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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF LINAGLIPTIN AND DAPAGLIFLOZIN IN ITS PURE AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

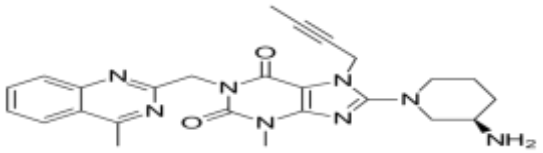
A Simple, precise, accurate, reproducible, robust and economical UV-Spectrophotometric method for simultaneous estimation of Linagliptin and Dapagliflozin in its pure and pharmaceutical dosage form have been developed. This method uses simultaneous equations based on the measurement of absorbances at two wavelengths 234nm and 230nm which are λmax of Linagliptin and Dapagliflozin respectively in ethanol solvent. These two drugs shows linearity at all the selected wavelengths and obey's Beer's Law in the concentration range of 6-14µg/ml for both the drugs. R^2 for both the drugs were found to be 0.999. Reproducibility and Inter-day precision were found less than 2% RSD. Accuracy for both the drugs were found within the limits. Statistical data shows that proposed work can be applied for the routine analysis of drugs in Quality Control Laboratory.

1. INTRODUCTION:

Simultaneous estimation plays an important role in pharmaceutical industry because it is very feasible and timesaving method. Specrophotometric methods and Chromatogaphic methods provide high degree of assurance that these techniques fit for the simultaneous estimationin the pharmaceutical dosage form [(1)]. Simultaneous equation method can be applicable for estimation of drugs whose spectra overlap properly. If sample contains two absorbing species and each species is absorbind at λmax of the other, it is possible to determine both the drugs by using simultaneous equation method. This method employs UV-Spectrophotometric method for the quantitative estimation of the drugs.

2. DRUG PROFILE:

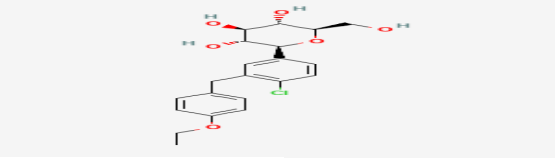
2.1. LINAGLIPTIN:



Molecular Formula: $C_{25}H_{28}N_8O_2$ $C_{25}H_{28}N_8$
Molecular Weight : 472.54
Appearance : White to yellow
Solubility: Soluble in ethanol, methanol, dimethyl formamide and slightly soluble in water.
Mechanism of action: Linagliptin is a competitive, reversible DPP-4 inhibitor. Inhibition of this enzyme slows the breakdown of GLP-1 and glucose-dependant

insulinotropic polypeptide (GIP). GLP-1 and GIP stimulate the release of insulin from beta cells in the pancreas while inhibiting release of glucagon from pancreatic beta cells. These effects together reduce the breakdown of glycogen in the liver and increase insulin release in response to glucose[(2)].

2.2.DAPAGLIFLOZIN:



Molecular Formula: $C_{24}H_{35}ClO_9$
Molecular Weight: 502.99
Appearance: White amorphous powder
Solubility: Soluble in ethanol, methanol, dimethyl formamide and slightly soluble in water.
Mechanism of action: Dapagliflozin inhibits the sodium-glucose cotransporter 2(SGLT2) which is primarily located in the proximal tubule of the nephron. SGLT2 facilitates 90% of glucose reabsorption in the kidneys and so its inhibition allows for glucose to be excreted in the urine. This excretion allows for better glycemic control and potentially weight loss in patients with type 2 diabetes mellitus[(3)].

3. PREPARATION OF STOCK SOLUTION

3.3.1.Preparation of standard stock solution of Linagliptin:

Weigh accurately 10mg of standard substance of Linagliptin and transfer it into 10ml volumetric flask and dissolve it in ethanol and make up the solution up to 10 ml with ethanol (Stock A-1000µg/ml).
Pipette out 1ml from the above solution and transfer into 10ml volumetric flask and dissolve it in ethanol and make up the solution up to 10 ml with ethanol(Stock B-100µg/ml).

3.3.2. Preparation of standard stock solution of Dapagliflozin:

Weigh accurately 10mg of standard substance of Dapagliflozin and transfer it into 10ml volumetric flask and dissolve it in ethanol and make up the solution up to 10 ml with ethanol (Stock A-1000µg/ml). Pipette out 1ml from the above solution and transfer into 10ml volumetric flask and dissolve it in ethanol and make up the solution up to 10 ml with ethanol (Stock B-100µg/ml).

3.3.3. Preparation of Sample Stock Solution of Linagliptin:

Transfer 10mg equivalent weight of tablet powder (400mg) into 10ml volumetric flask and dissolve it in ethanol and make up the solution up to 10 ml with ethanol(Stock A - 1000µg/ml). Pipette out 1ml from the above solution and transfer into 10ml volumetric flask and dissolve it in ethanol and make up the solution up to 10 ml with ethanol (Stock B-100µg/ml).

3.3.4. Preparation of Sample Stock Solution of Dapagliflozin:

Transfer 10mg equivalent weight of tablet powder (200mg) into 10ml volumetric flask and dissolve it in ethanol and make up the solution up to 10 ml with ethanol (Stock A - 1000µg/ml). Pipette out 1ml from the above solution and transfer into 10ml volumetric flask and dissolve it in ethanol and make up the solution up to 10 ml with ethanol (Stock B-100µg/ml).

4. RESULTS AND DISCUSSIONS:

4.1. Selection of wavelength:

The absorbance of the individual solution containing Linagliptin and Dapagliflozin at 10 µg/ml was determined in the UV range 200-400 nm using an Ethanol as blank. The maximum absorbance was found to be 238nm and 230 nm respectively and isobestic point was observed at 234 nm

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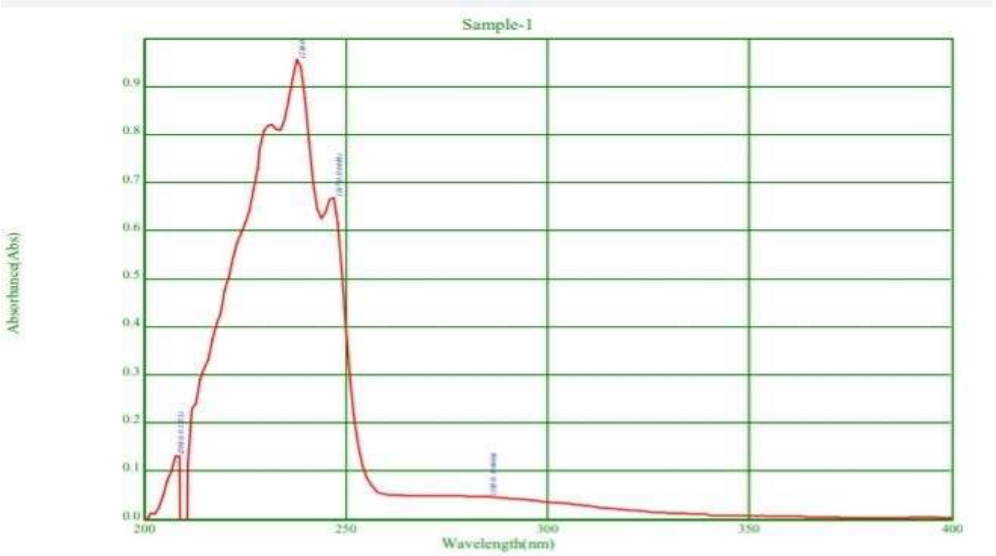

Fig no.1: Figure showing λ_{max} of Linagliptin

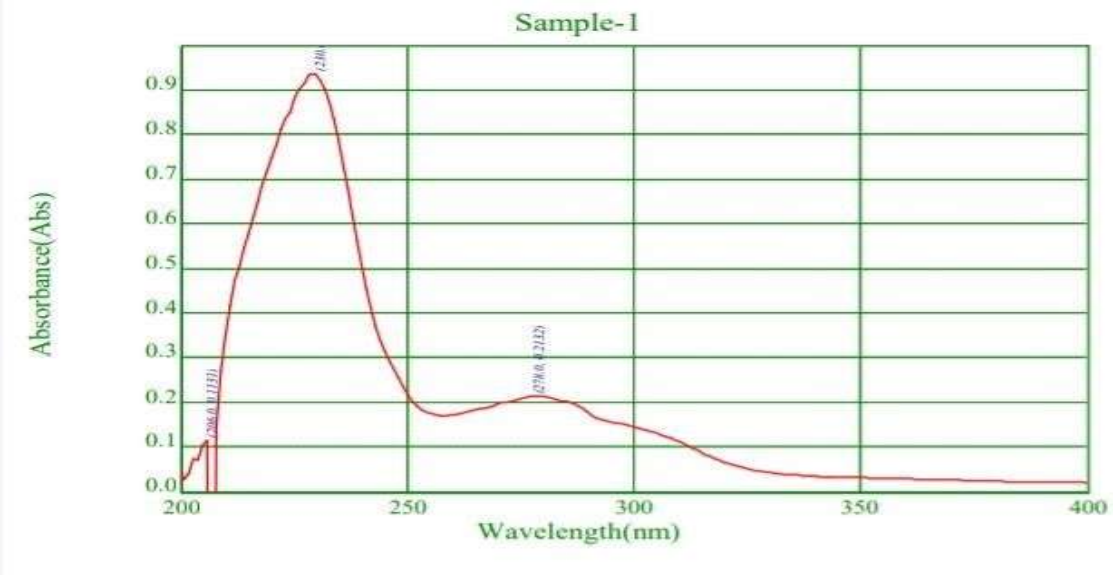

Fig no.2: Figure showing λ_{max} of Dapagliflozin

4.2. ASSAY BY SIMULTANEOUS EQUATION METHOD:

Table No.1:- Obsevation Values for Assay of Linagliptin

S.No	Drug Name	Conc.	Absorbance		Absorptive	
			238nm	230nm	238nm	230nm
1	Linagliptin	10µg/ml	0.9565	0.8071	0.09565 (ax_1)	0.08071 (ax_2)
2	Dapagliflozin	10µg/ml	0.6198	0.9364	006198 (ay_1)	0.09364 (ay_2)
3	Tablet formulation (Linagliptin)	Unknown C_X	1.071 (A_1)	0.911 (A_2)		
4	Tablet formulation (Dapagliflozin)	Unknown C_Y	0.8092 (A_1)	1.0826 (A_2)		

$$C_x = (A_2\,ay_1 - A_1\,ay_2) / (ax_2\,ay_1 - ax_1\,ay_2) = 9.96\mu g/ml$$
$$C_y = (A_1\,ax_2 - A_2\,ax_1) / (ax_2\,ay_1 - ax_1\,ay_2) = 9.8\mu g/ml$$

Assay

$$= \frac{\text{Concentration in mg/ml x average weight}}{\text{Weight of powder equivalent to 10mg x label claim}} \times 100$$

Assay of Linagliptin = 99.6%
Assay of Dapagliflozin = 98%

4.3. VALIDATION PARAMETERS

4.3.1. SPECIFICITY:

The blank solution i.e., ethanol was placed in the UV and spectrum was recorded.

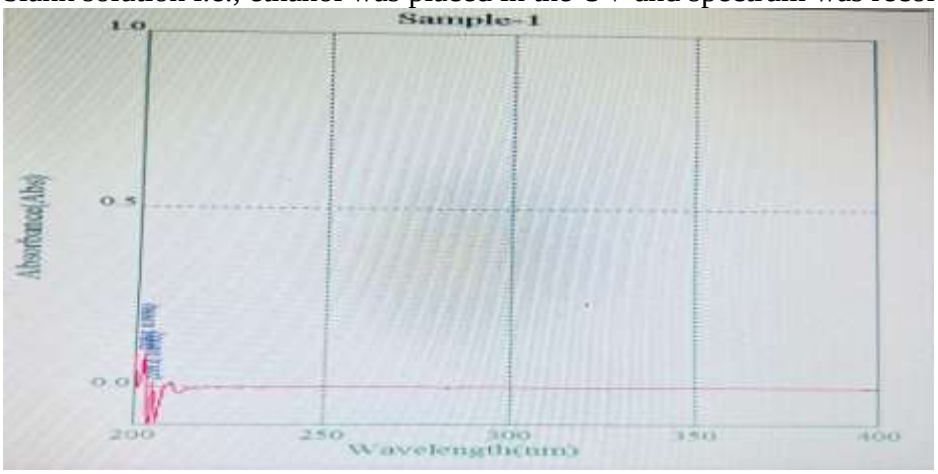

Fig No.3 : Spectrum of blank

Observation: From the spectrum we can conclude that excipients or solvents are not interfering in the spectrum of Linagliptin and Dapagliflozin.

4.3.2. LINEARITY:

Table No 2: Observation results of Linagliptin

Name of the Drug	Concentration [µg/ml]	Absorbance
Linagliptin	6	0.5009
	8	0.7480
	10	0.9565
	12	1.2410
	14	1.4082

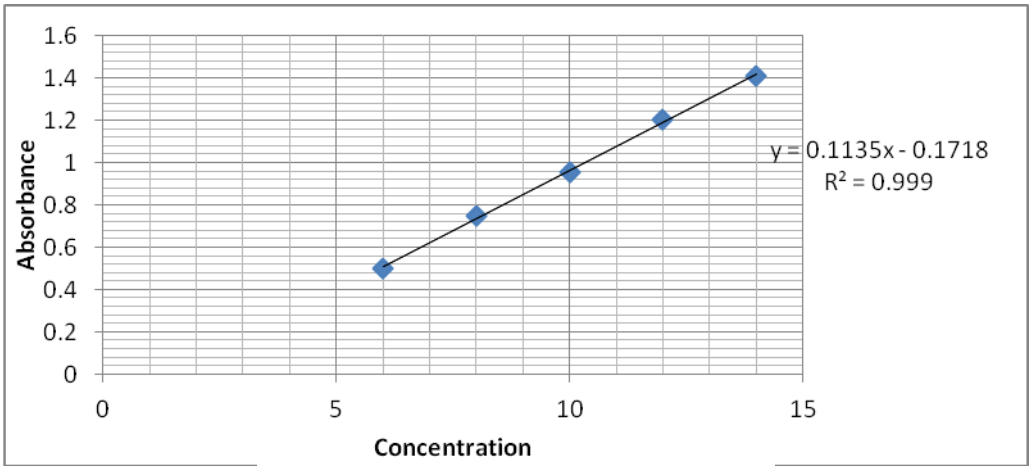

Fig No.4: Linearity graph of Linagliptin

Table No .3 : Observation table for Dapagliflozin

Name of the Drug	Concentration [µg/ml]	Absorbance
Linagliptin	6	0.5009
	8	0.7480
	10	0.9565
	12	1.2410
	14	1.4082

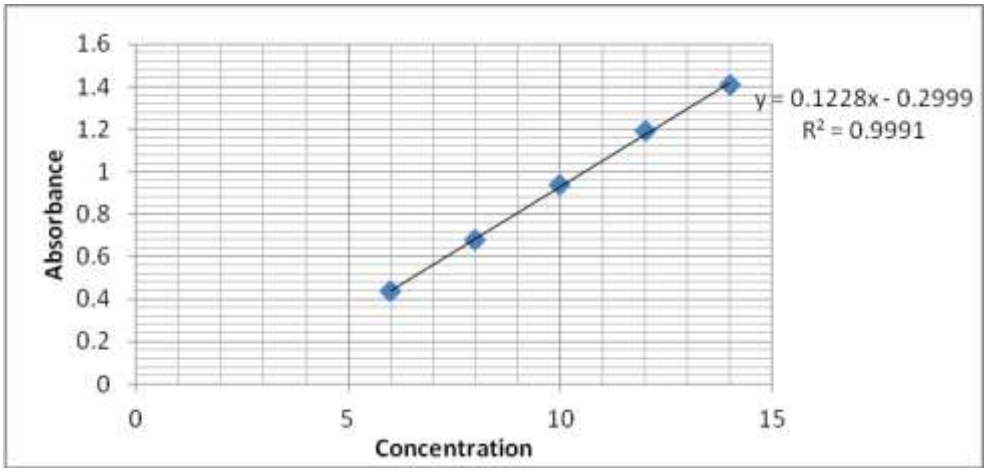

Fig No.5 : Linearity graph of Dapagliflozin

Acceptance Criteria: The Correlation Coefficient should be not less than 0.999.

4.3.3. ACCURACY:

Table no. 4:- Table showing results for accuracy of Linagliptin

S. No	Accuracy level	Conc.taken (ug/ml)	Absorbance	Conc. found	Recovery	% Mean
1	80	18	1.895	18.20	101	99.4
2	80	18	1.836	17.68	98.2	
3	80	18	1.853	17.83	99	
4	100	20	2.109	20.09	100.4	100.2
5	100	20	2.089	19.91	99.5	
6	100	20	2.314	20.15	100.7	

7	120	22	2.314	21.90	99.5	100.96
8	120	22	2.376	22.44	102.2	
9	120	22	2.358	22.28	101.2	

Table no. 5:- Table showing results for accuracy of Dapagliflozin

S. No	Accuracy level	Conc. taken (ug/ml)	Absorbance	Conc. found	Recovery	% Mean
1	80	18	5.334	17.78	98.7	99.1
2	80	18	5.330	18.1	100	
3	80	18	5.334	17.78	98.7	
4	100	20	6.032	102	102	100.8
5	100	20	5.984	101	101	
6	100	20	5.841	99.4	99.4	
7	120	22	6.359	21.6	98.1	100.2
8	120	22	6.520	22.17	100.7	
9	120	22	6.602	22.44	102	

Acceptance criteria: The Percentage recovery should lie between 98-102%.

4.3.4.PRECISION:

Table no 6: Repeatability results

Concentration	Sample Absorbance of Linagliptin	Sample Absorbance of Dapagliflozin
10 µg/ml	0.9565	0.9364
10 µg/ml	1.0321	0.8091
10 µg/ml	1.041	0.8112
10 µg/ml	0.9835	0.9465
10 µg/ml	1.034	0.8131
Average	1.0321	0.8131
SD	0.03744	0.0714806
%RSD	0.0362	0.087

Table no.7:-Results of Intermediate Precision

Concentration	Linagliptin		Dapagliflozin	
	Day-1	Day-2	Day-1	Day-2
10 µg/ml	0.9565	1.0353	0.9364	0.8137
10 µg/ml	1.0321	1.0327	0.8091	0.8131
10 µg/ml	1.041	1.0302	0.8112	0.9360
10 µg/ml	0.9835	1.0352	0.9465	0.8149
10 µg/ml	1.034	1.0344	0.8131	0.8139
Average	1.0321	1.0344	0.8131	0.8139
SD	0.03744	0.00214	0.0714806	0.0546086
%RSD	0.0362	0.0020	0.087	0.067

Acceptance Criteria: The % Relative Standard Deviation of Linagliptin and Dapagliflozin from the five sample preparations should be not more than 2.0%.

4.3.5. LIMIT OF DETECTION (LOD):

$$LOD = 3.3X\frac{\sigma}{S}$$

Table No.8:- Observation values for LOQ

Drug Name	Standard Deviation	Slope	LOD
Linagliptin	0.03744	0.1135	1.085
Dapagliflozin	0.07148	0.1228	1.9206

4.3.6. LIMIT OF DETECTION (LOD):

$$LOD = 10\,X\frac{\sigma}{S}$$

Table No.9:- Observation values for LOQ

Drug Name	Standard Deviation	Slope	LOQ
Linagliptin	0.03744	0.1135	3.29
Dapagliflozin	0.07148	0.1228	5.82

4.3.7. ROBUSTNESS

Table No.10: Robustness results of Linagliptin

S. No	Parameter	Absorbance
I	**Wavelength**	
1	236nm	0.9325
2	238nm	0.9565
3	240nm	0.9421
II	**Temperature**	
1	26^0C	0.9356
2	28^0C	0.9565
3	30^0C	0.9487

Table No.11: Robustness results of Dapagliflozin

S. No	Parameter	Absorbance
I	**Wavelength**	
1	228nm	0.8997
2	230nm	0.9364
3	232nm	0.9125
II	**Temperature**	
1	26^0C	0.9389
2	28^0C	0.9401
3	30^0C	0.9368

5. SUMMARY AND CONCLUSION

5.1. SUMMARY

A UV spectrophotometric method has been developed and validated for the simultaneous estimation of Linagliptin and Dapagliflozin in its pure and Tablet dosage form. The process was done by using simultaneous equation method with the detection wavelength set at 238nm and 230nm for Linagliptin and Dapagliflozin respectively**.** The method was linear with the correlation coefficient 0.999 in the concentration range of 6-14ug/ml. The limit detection were 1.085ug/ml and 1.9206ug/ml respectively. The limit of Quantification was found to be 3.29 ug/ml and 5.82 ug/ml respectively. The repeatability of inter day 1 and day 2 precision were satisfactory and the relative standard deviation did not exceed 2%. The accuracy of the method from the recovery studies is within the limits for both the drugs and the method is robusted. The method met the ICH regulatory requirements.

5.2. CONCLUSION:

Simple, accurate, precise, reproducible, robust and economical UV spectrophotometric method for simultaneous estimation of Linagliptin and Dapagliflozin in its pure and pharmaceutical dosage form have been developed. The method was developed by using ethanol as solvent. The developed method was validated for parameters viz accuracy, precision, linearity, robustness, limit of detection and limit of quantification as per ICH guidelines. All the parameters were found to be within the acceptance limits. The results indicated that the proposed method for simultaneous estimation of Linagliptin and Dapagliflozin is very accurate and cost effective and can be employed in routine sample analysis in its pure and pharmaceutical dosage form.

6. FUNDING: Self Funding

7. ACKNOWLEDGMENT: Our sincere thanks to our Principal Dr. T. Mangilal for providing us power and strength to overcome all the hurdles and hindrances that come in the way of doing project.

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