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Development and Validation of RP-HPLC Method for the Estimation of Linagliptin, Dapagliflozin and Metformine Hydrochloride in Combined Dosage Form

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Abstract: *To develop and validate a simple, accurate, precise, and robust Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) method for the simultaneous quantification of Metformin (METF), Linagliptin (LINA), and Dapagliflozin (DAPA) in their combined oral dosage form.Chromatographic separation was achieved on an ODS Hypersil C18 column (250 mm×4.6 mm,5 µm). The mobile phase consisted of 20 mM phosphate buffer (pH 3.5) and Methanol in a ratio of 80:20 (v/v), delivered isocratically at a flow rate of 1.0 mL/min. Detection was performed at 217 nm. The method was validated according to ICH guidelines for linearity, accuracy, precision, specificity, LOD, LOQ, and robustness.The method successfully resolved all three drugs with good peak symmetry. The linearity was excellent across the established ranges (e.g., METF:50−500 µg/mL) with high correlation coefficients (r2>0.991). The assay of the marketed formulation, 'Dapaglyn LM', showed high accuracy, with average drug recovery percentages of 100.57% (METF), 100.73% (LINA), and 101.70% (DAPA). Precision studies yielded low %RSD values (e.g., Method Precision RSD<0.9810), confirming reliability.The validated RP-HPLC method is efficient, sensitive, and meets all regulatory requirements for the simultaneous estimation of Metformin, Linagliptin, and Dapagliflozin. It is highly suitable for routine quality control and stability analysis in pharmaceutical manufacturing.*

Keywords: RP-HPLC, Pharmacokinetics, Bioavailability, Chromatography, Analytical Validation

I. INTRODUCTION

Linagliptin, Dapagliflozin, and Metformin Hydrochloride are critically important antidiabetic agents frequently prescribed together to achieve synergistic glycemic control. Linagliptin is a dipeptidyl peptidase-4 (DPP−4) inhibitor, Dapagliflozin is a sodium-glucose co-transporter 2 (SGLT2) inhibitor, and Metformin is a biguanide, all acting via distinct pharmacological pathways.[1,2] The increasing commercial availability and therapeutic importance of these three drugs in a fixed-dose combination dosage form necessitate the development of a highly selective, precise, and efficient analytical method for simultaneous quality control. The research gap stems from the need for a single, validated, and efficient RP-HPLC method capable of accurately and simultaneously quantifying all three compounds in the presence of common tablet excipients.[3,4] Prior analytical methods often focus on binary combinations or require complex, time-consuming sample preparation or gradient elution. Therefore, this study aimed to address this gap by developing a robust, simple, and rapid isocratic RP-HPLC method that complies with ICH guidelines, providing a superior analytical tool for pharmaceutical quality assurance.[5,6]

II. MATERIALS AND METHODS

Instrumentation

The analysis primarily utilized an Agilent Technologies HPLC system (Model: 1220), equipped with an ODS Hypersil C18 column and a Diode Array Detector (DAD) for detection. The precise wavelength of 217 nm was selected for optimal detection. A Shimadzu UV-Visible Spectrophotometer (Model: 1800) was also employed, likely for

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preliminary wavelength scanning or other assays. Weighing was performed using a Shimadzu Electronic Analytical Balance (Model: AUX220), and solution preparation was aided by an EIE Ultrasonic Cleaner (Model: EIE 808). Solution acidity was confirmed using a Toshcon Digital pH Meter. Essential labware included volumetric flasks, pipettes, filtration units, and 0.45 µm membrane filters.[7,8]

Chromatographic Conditions

The method was developed as a Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) technique. The stationary phase was an ODS Hypersil C18 column (250 mm×4.6 mm i.d., 5 µm particle size). The separation was carried out at ambient temperature. The mobile phase was a mixed solution of 20 mM phosphate buffer (pH 3.5) and Methanol in a ratio of 80:20 (v/v), ensuring appropriate polarity for drug separation. The system operated at a flow rate of 1.0 mL/min, and all analytes were detected at a specific wavelength of 217 nm.[9,10]

Sample Preparation

Before chromatographic injection, the pharmaceutical dosage form (tablets) containing the three drugs was finely pulverized and then dissolved in an appropriate solvent, such as methanol or phosphate buffer. To ensure complete dissolution and a homogeneous sample, the mixture was subjected to ultrasonication. Subsequent filtration through a 0.45 µm membrane filter was critical to remove insoluble excipients and particulates, thereby protecting the HPLC column and ensuring the clarity and reliability of the sample solution.[11,12]

Method Validation and Application

The developed HPLC method was subject to validation according to the ICH guidelines to confirm its suitability for the intended purpose. Key validation parameters evaluated included linearity, accuracy, precision, specificity, Limit of Detection (LOD), and Limit of Quantification (LOQ). Upon successful validation, the method was applied to commercial tablet formulations to accurately and precisely quantify Linagliptin, Dapagliflozin, and Metformin in the presence of tablet excipients. This final application ensures that the developed analytical procedure is robust and reproducible for routine quality control and regulatory compliance.[13,14]

III. EXPERIMENTAL WORK AND RESULTS

Preparation of Standard Solutions

Standard solutions for Metformin (METF), Linagliptin (LINA), and Dapagliflozin (DAPA) were systematically prepared in methanol. Stock solutions (A1, A2, A3) were created first, resulting in concentrations of 5 mg/mL for METF, 1 mg/mL for LINA, and 0.1 mg/mL for DAPA. These were subsequently diluted to produce working standard solutions (B1, B2, B3) of 200 µg/mL (METF), 40 µg/mL (LINA), and 4 µg/mL (DAPA). Finally, a single mixed working standard solution (B4) was prepared by combining aliquots of the stock solutions, achieving the same final working concentrations for all three drugs simultaneously.[15]

Optimization of Mobile Phase and Conditions

A 20 mM phosphate buffer was prepared by dissolving 2.7217 g of KH_2PO_4 in 1000 mL of water to serve as the aqueous component. Mobile phase optimization initially found that a 15:85 (v/v) mixture of buffer to methanol provided the best peak characteristics, yielding distinct retention times for METF (3.68 min), LINA (6.24 min), and DAPA (8.34 min). However, the final constant chromatographic conditions used for the procedure specified a mobile phase ratio of 80:20 (v/v) of 20 mM phosphate buffer (pH 3.5):Methanol, run isocratically on an ODS Hypersil C18 column at ambient temperature, with a flow rate of 1.0 mL/min and detection at 217 nm.[16]

Chromatographic Procedure and Results

The chromatographic system was equilibrated using the final optimized parameters. The individual working standard solutions (B1, B2, B3) were injected sequentially to accurately identify and confirm the retention time of each component. Subsequently, the mixed working standard solution (B4) was injected. The resulting chromatogram demonstrated that all three compounds—Metformin, Linagliptin, and Dapagliflozin—were successfully separated and detected at their respective retention times, exhibiting good resolution and peak symmetry, which validated the optimized HPLC method for the simultaneous determination of the drugs.[17]

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Application of the Proposed Method in Marketed Formulations

The developed RP-HPLC method was successfully applied to quantify the three active pharmaceutical ingredients in a commercially available tablet. The formulation analyzed was marketed under the Trade Name: Dapaglyn LM, manufactured by Torrent Pharma, with an average tablet weight of 1.0642 g. The label claim composition was 500 mg of Metformin, 5 mg of Linagliptin, and 10 mg of Dapagliflozin. For the analysis, the B4 Mixed Working Standard Solution was used as the reference standard. The preparation of the sample solution began by accurately weighing and powdering twenty tablets. A portion of this powder, weighing 98.05 mg (equivalent to the labeled ratio of 50.0 mg METF,10.0 mg LINA, and 1.0 mg DAPA), was extracted into a 10.0 mL volumetric flask using methanol and sonication for 10 min to ensure complete extraction. After cooling and filtration, a 0.4 mL aliquot of this solution was further diluted to 10.0 mL with the mobile phase. This final dilution yielded a sample solution with theoretical concentrations of 200 µg/mL (METF), 40 µg/mL (LINA), and 4 µg/mL (DAPA), ready for HPLC injection.[18-20]

Linearity

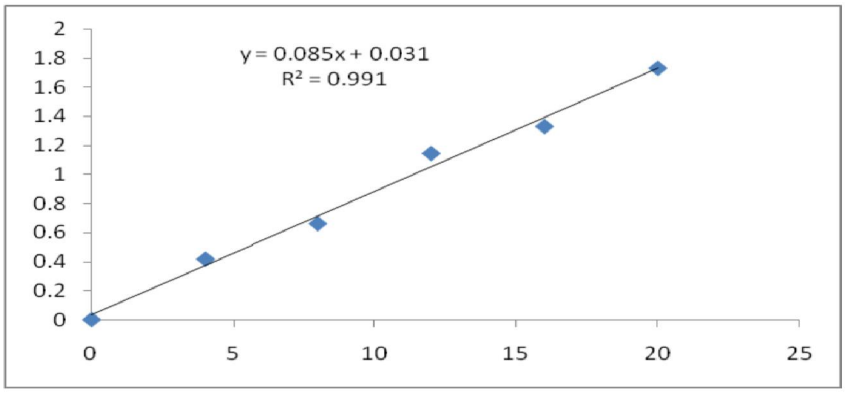

Fig 1: Calibration Curve of Metformin

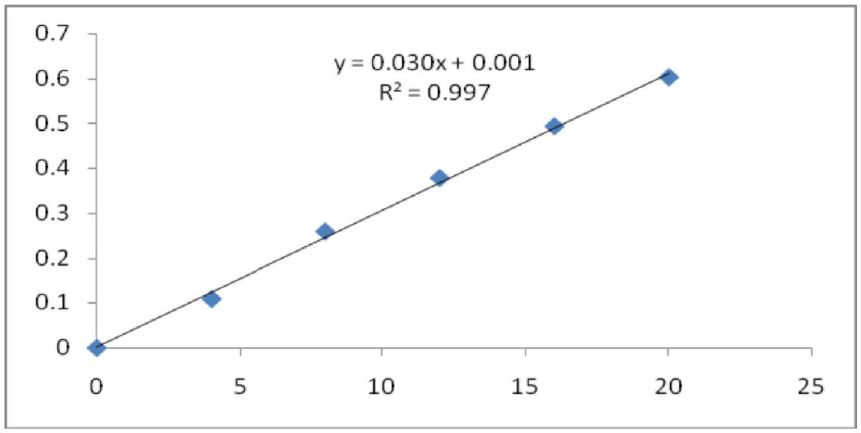

Fig 2: Calibration Curve of Linagliptin

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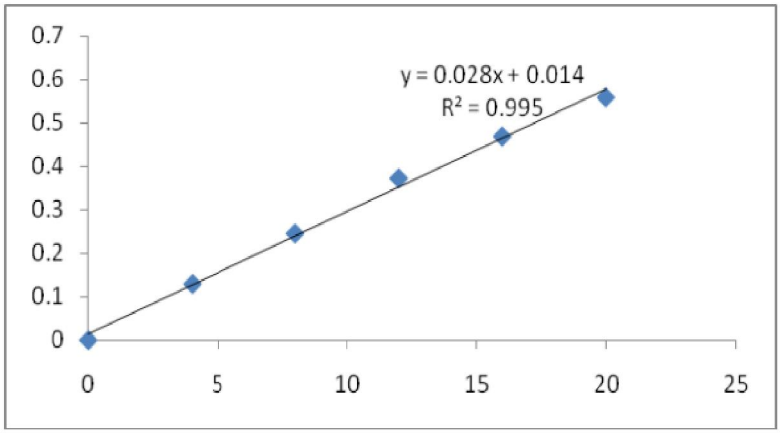

Fig 3: Calibration Curve of Dapagliflozin

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ determination were done with method based on standard deviation of the response and the slope of calibration curve.

Table 1: Analytical performance data

Parameters	METF	LINA	DAPA
Linear dynamic range (µg/ml)	50-500	10-100	1-10
Slope	0.085	0.030	0.028
Correlation coefficient (r^2)	0.991	0.997	0.995
LOD (µg/ml)	0.097	0.275	0.295
LOQ (µg/ml)	0.294	0.833	0.893

Estimation of Drugs in Marketed Formulation

Sample Preparation

Six replicate samples of powdered tablet equivalent to **50.0 mg of Metformin (METF)**, **10.0 mg of Sitagliptin (SITA)**, and **1.0 mg of Dapagliflozin (DAPA)** (total powder weight: **98.05 mg**) were accurately weighed and transferred into **10.0 mL volumetric flasks**. Each sample was dissolved in a small volume of methanol, sonicated to ensure complete dissolution, and the volume was made up to 10 mL with methanol to obtain homogeneous sample solutions.

Table 2: Analysis of Marketed Formulation

Prep No.	Wt. of sample (mg)	LINA			METF			DAPA		
		Areaof sample	Area of standard	% Assay	Areaof sample	Area of standard	% Assay	Area of sample	Area of standard	% Assay
1	106.4	157120	158136	101.25	3008995	3034297	99.41	103482	104166	101.46
2	106.4	156100	158136	100.67	3047875	3034297	100.32	103221	104166	102.11
3	106.4	154650	158136	99.77	3089204	3034297	101.78	102599	104166	101.21
4	106.4	155450	158136	100.27	3054211	3034297	100.58	103530	104166	101.76

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5	106.4	157000	158136	101.43	3071032	3034297	101.32	103498	104166	101.84
6	106.4	156500	158136	101.00	3034591	3034297	100.01	103527	104166	101.82
			Average	100.73		Average	100.57		Average	101.70
			SD[±]	0.7364		SD[±]	0.8353		SD[±]	0.3208
			%RSD	0.7311		%RSD	0.8305		%RSD	0.2686

Table 3: Results of specificity study

Formulation	Normal	Acid	Alkali	Neutral	Oxide	Heat	Photo
METF[%]	100.55	100.02	100.63	100.51	96.45	100.75	100.61
LINA[%]	99.87	97.53	97.89	100.13	100.75	99.56	99.29
DAPA[%]	99.90	99.45	98.91	100.43	100.11	100.56	99.86

Accuracy

Accuracy of proposed method was ascertained on the basis of recovery studies performed by standard addition method at three different levels of labeled claim (i.e. 80, 100 and 120% of labeled claim).

Precision

The results of system, method and intermediate precision studies are shown.

Table 4: Results of system, method and intermediate precision

Validation Parameters		LINA			METF			DAPA		
		Mean	SD [±]	RSD [%]	Mean	SD [±]	RSD [%]	Mean	SD [±]	RSD [%]
System Precision [a)]		159218	625.32	0.3927	3036545	13117.32	0.4319	107059	1579.12	1.4749
Method Precision [a)]		100.71 %	0.8395	0.8335	100.51 %	0.9861	0.9810	101.72 %	0.2722	0.2676
Intermediate precision	Intraday [b)]	99.30 %	0.1903	0.1916	99.76 %	0.1322	0.1326	101.67 %	0.2196	0.2160
	Interday[b)]	99.14 %	0.3007	0.3033	99.79 %	0.0709	0.0710	101.67 %	0.2645	0.2602
	Different Analyst [b)]	100.89 %	0.3412	0.3382	101.63 %	0.2212	0.2078	101.41 %	0.1789	0.1764

Mean from six analyses(n=6)
Mean from 3 analyses(n=3)

Robustness

Robustness was checked by analysis of sample solutions with standard on same plate, after making small changes in method parameters.

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Table 5: Parameters for robustness study

Parameter	- Level	Nominal	+ Level
Change in Scanning Wavelength (nm)	228 nm	230 nm	232 nm
Change in methanol content in total mobile phase (ml)	81.0 ml	86.0 ml	89.0 ml
Change in flow rate (ml)	0.9	1.1	1.3

Table 6: Robustness study

Condition		METF By peak area*		LINA By peak area*		DAPA By peak area*	
		Amount estimated [%] ± SD	RSD [%]	Amount estimated [%] ± SD	RSD [%]	Amount estimated [%] ± SD	RSD [%]
Change in wavelength (±2 nm)	228 nm	98.47 ± 0.5784	0.5762	100.14 ± 0.2422	0.2119	101.71 ± 0.2548	0.2502
	232 nm	99.27 ± 0.3478	0.3297	99.41± 0.0129	0.0510	101.70 ± 0.1253	0.1230
Change in mobile phase composition (±2.0 ml)	Methanol: 20mM phosphate solution 83.0: 17.0 (v/v)	98.43 ± 0.3778	0.4121	100.09 ± 0.0457	0.0746	101.74 ± 0.2396	0.2353
	Methanol: 20mM phosphate solution 87.0: 13.0 (v/v)	98.24 ± 0.3160	0.3124	99.44 ± 0.1419	0.1522	101.89 ± 0.0200	0.0196
Change in flow rate (± 0.2 ml/min)	0.8	98.16 ± 0.3143	0.3003	99.51 ± 0.1167	0.1269	100.22± 0.1197	0.1196
	1.0	98.85 ± 0.1700	0.1719	99.53 ± 0.0910	0.0942	99.97 ± 0.1076	0.1076

IV. CONCLUSION

The developed RP-HPLC method is validated as per ICH guidelines and can be reliably used for the routine quality control analysis of Metformin Hydrochloride, Linagliptin, and Dapagliflozin in bulk and in pharmaceutical dosage forms. It offers significant advantages in terms of simplicity, sensitivity, accuracy, precision, and robustness, making it an efficient analytical tool in pharmaceutical research and industry.

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