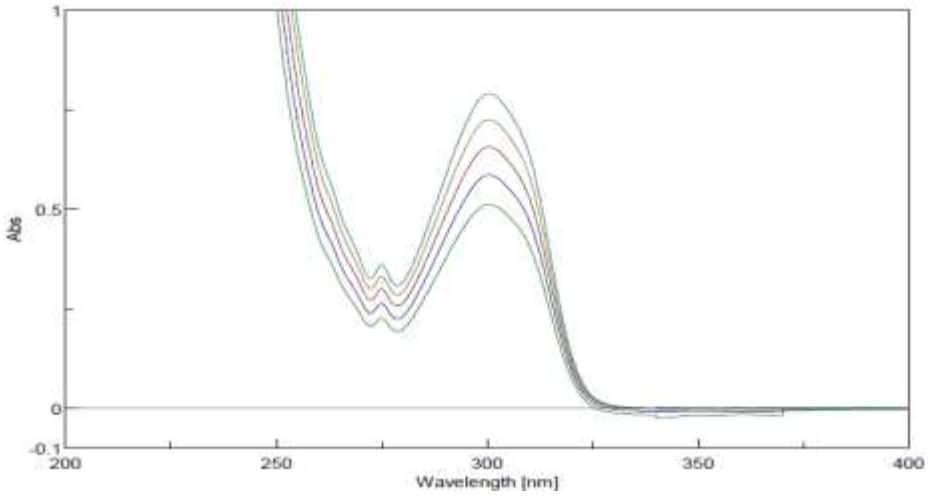

Figure 9: Overlay of spectrum for glyburide linearity levels from 80% to 120%

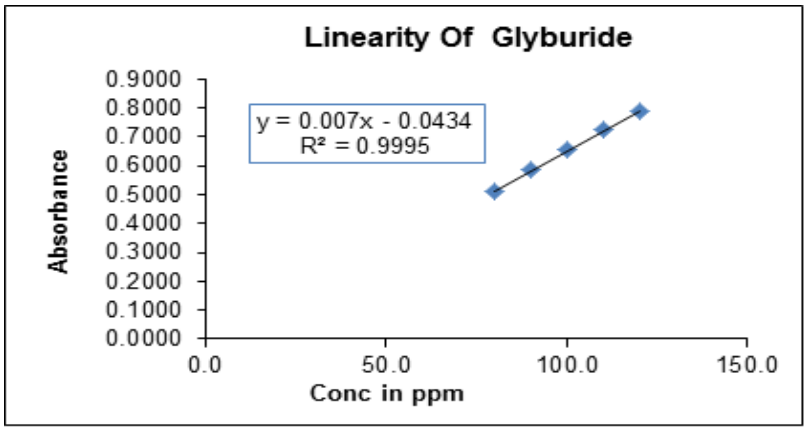

Figure 10: Linearity plot for Glyburide-Absorbance vs. concentration

From the linearity data and the subsequent statistical analysis, it was found that the correlation coefficient (R) was 0.999, slope was found to be 0.007 and Y-Intercept was -0.0434 (Figure 10). Hence it is concluded that the method is Linear within the working range of 80-120 ppm.

Accuracy

Accuracy was determined by recovery study of glyburide at three levels in the range of 80%-120%. The percentage recovery was found to be 100.0%, 99.6% and 100.2% respectively at each level. The mean recovery of all the three levels was found to be 99.9%. Hence it is proved that the method is accurate for recovery of glyburide.

Robustness

Robust nature of method for glyburide was proved by small deliberate change in the experimental condition. Being a spectrophotometric method, the wavelength of detection was changed by ± 1 nm and the assay was determined at these wavelengths. These results were compared with the results of as such condition. For a method to be robust, the absolute difference between as such condition and deliberately changed condition should not be more than 2%. It was found that %difference in assay value at 302 nm was 0.47% and at 300 nm was 0.44%. As results were within the acceptable criteria even after deliberate change to the experimental conditions, it is proved that the method remained unaffected by small variations of parameter.

CONCLUSION

Analytical methods for the determination of Assay of Metformin and Glyburide in combination tablet dosage form using UV-Visible spectrophotometer were developed and validated. The developed methods werefound to bespecific, accurate, precise, linear and robust for its intended use.

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