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Development and Validation for Simultaneous Estimation of Alogliptin and Metformin in Combined Dosage form by UV Spectroscopy Method

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Abstract

The method used was successfully applied for the determination of both the drugs which are alogliptin and metformin in combined dosage form. The method used in the study was UV spectroscopy method. Linearity, accuracy, precision and stability have been studied. The results of the analysis have been validated statistically and by recovery studies which were according to ICH guidelines.

Keywords: Alogliptin, metformin, UV spectrophotometry, ICH guidelines.

INTRODUCTION:

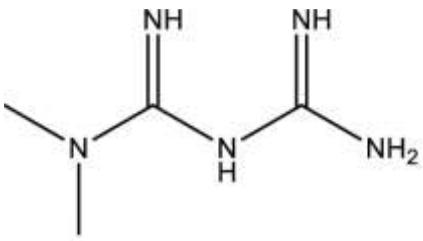
Drug Profile:

Drug Profile of Metformin Hcl

Proper Name: Metformin hydrochloride

Chemical Name: 1,1-Dimethylbiguanide hydrochloride

Chemical Structure:



. HCl

Description: Metformin Hydrochloride is the hydrochloride salt of the biguanide metformin with antihyperglycemic and potential antineoplastic activities. Metformin inhibits complex I (NADPH:ubiquinone oxidoreductase) of the mitochondrial respiratory chain, thereby increasing the cellular AMP to ATP ratio and leading to activation of AMP-activated protein kinase (AMPK) and regulating AMPK-mediated transcription of target genes. This eventually prevents hepatic gluconeogenesis, enhances insulin sensitivity and fatty acid oxidation and ultimately leads to a decrease in glucose levels. Metformin may exert antineoplastic effects through AMPK-mediated or AMPK-independent inhibition of mammalian target of rapamycin (mTOR), which is up-regulated in many cancer tissues. Furthermore, this agent also inhibits tumor cell migration and invasion by inhibiting matrix metalloproteinase-9 (MMP-9) expression which is mediated through the suppression of transcription activator protein-1 (AP-1) activation.

Empirical Formula: $C_4H_{12}ClN_5$

Molecular Weight: 165.62

Physical Form: It is a white crystalline powder.

Solubility: Freely soluble in water; slightly soluble in alcohol. Practically insoluble in ether, chloroform, acetone, methylene chloride.

pKa: 12.4

log Kow = -2.64 at 25 °C

Contra indications: Kidney disorders, lung diseases, liver diseases.

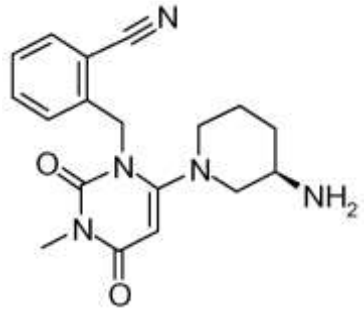
Pharmacological action: Oral hypoglycemic agent. It is used in the first line therapy in the treatment of type –ii diabetes mellitus. It decreases blood glucose levels by decreasing hepatic glucose production and improving insulin sensitivity by increasing peripheral glucose uptake and utilization.

Adverse drug reaction : gastrointestinal irritation including diarrhoea, nausea, vomiting.

Storage: Store at room temperature.

Drug Profile of Alogliptin

Chemical Structure:



Molecular weight: 339.39 g/mol

IUPAC name: 2-({6-[(3r)-3-aminopiperidin-1-yl]-3-methyl-2,4- dioxo-3, 4-dihydropyrimidin-ethyl) benzonitrile

Empirical formula: $C_{18}H_{21}N_5O_2$

Pharmacopeial status: Official in USP.

Description: Colourless fine powder.

Solubility: Soluble in methanol, sparingly soluble in water.

Indications: Indicated as an adjunct diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus.

Pharmacological action:

Novel hypoglycemic drug belongs to dipeptidyl peptidase-4 inhibitor group, which normally degrades the incretins glucose dependant insulin tropic polypeptide (git) and glucagon like peptide.

Adverse drug reaction: Hypoglycaemia, joint pain.

Storage: store in cool temperature.

Presentation: film coated tablet.

Trade name: Kazano [Metformin 500mg, Alogliptin 12.5mg]

MATERIALS AND METHODS:

Drug Sample

Metformin HCl was received as gift sample from Cadila pharmaceutical, Ahmedabad, Gujarat. Alogliptin was purchased from Vivan Life sciences, Thane, Maharashtra. The formulation KAZANO® (Metformin and Alogliptin) 500-12.5mg tablets (Takeda, Canada) were purchased from Syras Pharmaceuticals., Tumkur, Karnataka

Selection of Solvent

Preparation of Metformin hydrochloride in 0.1 N NaOH

100mg of metformin hydrochloride was weighed and transferred to a 100 ml standard flask and dissolved in 0.1N sodium hydroxide solventand made up to the volume (Conc 1mg/ml). 1ml of the stock solution was pipetted out into separate 10ml standard flask and made upto the volume using 0.1N sodium hydroxide solvent (Conc 100µg/ml). From this resulting solution, dilutions in the range of 40 µg/ml of the drug were prepared with 0.1N sodium hydroxide solvent as solvent.

Preparation of Metformin hydrochloride in Acetonitrile

100mg of metformin hydrochloride was weighed and transferred to a 100 ml standard flask and dissolved in Acetonitrile solvent and made up to the volume (Conc 1mg/ml). 1ml of the stock solution was pipetted out into separate 10ml standard flask and made upto the volume using Acetonitrile solvent (Conc 100µg/ml). From this resulting solution, dilutions in the range of 1 µg/ml of the drug were prepared with Acetonitrile solvent as solvent.

Preparation of Metformin hydrochloride in Methanol

100mg of metformin hydrochloride was weighed and transferred to a 100 ml standard flask and dissolved in methanol solvent and made up to the volume (Conc 1mg/ml). 1ml of the stock solution was pipetted out into separate 10ml standard flask and made upto the volume using methanol solvent(conc 100µg/ml). From this resulting solution, dilutions in the range of 40 µg/ml of the drug were prepared with methanol solvent as solvent.

Preparation of Alogliptin in 0.1 N NaOH

100mg of Alogliptin was weighed and transferred to a 100ml standard flask and dissolved in 0.1N sodium hydroxide solvent and made up to the volume (conc 1mg/ml). 1ml of the stock solution was pipetted out into separate 10ml standard flask and made upto the volume using 0.1N sodium hydroxide solvent (Conc 100µg/ml). From this resulting solution, dilutions in the range of 10 µg/ml of the drug were prepared with 0.1N sodium hydroxide solvent as solvent.

Preparation of Alogliptin in Acetonitrile

100mg of Alogliptin was weighed and transferred to a 100ml standard flask and dissolved in Acetonitrile solvent and made up to the volume (Conc 1mg/ml). 1ml of the stock solution was pipetted out into separate 10ml standard flask and made upto the volume using Acetonitrile solvent (Conc 100µg/ml). From this resulting solution, dilutions in the range of 10 µg/ml of the drug were prepared with Acetonitrile solvent as solvent.

Preparation of Alogliptin in Methanol

100mg of Alogliptin was weighed and transferred to a 100ml standard flask and dissolved in methanol solvent and made up to the volume (Conc 1mg/ml). 1ml of the stock solution was pipetted out into separate 10ml standard flask and made upto the volume using methanol solvent (Conc 100µg/ml). From this resulting solution, dilutions in the range of 10 µg/ml of the drug were prepared with methanol solvent as solvent.

The UV spectrum of the solution recorded and overlain spectra of metformin and alogliptin in the solutions shown in the figures.

Selection of Wavelength

The Absorbances of both the drugs Metformin hydrochloride and Alogliptinwere analysed for higher and sharp peak in various solvents through software.

Analysis of Formulation

Preparation of Standard Solution

Stock solutions of metformin (400 µg/ml) and alogliptin (10 µg/ml) are prepared in methanol were prepared in 100ml volumetric flask seperately.

Then a final solution of metformin (40 µg/ml) and alogliptin (1 µg/ml) and mixture of metformin and alogliptin (40 µg/ml and 1 µg/ml) were prepared in methanol.

The standard curve was plotted using different concentration of

- Metformin (2 µg/ml to 10 µg/ml)
- Alogliptin (0.05 µg/ml to 0.25µg/ml) and
- Mixture of metformin and alogliptin (2.05 µg/ml to10.25 µg/ml) were prepared from respective stock solution

The absorbance's were noted at selected wavelengths and shown in tables.

Preparation of Sample Solution

20 tablets containing 500 mg of metformin and 12.5 mg of alogliptin (Label claimed) were weighed. The quantity equivalent to 40 mg of metformin and 1 mg of alogliptin were taken and dissolved in Methanol. Then the resulting solution is sonicated for 20 mins and makes upto the mark by using same solvent. The solution was filtered and absorbances were noted at 237 nm and 225 nm and amount of metformin and alogliptin were calculated using simultaneous equation method, formula is given below.

$$c_x = \left(\frac{A_2 a_{y1} - A_1 a_{y2}}{a_{x2} a_{y1} - a_{x1} a_{y2}}\right) \quad \text{And} \quad c_y = \left(\frac{A_1 a_{x2} - A_2 a_{x1}}{a_{x2} a_{y1} - a_{x1} a_{y2}}\right)$$

Where,

A1 and A2 are absorbances of formulation at 237 and 225 respectively,

Cx and Cy are the concentration of metformin and alogliptin respectively,

ax1 and ax2are absorptivities of metformin at 237 and 225 respectively,

ay1 and ay2 are absorptivities of alogliptin at 237 and 225 respectively,

% Label claim and estimated amount had shown in Table.

Validation

Linearity

- **Linearity for Metformin HCl**

Linearity of the Metformin was analyzed by preparing the dilution in the range of 2-20 µg/ml. The absorbance of this solution was noted at the wavelength 237nm and 225nm and calibration curve were plotted using concentration Vs absorbance shown in figures.

- **Linearity for Alogliptin**

Linearity of the Alogliptin was analyzed by preparing the dilution in the range of 0.05-0.50 µg/ml. The absorbance of this solution was noted at the wavelength 237nm and 225nm and calibration curve were plotted using concentration Vs absorbance shown in figures.

Accuracy

To study the reliability, suitability and accuracy of the method recovery experiments were carried out. To the formulation equivalent to 40 mg of metformin and 1 mg of alogliptin at the level of 80% and 100% pure metformin and alogliptin were added and suitably diluted. The contents were determined from respective chromatogram and table.

Precision

The following Precision studies were carried out,

1. Intraday precision
2. Interday precision

- **Intraday precision**

Intraday precision was carried out by analyzing the standard drug solution at three different concentrations in the linearity range for both the drugs, for three times on the same day and % RSD was calculated.

- **Interday precision**

Interday precision was carried out by analyzing the standard drug solution at three different concentrations in the linearity range for both the drugs, for three times on the same day and % RSD was calculated.

Stability

The sample solution was subjected to stability studies at (RT) room condition. Stability was studied to find any change in absorbance and peak shape of UV spectra when compared to the freshly prepared solution.

RESULTS AND DISCUSSIONS:

Selection of Solvent

Spectra and Interpretation of Metformin hydrochloride and Alogliptin in 0.1 N NaOH

The solutions were scanned in UV region (200 to 400 nm) and characteristic of absorbance and the λmax was analysed. The following Fig represents the Spectra of Metformin hydrochloride and Alogliptin in 0.1 N NaOH. A well resolved peak was not obtained in this solvent shown in Figure No. 1.

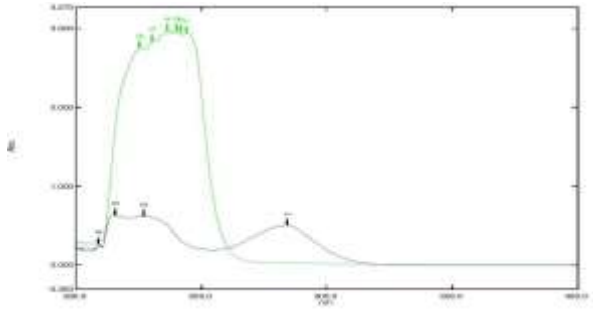


Figure No. 1. : UV Spectra of Metformin hydrochloride and Alogliptin in 0.1 N NaOH

Spectra and Interpretation of Metformin hydrochloride and Alogliptin in Acetonitrile

The solutions were scanned in UV region (200 to 400 nm) and characteristic of absorbance and the λmax was analysed. The following Fig represents the Spectra of Metformin hydrochloride and Alogliptin in Acetonitrile, which can be used as solvent in HPLC also. A well resolved peak was not obtained in this solvent also shown in Figure No. 2.

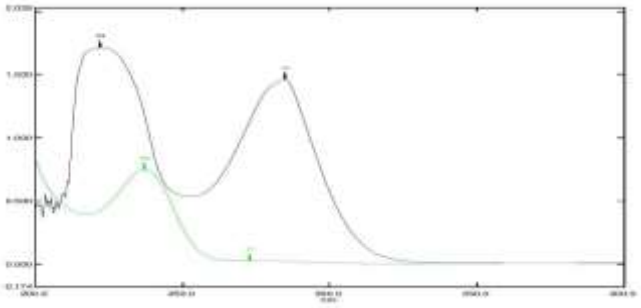


Figure No. 2. : UV Spectra of Metformin hydrochloride and Alogliptin in Acetonitrile

Spectra and Interpretation of Metformin hydrochloride and Alogliptin in Methanol

The solutions were scanned in UV region (200 to 400 nm) and characteristic of absorbance and the λmax was analysed. The following Fig represents the Spectra of Metformin hydrochloride and Alogliptin in Methanol, which can be used as solvent in HPLC also. The detection wavelength λmax which can selected is 237 nm for metformin and 225 nm for alogliptin because of clear and definite peak was observed and shown in Figure No. 3.

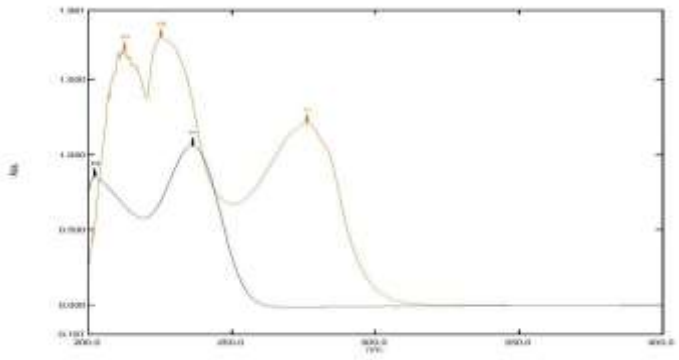


Figure No. 3. : UV Spectra of Metformin hydrochloride and Alogliptin in Methanol

Selection of Wavelength

Absorbance's of both the drugs were higher and gave good sharp peak in methanol, so it was decided to prepare drug solution in methanol for further studies. The λ_{max} of metformin and alogliptin were found to be 237 nm and 225 nm respectively shown in Figure No. 4.

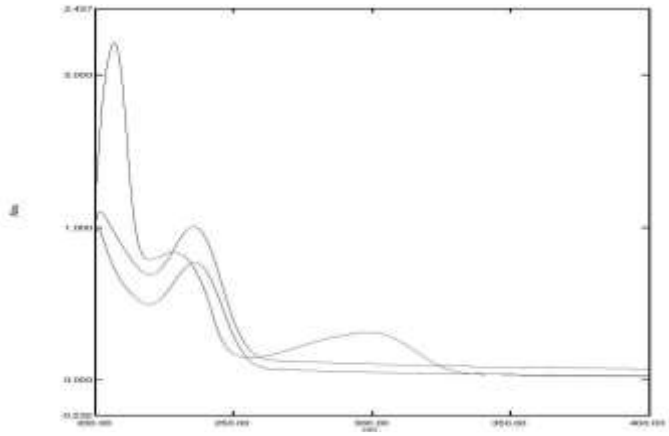


Figure No. 4. : Overlain spectra of Metformin, Alogliptin and Mixture of Metformin and Alogliptin in Methanol

Analysis of Formulation

Preparation of Standard Solution of Metformin

The standard curve of Metformin was plotted using different concentration of (2 µg/ml to 10 µg/ml) is shown in Table No.1. and Figures No. 5 & 6.

Table No. 1: Absorbance of metformin at selected λ_{max} 237nm and 225nm

S. No.	Concentration (µg/ml)	Absorbance at λ_{max} 237nm	Absorbance at λ_{max} 225nm
1	2	0.223	0.173
2	4	0.419	0.267
3	6	0.637	0.421
4	8	0.812	0.565
5	10	1.026	0.697

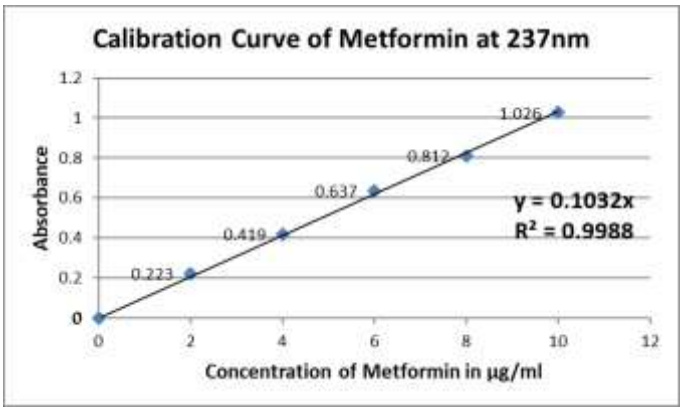


Figure No. 5. : Standard Curve of Metformin at λ_{max} 237nm

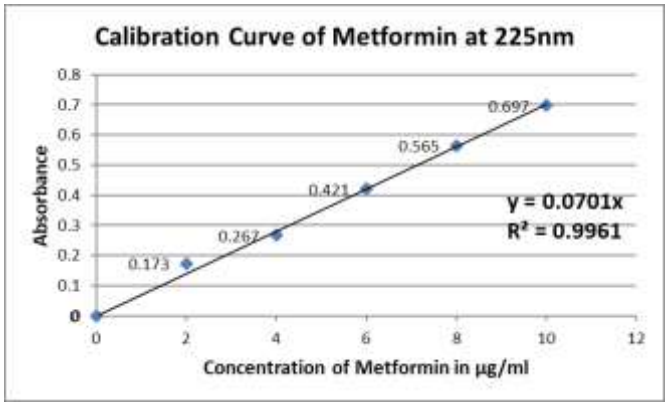


Figure No. 6. : Standard Curve of Metformin at λ_{max} 225nm

Preparation of Standard Solution of Alogliptin

The standard curve of Alogliptin was plotted using different concentration of (0.05 µg/ml to 0.25µg/ml) is shown in Table No. 2 and Figure No. 7 & 8.

Table No. 2. : Absorbance of Alogliptin at selected λ_{max} 237nm and 225nm

S. No	Concentration (µg/ml)	Absorbance at λ_{max} 237nm	Absorbance at λ_{max} 225nm
1	0.05	0.014	0.021
2	0.10	0.019	0.041
3	0.15	0.026	0.061
4	0.20	0.036	0.078
5	0.25	0.042	0.097

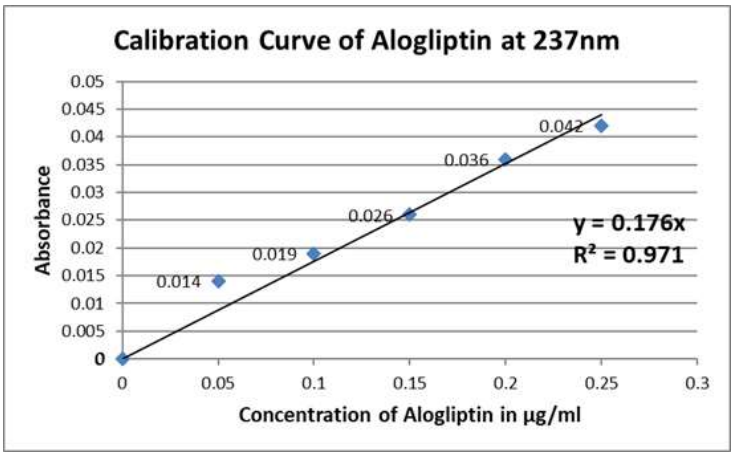


Figure No. 7. : Standard Curve of Alogliptin at λ_{max} 237nm

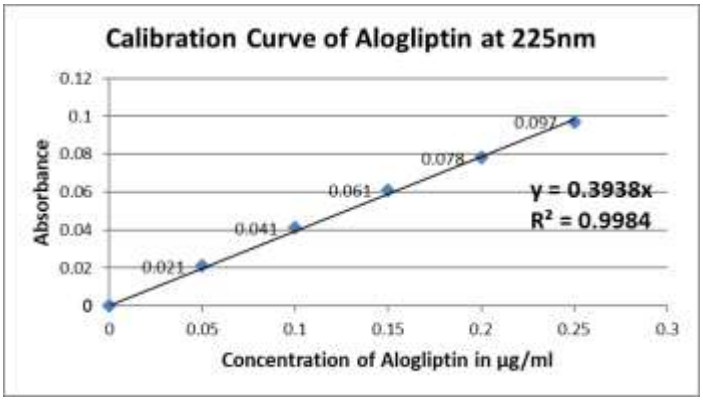


Figure No. 8: Standard Curve of Alogliptin at λ_{max} 225nm

Preparation of Sample Solution

The results of the tablet formulation solution was analysed at 237 nm and 225 nm for the amount of metformin and alogliptin were calculated using simultaneous equation method is given in the following Table No. 3.

Table No. 3. : Analysis of formulation

S. No.	Drug	Amount(mg)		%Label claim	%RSD*
		Labeled	Estimated		
1	Metformin	500	498.45	99.7	0.34
2	Alogliptin	12.5	12.16	97.28	0.46

*RSD (n=5)

Validation

Linearity

- **Linearity for Metformin HCl**

Linearity of the Metformin was analyzed by preparing the dilution in the range of 2-20 µg/ml. The absorbance of this solution was noted at the wavelength 237 nm and 225 nm as shown in Figures No 9 & 10.

The linearity was found in the range of 2-10 µg/ml at both the wavelength. At 237 nm the R^2 value was 0.998 and at 225 nm the R^2 value was 0.996.

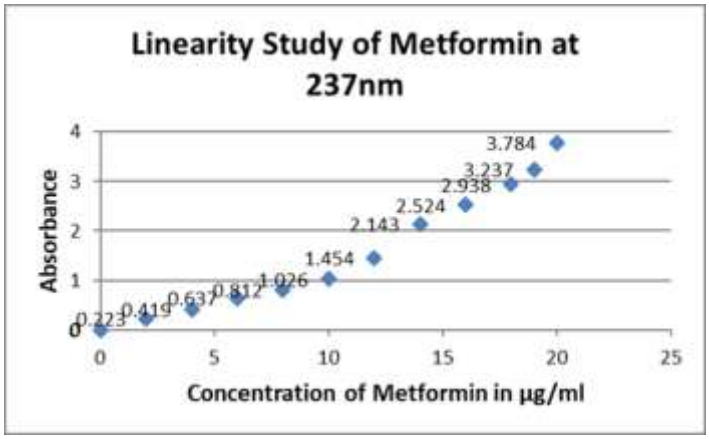


Figure No. 9: Linearity Study of Metformin at λ_{max} 237nm

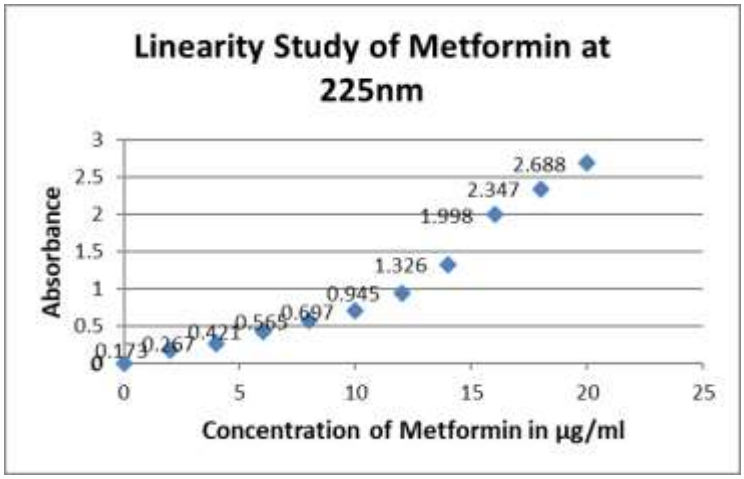


Figure No. 10. : Linearity Study of Metformin at λ_{max} 225nm

- **Linearity for Alogliptin**

Linearity of the Alogliptin was analyzed by preparing the dilution in the range of 0.05-0.50 µg/ml. The absorbance of this solution was noted at the wavelength 237 nm and 225 nm shown in Figures No. 11 & 12**.**

The linearity was found in the range of 2-10 µg/ml at both the wavelength. At 237 nm the R^2 value was 0.971 and at 225 nm the R^2 value was 0.998.

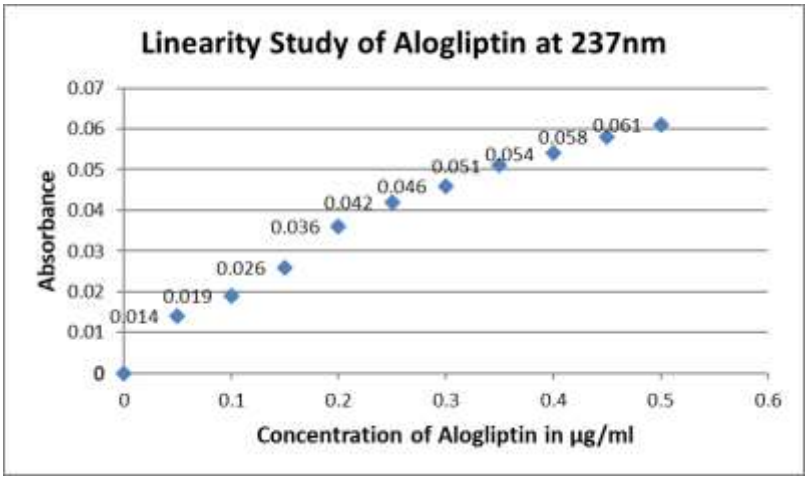


Figure No. 11. : Linearity Study of Alogliptin at λ_{max} 237nm

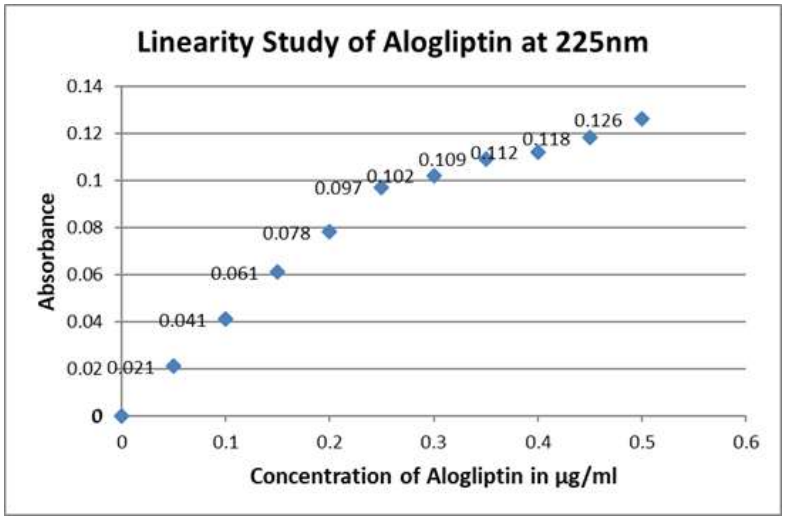


Figure No. 12. : Linearity Study of Alogliptin at λ_{max} 225nm

Accuracy

The recovery studies and the contents were determined from respective chromatogram and shown in Table No. 4.

Table No. 4: Accuracy study of Metformin and Alogliptin

S. No.	Level	% Recovery		% RSD	
		Metformin	Alogliptin	Metformin	Alogliptin
1	80%	99.83	98.73	0.201	0.260
2	120%	98.79	101.43	0.106	0.424

% RSD (n=5)

Precision

The intraday and interday precision study doesn't show change in % RSD more than 0.5% shown in Table No. 5 & 6. So the Procedure can be effectively used for analysis.

Table No. 5. : Intraday Study of Metformin and Alogliptin

S. No.	Drug	Amount(mg)		%Label claim	%RSD*
		Labeled	Estimated		
1	Metformin	500	499.34	99.86	0.24
2	Alogliptin	12.5	12.32	98.56	0.36

*RSD (n=3)

Table No. 6. : Interday Study of Metformin and Alogliptin

S. No	Drug	Amount(mg)		%Label claim	%RSD*
		Labeled	Estimated		
1	Metformin	500	498.87	99.77	0.34
2	Alogliptin	12.5	12.47	99.76	0.46

*RSD (n=5)

Stability

Stability was studied shown that there is no change in absorbance and peak shape of UV spectra when compared to the freshly prepared solution given in Table No. 7.

Table No. 7: Stability Study of Metformin and Alogliptin

S. No.	Drug	Absorbance Freshly Prepared		Absorbance after 3 hrs	
		λ_{max} 237 nm	λ_{max} 225 nm	λ_{max} 237 nm	λ_{max} 225 nm
1	Metformin (10 µg/ml)	1.026	0.697	1.024	0.699
2	Alogliptin (2.0 µg/ml)	0.036	0.078	0.036	0.076

CONCLUSION:

The new UV-spectrophotometric technique is found to be quite straightforward, accurate, exact, repeatable, sensitive, and affordable based on the results obtained. Without any prior component separation, they can be used as an efficient analytical instrument for routine quality control of pharmaceutical dosage forms containing a combination of metformin and alogliptin.

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