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Rp-Hplc Method Development And Validation For Simultaneous Estimation Of Dapagliflozin And Linagliptin Bulk Drug And It's Pharmaceutical Formulation

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➢ **Abstract:**

A novel, precise, and accurate reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Dapagliflozin and Linagliptin in bulk drug and pharmaceutical formulation. The chromatographic separation was achieved using a C18 column with a mobile phase consisting of [Mobile Phase Composition] at a flow rate of [Flow Rate]. The detection wavelength was set at [Detection Wavelength]. The method was validated according to ICH guidelines, and the results showed good linearity, accuracy, precision, and specificity. The retention times for Dapagliflozin and Linagliptin were found to be [Retention Time] and [Retention Time], respectively. The percentage recovery for both drugs was within the acceptable limits. The developed method was successfully applied to the analysis of Dapagliflozin and Linagliptin in pharmaceutical formulation, and the results were in good agreement with the label claim. The proposed method can be used for routine quality control analysis of these drugs in pharmaceutical industries. The developed method is simple, rapid, and cost-effective, making it suitable for routine quality control analysis in pharmaceutical industries. The method's specificity, accuracy, and precision ensure reliable quantification of Dapagliflozin and Linagliptin, supporting its use in pharmaceutical development and quality control.

Key Words: RP-HPLC, Dapagliflozin, Linagliptin,Method Development, Validation, Simultaneous Estimation.

1) AIM & OBJECTIVE

❖ **AIM:**

RP-HPLC Method Development and Validation For Simultaneous Estimation of Dapagliflozin and Linagliptin Bulk Drug and It's Pharmaceutical Formulation

❖ **OBJEVTIVES:**

- To select suitable drug i.e. Dapagliflozin and Linagliptin combination and drug formulation
- To apply suitable analytical techniques
- To optimize the analytical techniques employed
- To validate the method as per ICH guidelines
- To select drug or drug combinations and to develop analytical methodology for estimation of drug

4. PLAN OF WORK

Procurement of pure drug samples for Dapagliflozin and Linagliptin and their marketed formulation in combination. Analysis of Pure sample by reported methods. Trial of the instrumental methods on pure drug samples which includes the following steps-

SPECTROPHOTOMETRY

- Determination of scanning wavelength

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

- Selection of mobile phase.
- Selection of column.
- Selection of chromatographic conditions.
- Linearity range.
- System suitability parameters.

The efforts will also be made to develop the most reliable and highly sensitive High Performance Liquid Chromatographic method for the combination,

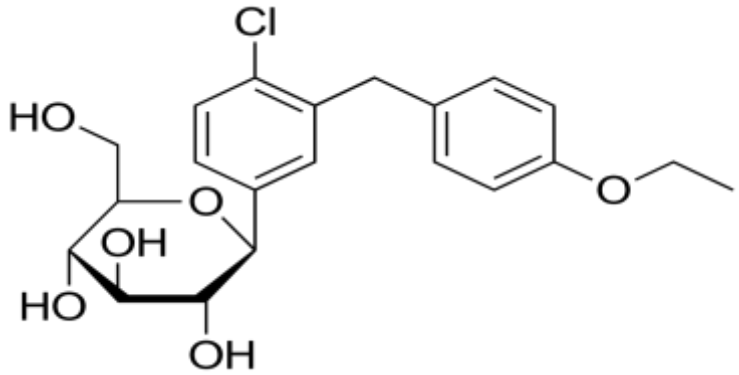
The steps in Method development will as follows-

- Analysis of standard laboratory mixture to see feasibility of the proposed methods.
- To adopt the selected methods on marketed formulation.
- Recovery studies.
- Validation of the proposed methods.\

PARAMETER FOR VALIDATION TO BE STUDIES

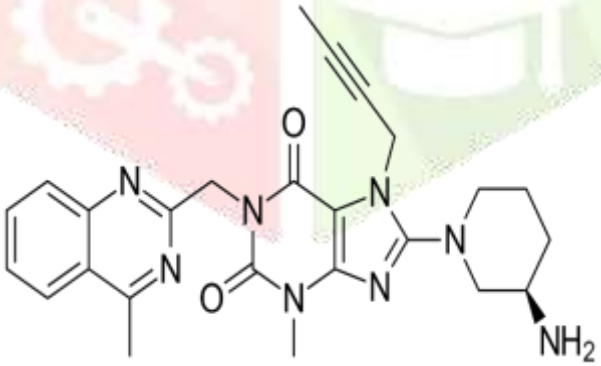
- Accuracy
- Precision
- Specificity
- Ruggedness
- Robustness
- Limit of detection
- Limit of quantitation
- Linearity and range
- **2. DRUG PROFILE**
- **2.1 DAPAGLIFLOZIN**

- **Structure: -**



- *Chemical Name : (2S,3R,4R,5S,6R)-2-[4-Chloro-3-(4-ethoxybenzyl)phenyl]-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol*
- **Description** : - White to off-white powder..
- **Molecular formula** : - $C_{21}H_{25}ClO_6$
- **Molecular weight** : - 408.88
- **Solubility** : - It is soluble in organic solvents such as ethanol, DMSO,& dimethyl formamide.
- **Category** :- Dapagliflozin is in a class of medications called sodium-glucose co-transporter 2 (SGLT2) inhibitors. It lowers blood sugar by causing the kidneys to get rid of more glucose in the urine.
- **Uses: -** Dapagliflozin is mainly used to treat type 2 diabetes. It can also be used to treat heart failure and chronic kidney disease (CKD). Dapagliflozin is usually prescribed if: you have type 2 diabetes and cannot take metformin.

- **2.2 LINAGLIPTIN:**
- **Structure: -**



- **Chemical Name:** - 8-[(3R)-3-Aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-methylquinazolin-2-yl)methyl]-3,7-dihydro-1H-purine-2,6-dione
- **Description** : - White or white to yellowish powder
- **Molecular formula** : - $C_{25}H_{28}N_8O_2$
- **Molecular weight** : - 472.553
- **Solubility** : - It is soluble in organic solvent such as ethanol, DMSO, dimethyl Formamide
- **Category** : - Linagliptin belongs to a class of drugs called DPP-4 inhibitors. used for type IIdiabetes mellitus

- **Uses: -** It used to treat type II diabetes. Type II diabetes is a condition where the body does not make enough insulin, or the insulin that it makes does not work properly.

3. MATERIALS & METHODS

3.1. Materials

The drug used for present investigation was obtained from MG Lab Hyderabad as gift sample.

➢ **Details of Pure Drug:**

Table No.01: Details of API

Sr. No.	Drug	Supplied by	Quantity	Purity (Assay)
01	Dapagliflozin	MG Lab India	10g	99.7
02	Linagliptin	MG Lab India	10g	99.8

➢ **Reagents and Chemicals:**

All reagents and chemicals used were of AR grade and HPLC grade.

Table No.02: List of Reagent and Chemicals used.

Sr. No.	Name of chemicals	Manufacturer
1.	Acetonitrile HPLC Grade	Merck Ltd., India
2.	Methanol HPLC grade.	Merck Ltd., India
3.	Ortho-phosphoric acid.	Merck Ltd., India
4.	Water HPLC grade.	Merck Ltd., India

3.2 METHODS & PROCEDURE

➢ **Identification and characterization of drug**

Previous to commenced the experimental work it is necessary to determine the different physical and chemical property of the drug which provide information regarding the purity and nature of drug. This will help in selection of solvents and procedure for the robust and stable analytical method development.

➢ **Selection and procurement of drug**

Dapagliflozin (DAP) & Linagliptin (LIN) were selected as model drug candidate for method development and validation. The drugs were kindly gifted from Pharmaceutical industry India. The procured drug was analyzed for different physical properties viz. color, odor, melting point, etc.

➢ **FT-IR analysis:**

The IR absorbance spectrum of DAP & LIN was recorded using FTIR 8400S spectrometer (Shimadzu) over range of 4000 to 400 cm-1.

FTIR stands for Fourier Transform Infrared Spectroscopy. The IR spectroscopy theory utilizes the concept that molecules tend to absorb specific frequencies of light that are characteristic of the corresponding structure of the molecules. The energies are reliant on the shape of the molecular surfaces, the associated vibronic coupling, and the mass corresponding to the atoms. It is a technique that uses infrared light to identify chemical properties and molecular structure of materials. FTIR is a modern and preferred method of infrared spectroscopy.

The IR absorbance spectrum of DAP & LIN was recorded using FTIR 8400S spectrometer (Shimadzu) over range of 4000 to 400 cm-1.

- **UV Spectroscopy Analysis**

The ultraviolet absorption spectrum of DAP & LIN were obtained using Shimadzu1800- UV visible spectrophotometer and 1cm quartz cells, over a wavelength range of 400 to 200 nm. The wavelength maxima (λmax) were analyzed showed in table no. 09

6. Validation parameters:

a**) Accuracy:**

It was ascertained on the basis of recovery studies performed by standard addition method.

b) Precision:

Precision of an analytical method is expressed as S.D or R.S.D of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method.

c) Ruggedness:

The studies of ruggedness were carried out under two different conditions-

1) Days
2) Analyst.

i) Interday (Different days):

Same procedure was performed as under marketed formulation analysis on different days. The % label claim was calculated**ii) Intraday:**

It was performed by using same procedure as under marketed formulation analysis and absorbance recorded at 3 hrs. interval within a day. The percent label claim was calculated using formula . **iii) Different analyst:**

The sample solution was prepared by two different analysts and same procedure was followed as described earlier. The % label claim was calculated as done in marketed formulation estimation.

d) Specificity:

Specificity was measured as ability of the proposed method to obtain well separated peak for DAP and LIN without any interference from component of matrix.

Mean retention time for –

DAP – 7.128

LIN – 3.226

The values obtained were very close to that in standard laboratory mixture indicates no interference from the component of matrix.

e) Linearity and range:

According to USP tablet powder equivalent to 80, 90, 100, 110, 120 % of label claim was taken and dissolved & diluted appropriately with mobile phase to obtain a concentration in the range of 80%-120% of the test concentration. The chromatograms of the resulting solutions were recorded. DAP and LIN marketed formulation was found to be linear in the range ± 20% of the test concentration of the respective drug.

f) Robustness

Robustness and ruggedness - the ability of an analytical method to remain unaffected by small variations in method parameters and influential environmental factors and characterize its reliability during normal usage. No change of the detected amount of the analyte in a certain sample in spite of the variation of the method parameter.The robustness study indicated that the factors selected remained unaffected by small variation of organic composition of mobile phase, wavelength and the flow rate. The system suitability results should lie within the limit. Hence the method was robust

g) Limit of Detection (LOD) and Limit of Quantitation (LOQ):

Limit of detection is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated an exact value.

Limit of quantitation is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision accuracy.

As per ICH guideline both LOD & LOQ were performed on the basis of standard deviation of the response and slope and expressed by following formulae (4) and (5) respectively.

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

$$LOQ = \frac{10\,\sigma}{S}$$

Where,

σ = The standard deviation of the response

S = The slope of the calibration curve

4. RESULT & DISCUSSION

Now a day's drugs are commonly used clinically and analyst is required to develop suitable method for their analysis. A fixed dose combination containing Dapagliflozin & Linagliptin is recently available in market as tablet dosage form.

Percent purity of above mentioned drugs were reported by Supplier Company as follows-

1) Dapagliflozin - 99.7 %.
2) Linagliptin - 99.8 %

Primary this was not analysed in our study and the % purity stated by the suppliers was taken as standard for comparison studies

The physico-chemical characterization of drug molecule is important with regard to its purity, identification in development and validation of analytical method. The various tools used for characterization of drug molecules include melting point, UV spectroscopy, solubility study, etc. The solubility study, melting point analysis, UV spectroscopy of the drug was done.

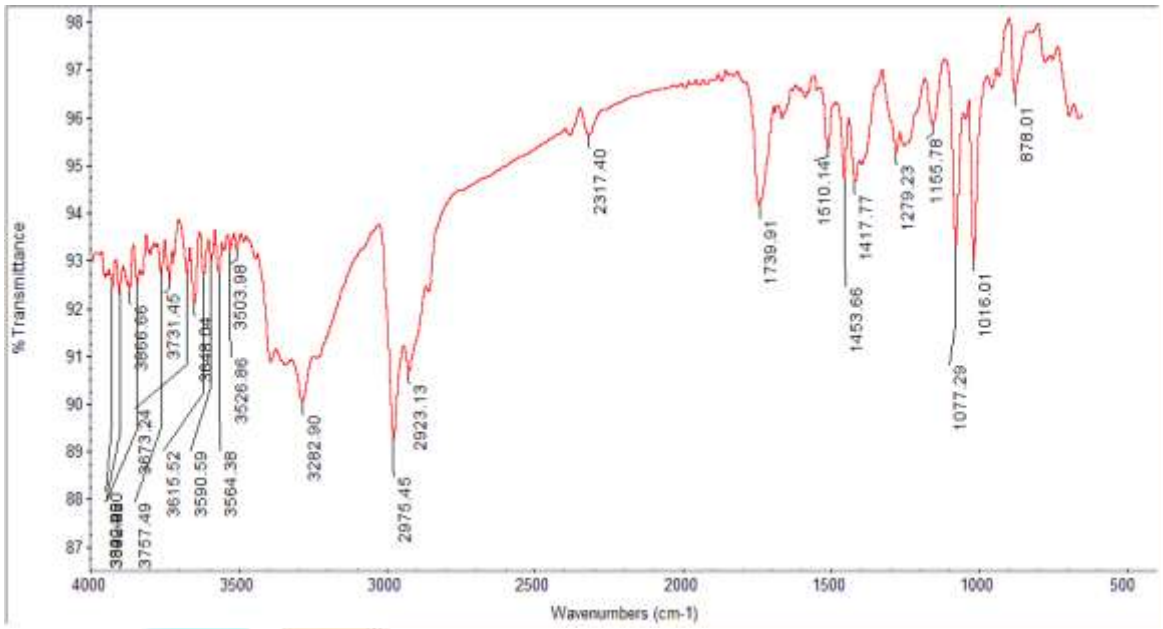


Fig no 2: FT-IR Spectra of LIN

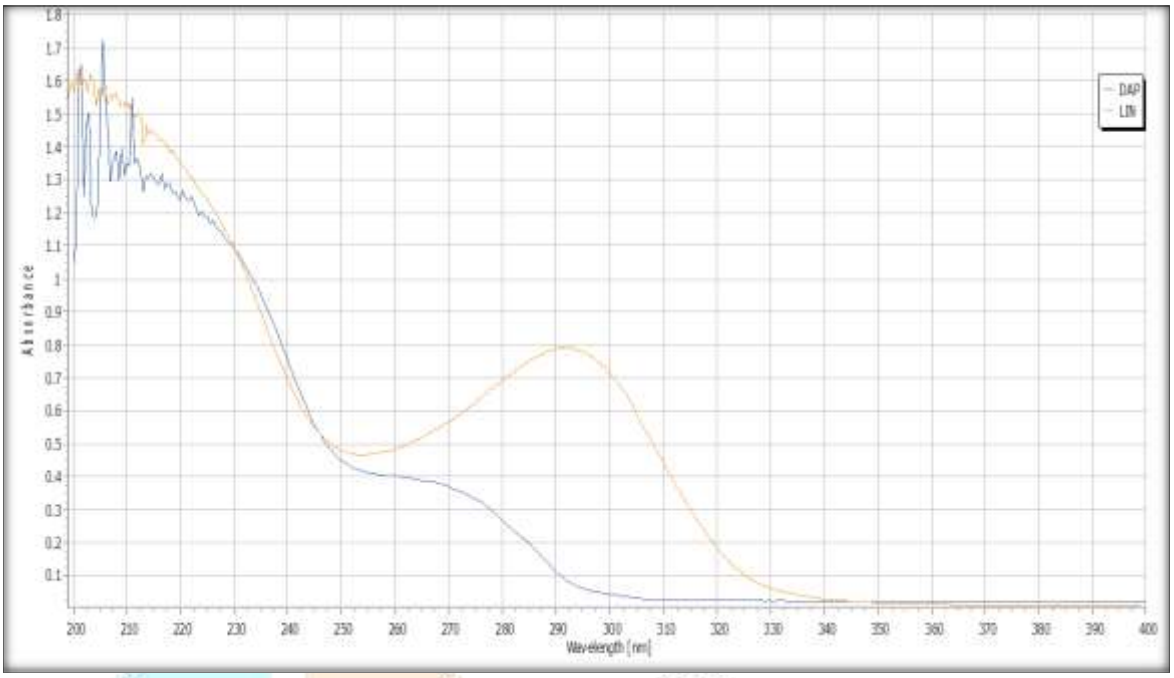


Fig. No. 3: - Overlain of UV Spectra of DAP & LIN

a. Validation parameters:

a) Accuracy:

It was ascertained on the basis of recovery studies performed by standard addition method. The results of recovery studies and statistical data are recorded in Table No. 15

Table No.6: Results and statistical data for Recovery study of DAP and LIN

Sr. No.	wt. of formul ation (mg)	Amount of Drug Added in (µg/ml).		Peak Area of stand.		Peak Area of sample		% Recovery	
		DAP	LIN	DAP	LIN	DAP	LIN	DAP	LIN
1		1	1			480371.3	201392.7	100.3	100.4

2	125	1	1	478934.5	200590.3	480850.2	199386.8	100.4	99.4
3		1	1			481329.2	200189.1	100.5	99.8
4		2	2			483723.8	199988.5	101	99.7
5		2	2			484202.8	199787.9	101.1	99.6
6		2	2			475582.0	202195.0	99.3	100.8
7		3	3			481808.1	202395.6	100.6	100.9
8		3	3			482766.0	202796.8	100.8	101.1
9		3	3			483244.9	203599.2	100.9	101.5
						Mean		100.54	100.36
						S.D.		0.541	0.757
						C.V		0.005	0.008

***Results are mean of three replicates**

b) Precision:
Precision of an analytical method is expressed as S.D or R.S.D of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method.

c) Ruggedness:
The studies of ruggedness were carried out under two different conditions-

1) Days
2) Analyst.

d) Specificity:
Specificity was measured as ability of the proposed method to obtain well separated peak for DAP and LIN without any interference from component of matrix.

Mean retention time for –

DAP – 7.825

LIN – 3.914

The values obtained were very close to that in standard laboratory mixture indicates no interference from the component of matrix.

Typical chromatogram is shown in the Fig. No. 22

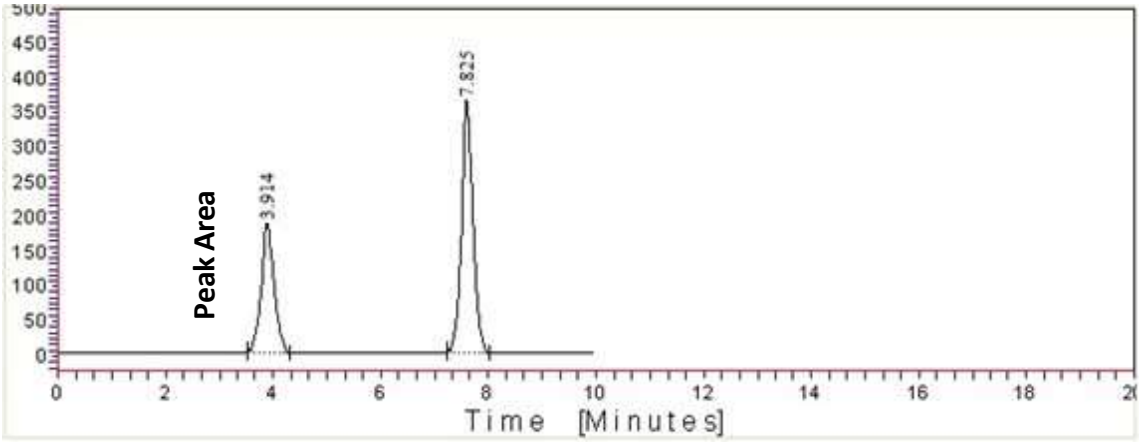


Fig. No.6: Chromatogram obtained by formulation of DAP & LIN

e) Linearity and range:

According to USP tablet powder equivalent to 80, 90, 100, 110, 120 % of label claim was taken and dissolved & diluted appropriately with mobile phase to obtain a concentration in the range of 80%-120% of the test concentration. The chromatograms of the resulting solutions were recorded. DAP and LIN marketed formulation was found to be linear in the range ± 20% of the test concentration of the respective drug.

(f) Robustness: -

Robustness is a measure of how well an analytical method can produce reliable results when there are small changes to the experimental conditions. The robustness study for DAP and LIN shown in following table

f) Limit of Detection (LOD) and Limit of Quantitation (LOQ):

Limit of detection is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Limit of quantitation is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision accuracy. The result shown in following table

Table 8: LOD & LOQ of DAP & LIN

Sr. No.	Drug Name	LOD g/ml	LOQ g/ml
1	DAP	0.781	2.01
2	LIN	0.487	1.19

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