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Validated Method for Simultaneous Estimation of Linagliptin and Dapagliflozin

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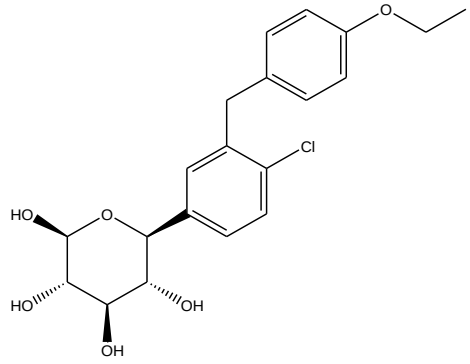
ABSTRACT:
The objective of research was development of analytical method for simultaneous estimation of two drugs in combination; that is available commercially as tablets. Various approaches were tried for the combination. When spectrophotometric, colorimetric, fluorimetric, spectro-fluorimetric methods did not show any promise, the planar chromatographic technique was successfully developed and validated. The details of development work, difficulties encountered, method development, optimization and validation is discussed.

KEYWORDS: HPTLC, Simultaneous estimation, Validation

I. INTRODUCTION

Drugs used in management of diabetes are called as anti-diabetic drugs. Dapagliflozin(DAPA) is oxane derivative with triol moiety and chemically it is represented as (2S, 3R, 4R, 5S, 6R)-2-(4-Chloro-3-(4-ethoxybenzyl)-phenyl)-6-(hydroxymethyl)tetrahydro-2H-pyran-3, 4, 5-triol. It acts as Sodium–Glucose cotransporter-2 inhibitor (SGLT-2) in kidney.Linagliptin(LIN) is a dipeptidyl peptidase-4 (DPP-4) inhibitor type of anti-diabetic drug, DPP-4 is a enzyme which is responsible for degradation of incretin hormone. Chemically linagliptin is a 8-[(3R)-3-aminopiperidin-1-yl]-7-(but-2-yn-1-yl)-3-methyl-1-[(4-methylquina zolin-2-yl)-methyl]-2, 3, 6, 7-tetrahydro-1H-purine-2, 6-dione**[1-3]**. The combination of both drugs as 5mg linagliptin and 10mg dapagliflozin provides synergistic effect on activity of dose**[4].**The estimation of combination is based on the separation technique using chromatography **[5]**.

Literature survey revealed several analytical methods like UV spectroscopy, colourimetry, fluorometry, HPTLC, etc. The various analytical methods are chosen so as to determine the accurate estimation of content and errors can be minimized by comparative analysis. Each method can be as efficient as the other therefore the aim was to establish and validate robust analytical method for simultaneous estimationof linagliptin and dapagliflozin **[6-8]**.



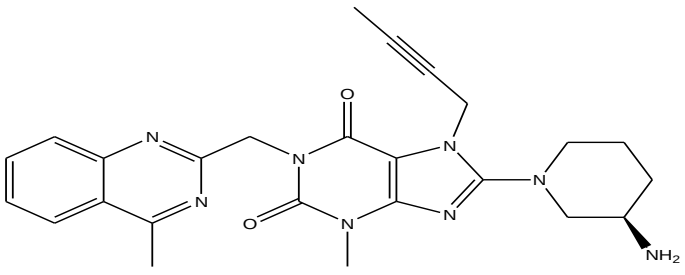


Fig 1.(i) Dapagliflozin **(ii) Linagliptin**

II. MATERIALS AND METHODS

Chemicals and reagents:

Linagliptin and Dapagliflozin were received as gift sample from pharmaceutical industry. Methanol AR grade, Ethyl acetate, Hexane was purchased from Lobachemie. Pvt. Ltd

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Instrumentation:

UV-spectral analysis was performed on UV- Visible spectrophotometer (Make: JASCO, Model- V730).

The HPTLC system(make CAMAG), featuring the Linomat 5 sample applicator, was operated with a gentle stream of nitrogen and paired with a Hamilton microliter syringe (100µL). The TLC SCANNER 3 was controlled by Win CATS software version 1.4.3.

Preparation of standard stock solution:

10 mg of drug was weighed and dissolved in 10 ml methanol to obtain 1000 ug/ml stock solution. Further, 1 ml of solution was taken from stock solution and diluted with methanol to make up the 10 ml volume in volumetric flaskto obtain solution of strength 100 ug/ml.

Preparation of sample solution:

The sample solution was prepared weighing and diluting the marketed formulation of this drug combination. Marketed tablet of LIN and DAPA were taken (linadapa) having 5mg LIN and 10mg DAPAper tablet.

Spectroscopic Techniques tried for simultaneous estimation:

The UV absorbance of each drug was noted in the range of 200 nm to 400 nm. The overlain spectra are as shown in Fig. 2

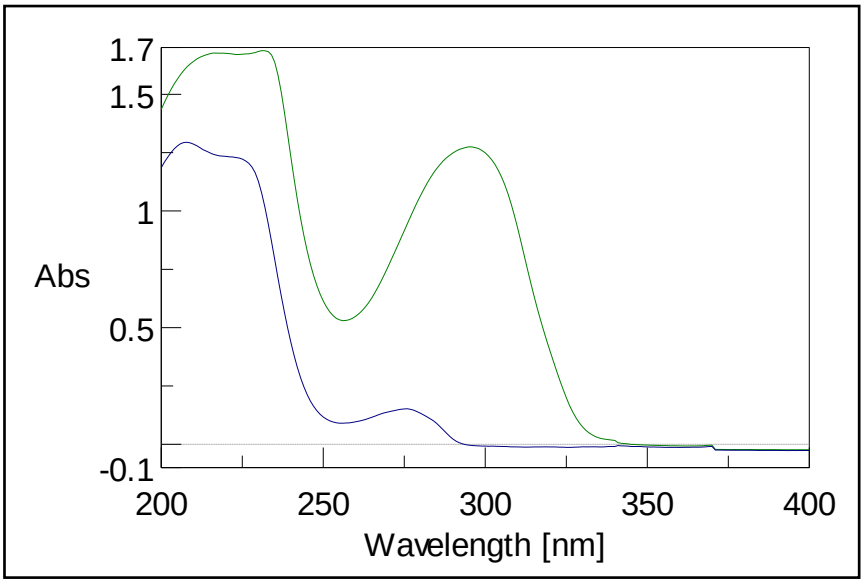

Fig. 2: Overlain UV spectra of Linagliptin (20ug/ml) and Dapagliflozin (20 ug/ml)

Since DAPA appeared to have negligible absorbance at wavelengths above 293 nm, a method "Absorbance corrected for Interference" was tried for this combination using 225 nm and 296 nm as the two wavelengths. But there was lack of reproducibility of results.

The second approach tried was based on converting Linagliptin to fluorescent derivative by the reaction of its primary amine group with the reagents orthophthalaldehyde and 2-mercaptoethanol. Linagliptin upon derivatisation showed fluorescence with 332 nm as excitation wavelength and 449 nm as emission wavelength. But this approach did not work for the combination because DAPA also showed fluorescence with 346 nm as excitation wavelength and 446 nm as emission wavelength.

Hence finally, a planar chromatographic separation was attempted.

Mobile phase development:

To optimize the mobile phase for acceptable resolution, various trails were taken such as Hexane:Ethyl acetate(5:5 v/v), Hexane:Methanol:Ethyl acetate(4:2:4 v/v/v),Chloroform:Methanol:Triethylamine (5:5:0.2 v/v/v) but retardation factor (Rf) as well as peak shape, was not satisfactory. The mobile phase composed ofHexane:Methanol:Ethyl acetate: Triethylamine (4:2:4:0.2 v/v/v/v) has resulted in considerable improvement of Rf and peak shape.

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Chromatographic condition:

Chromatographic separation was performed on aluminium plates precoated with silica gel $60F_{254}$, (10×10 cm with 250 µm layer thickness). Samples were applied on the plate as a band of 6 mm width using a 100 µl syringe with a Linomat applicator. The mobile phase was composed of Methanol: Hexane: Ethyl acetate: Triethylamine (2:4:4:0.2v/v/v/v). A well-separated band was observed with a Rf value of 0.33±0.02 for LIN and 0.72± 0.02 for DAPA.

METHOD VALIDATION

The validation of methods was performed with respect to validation parameters given by International Conference on Harmonization (ICH) guideline Q2 (R1).

Specificity

To evaluate specificity blank, standard solution containing LIN and DAPA and sample solution were applied on TLC plate. Any interference from blank, sample excipients and mobile phase to the peak of interest was checked.

Linearity

The linear curves were obtained over a concentration range of 200 – 1200 ng/band for LIN and 1250 – 7500 ng/band for DAPA. Standard solutions of LIN and DAPA were applied to the plate in specific aliquots. The values were plotted as the amount of drug spotted (ng/band) against the peak area to obtain a calibration curve.

Precision

This parameter is indicative of method's reproducibility. Precision evaluations were conducted through both interday and intraday studies by mixed standard solutions of LIN and DAPA that was applied in six replicates onto TLC plate, using the syringe. Peak area and the relative standard deviation as a Percentage Relative Standard Deviation (% RSD) were then computed.

Assay

The 10 tablets were weighed and average weight was calculated. After that all tablets were crushed properly to obtain fine powder. The weight equivalent to 10 mg DAPAwas taken and dispersed in 5 ml methanol followed by shaking (10 min), sonication (5 min) and then volume was made up to 10 ml. This solution was diluted appropriately to obtain two separate solutions one containing 40 ug/ml of LIN and the other containing 250 ug/ml of DAPA. The resultant solution was applied to the TLC plate and developed using an optimized mobile phase. The amount of each drug was calculated by extrapolating the peak area in the linear regression equation.

Accuracy

Accuracy assessment was performed through the standard addition method by determining the recoveries of LIN and DAPA. Known quantities of a mixed standard solution containing LINand DAPAwere added to pre-quantified sample solutions of the test substances. The determination of LIN and DAPA amounts was accomplished by substituting the peak area values in the regression equations derived from the calibration curve.

Robustness

By systematically changing the saturation time in the development chamber , detection wavelength , and distance travelled till the solvent front, the robustness was evaluated. Three relicates of a single standard concentration were used in the evaluation. Mean area values and the percentage relative standard deviation (% RSD) were calculated, and the observed effects of parameter changes were reported.

Limit of detection and limit of quantification

The LOD and LOQ was determined using following formula:

$LOD = 3.3 \times SD/S$

$LOQ = 10 \times SD/S$

Where, SD is the response at the lowest concentration and 'S' is the slope of the corresponding calibration curve

III. RESULTS AND DISCUSSIONS

Optimization of HPTLC chromatographic conditions

The goal of obtaining high-resolution and repeatable peaks for HPTLC tests was investigated by experimenting with different mobile phase proportions. Using methanol: hexane: ethyl acetate: triethylamine (0.2: 0.4: 0.4: 0.1 v/v/v/v) as the mobile phase allowed for the successful achievement of this goal. The studies used a solvent migration distance of 80 mm and chamber saturation duration of 15 minutes at room temperature (25 ± 2ºC). With symmetrical and sharp peaks and Rf values of 0.33±0.02 and 0.72±0.02for LIN and DAPA respectively, the method showed effective resolution.

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Specificity

The Rf values corresponding to the chromatographic peaks of DAPA and LIN in the sample matched those in the standard chromatogram for DAPA and LIN. These experimental results of peak purity values demonstrate the purity of the chromatographic peaks for DAPA and LIN in both the sample and standard.

Linearity

As Figure 3 indicates, the linear regression data derived from the calibration curves demonstrated a good linear correlation within the concentration range of 200 – 1200 ng/band for LIN and 1250 – 1750 ng/band for DAPA. The regression equations, along with the coefficient of correlation (R^2) values, demonstrated strong linearity with R^2 values of 0.890 and 0.992 for LIN and for DAPA respectively.

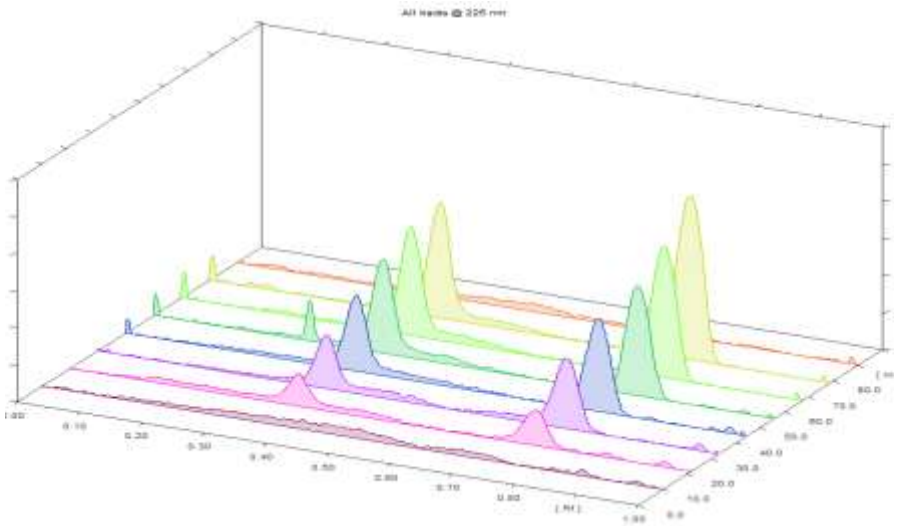

Fig.3 Representative densitogram of Linearity for mixture LIN(200 – 1200 ng/band) and DAPA(1250 – 1750 ng/band)

Precision

The repeatability of the method was evaluated. A standard mixture applied in six replicates was used to measure intra- and inter-day variations for LIN and DAPA. The percentage relative standard deviation (% RSD) was determined to be less than 2%.

Assay

The percentages assay of LIN and DAPA in the marketed tablets was found to be 99.5%and 98.2% respectively.

Accuracy

The recovery findings were within the acceptable range as defined by the ICH guideline.As shown, recovery values were calculated at 80%, 100% and 120% levels of standard addition.

Table 1. Accuracy study of LINA and DAPA

DRUG	LEVEL (%)	Amount of sample (ng/band)	Amount of std. spiked (ng/band)	Total amount spotted	Amount Recovered	% Recovery (Average)
	80	187.5	160	347.5	344.8	99.23
LINA	100	187.5	200	387.5	389.0	100.41
	120	187.5	240	427.5	423.59	99.08
	80	1250	1000	2250	2231.26	99.16
DAPA	100	1250	1250	2500	2455.8	98.23
	120	1250	1500	2750	2753.0	100.1

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LOD and LOQ

The method's sensitivity was evaluated through the determination of LOD and LOQ values which were obtained as 45ng/bandand 150ng/bandfor Linagliptin and 300ng/band and 999ng/band for Dapagliflozin respectively.

Robustness

A percentage relative standard deviation (% RSD) of less than 2% was obtained by calculating the standard deviation of peak areas for each parameter.

Table 2. Robustness

Parameter	Condition	%RSD
Saturation Time (20 min)	15 min	1.68
	25 min	1.82
Wavelength	Immediate	0.91
	2 hrs	0.65
Time from Development to Scanning	Immediate	1.53
	2 hrs	1.24

A summary of validation results is mentioned in Table 3.

Table 3. Method Validation Summary

Sr no.	**Validation parameter**	**Linagliptin**	**Dapagliflozin**
1	Linearity Range (lin – 200-1200 ng/band) (dapa –1250-7000 ng/band)	y =8.2939x + 2737.4 R^2 = 0.8907	y = 2.3734x + 1429.2 R^2 = 0.9925
2	Precision	1.25	0.96
3	Assay	99.5%	98.2%
4	Accuracy	99.57%	99.16%
5	LOD	45ng/band	300ng/band
6	LOQ	150ng/band	999ng/band
6	Robustness	Robust	
7	Specificity	Specific	

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IV. CONCLUSIONS:

The developed HPTLC method facilitated rapid separation within a brief timeframe, enabling the simultaneous measurement of Linagliptin and Dapagliflozin with good accuracy, sensitivity, and precision. This characteristic is particularly vital in quality control processes. The method, was successfully validated as per ICH guidelines. This method may be used for routine quality control of these drugs in combination.

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