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Eco-Friendly LC-MS/MS Method for Quantification of Dapagliflozin and Linagliptin in Combined Dosage Form: Development, Validation and AGREE Assessment

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Abstract

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder that requires effective therapeutic monitoring. Dapagliflozin and linagliptin, two commonly prescribed antidiabetic agents, act via distinct mechanisms—dapagliflozin inhibits sodium-glucose co-transporter-2 (SGLT-2), while linagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor. This study aimed to develop and validate a sensitive, precise, and green LC-MS/MS method for the simultaneous quantification of dapagliflozin and linagliptin in pharmaceutical dosage forms. Chromatographic separation was achieved on a Phenomenex Gemini C18 column (50 mm × 4.6 mm, 5 µm) using an isocratic mobile phase of methanol and 0.1% formic acid (80:20, v/v) at a flow rate of 0.5 mL/min. Detection was carried out using a mass spectrometer equipped with electrospray ionization (ESI) in multiple reaction monitoring (MRM) mode, monitoring transitions at m/z 426.3→135.1 for dapagliflozin and 473.3→420.3 for linagliptin. The method exhibited excellent linearity over the range of 5–500 ng/mL for dapagliflozin ($r^2 \geq 0.9996$) and 10–1000 ng/mL for linagliptin ($r^2 > 0.9993$). It demonstrated acceptable precision, accuracy, and stability in accordance with regulatory guidelines. Additionally, the AGREE metric yielded a score of 0.76, indicating good compliance with green analytical chemistry principles. The developed LC-MS/MS method is reliable, eco-friendly, and suitable for routine quality control of dapagliflozin and linagliptin in combined formulations. Its robustness also supports potential future applications in pharmacokinetic and bioequivalence studies.

Keywords: Dapagliflozin, Linagliptin, Liquid Chromatography, Mass spectroscopy, Method development, Validation, AGREE.

Introduction

Type II diabetes (NIDDM) is frequently treated with dapagliflozin and linagliptin. They target different metabolic pathways and have complementary mechanisms of action, which enhance therapeutic efficacy and reduce adverse interactions. Dipeptidyl peptidase- 4 (DPP- 4) and sodium-glucose co-transporter-2 (SGLT 2) blockers are used together to optimize glycemic management, minimize weight, and lowering the level of systolic blood pressure (1-3).

Dapagliflozin (Figure 1) helps control blood sugar levels by selectively and permanently blocking SGLT-2 in the kidneys, which reduces the reabsorption of glucose and promotes its elimination through urine. It is categorized as a class III chemical under the Biopharmaceutical Classification System (BCS),

suggesting that it has minimal permeability but excellent solubility (4).

Linagliptin (Figure 2) is a reversible DPP-4 blocker that improves glycemic control in Type II diabetes by lowering glucagon release while boosting insulin production through blocking the breakdown of GLP-1 and GIP (5). To help T2DM patients better regulate their blood sugar, linagliptin can be taken either by itself with lifestyle modifications or in conjunction with other drugs like metformin or thiazolidinedione (6,7).

A comprehensive review of the literature indicates that a variety of analytical techniques have been established for the quantification of linagliptin (LINA) and dapagliflozin (DAPA), both alone and in combination with other antidiabetic drugs. In pharmaceutical formulations, a number of methods have been reported for simultaneous determination, such as RP-HPLC and HPTLC (8,9,10,11). For example, presented a two-tiered analytical method that uses RP-HPLC to quantify drugs and LC-MS/MS to identify degradation products Prajapati et al (12). These procedures are efficient, but they necessitate complicated sample handling, more instruments, and longer analysis times.

In this work, a completely integrated LC-MS/MS approach for the simultaneous measurement of linagliptin (LINA) and dapagliflozin (DAPA) in a single analytical run is presented. Compared to previously documented multi-step approaches, this not only improves analytical throughput but also lowers solvent usage and streamlines operational procedures. The suggested method's improved environmental performance was further demonstrated by a comparative greenness evaluation using the AGREE tool, highlighting its compatibility with the principles of Green Analytical Chemistry (13,14).

The AGREE tool provides a thorough evaluation of a method's sustainability using a circular pictogram and a numerical score that ranges from 0 to 1. It is based on the 12 principles of green analytical chemistry. AGREE is a useful and contemporary indicator for the development of pharmaceutical quality control methods since higher scores indicate greater environmental compatibility (15).

With the goal to ensure the fit for regular pharmaceutical analysis with an emphasis on regulatory compliance and green chemistry principles, this work attempts to develop a sensitive, effective, and ecologically sustainable LC-MS/MS method for the simultaneous estimate of DAPA and LINA.

Materials and Methods

Materials

Reference standards of Dapagliflozin and Linagliptin were provided as gift sample from Morepen Laboratories Ltd. HPLC grade methanol and formic acid is used during development and validation method.

Equipment and chromatographic condition

Utilizing Lab Solution software, a Shimadzu 8030 mass spectrometer (Tokyo, Japan) equipped with an electrospray ionization interface, SIL-20AC autosampler, CTO-20AC column oven, CBM-20A controller, and LC-20AD pump was combined with a liquid chromatography system.

Various chromatographic conditions and buffers were tested for optimal separation. While formic acid improved analyte separation, Dapagliflozin showed a weaker response. Following several experiments, a Phenomenex Gemini C18 column (150 × 4.6 mm, 5 µm) containing methanol (A) and 0.1% formic acid in water (B) (80:20, v/v) at a flow pace of 0.5 mL/min and an injection volume of 5 **µL** was used to optimize an isocratic technique. With a 4.0-minute total run time, Dapagliflozin and Linagliptin eluted at approximately 1.8 and 0.8 minutes, respectively. The auto tester and column were kept at 5 ± 3°C and 40 ± 0.3°C, respectively, to ensure the best possible separation and detection.

Selection of mass range

To determine the precursor ion and product ion, the medication solution containing dapagliflozin and linagliptin was directly injected into the LC-MS/MS and scanned throughout the 100–450 range (figures 1 and 2). The detection process was performed in MRM by analyzing the m/z of 473.3→420.3 for linagliptin and 426.3→135.1 for dapagliflozin. Figures 3 and 4 display the MRM chromatograms for dapagliflozin and linagliptin, correspondingly.

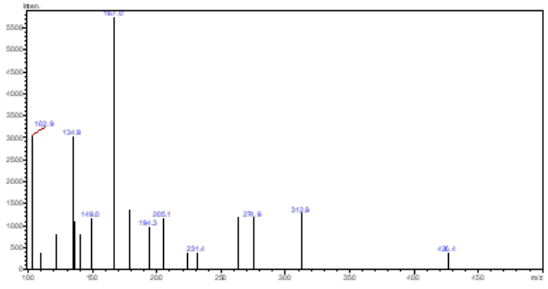


Figure 1. Structure and Mass spectra of Dapagliflozin

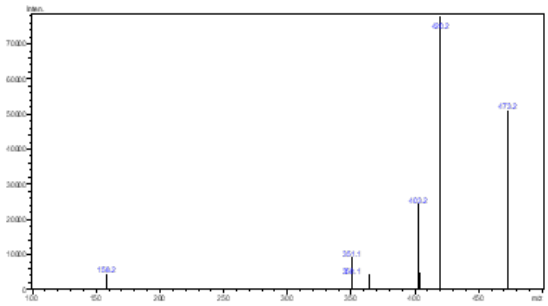


Figure 2. Structure and Mass spectra of Linagliptin

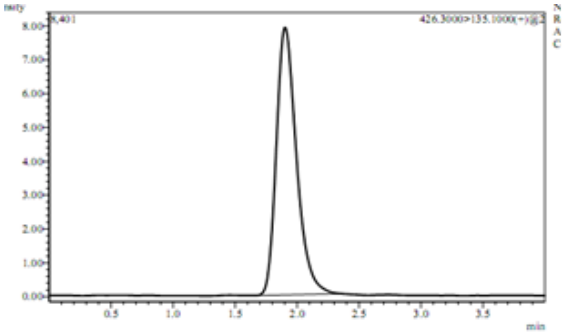


Figure 3. MRM chromatogram of Dapagliflozin

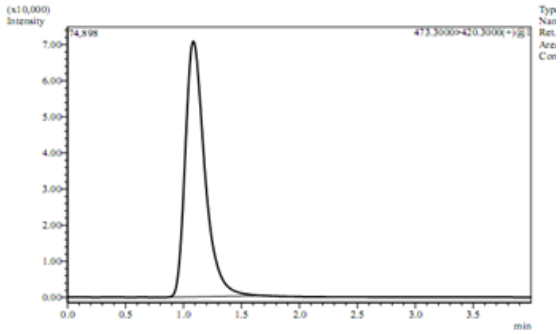


Figure 4. MRM chromatogram of Linagliptin

Standard solution preparation

Precisely weighed quantities of 10 mg DAPA and 10 mg LINA were placed in 100 ml volumetric flasks, dissolved using sonication in the appropriate diluent, and diluted to the necessary level in order to create standard stock solutions. One milliliter of each of these stock solutions was put into a different 10-milliliter volumetric flask and used the same diluent to dilute it to volume. Additional serial dilutions were used to create working standard solutions with concentrations between 5 and 500 ng/mL for dapagliflozin and 10 and 1000 ng/mL for linagliptin.

Validation of method

The new method was validated in accordance with the Q2 R1 requirements for analytical method validation published by the International Council for Harmonization of Technical Requirements of Pharmaceuticals for Human Use (ICH) (16,17). The reliability, precision, the concept of linearity, robustness, LOD and LOQ of the current LC-MS/MS method were all validated.

Linearity

Linearity for DAPA and LINA was assessed using a standard calibration range of 5–500 ng/mL and 10–1000 ng/mL, respectively. The linearity parameter was evaluated by measuring six equidistant concentration levels (n=6). A calibration curve was plotted for each analyte, representing drug concentration versus

peak area, and the corresponding regression equation was determined.

Precision

Intraday precision

Three concentration levels were evaluated for three replicates on the same day in order to determine the intraday precision.

Inter day precision

Similar to intraday precision, interday precision was calculated by taking into account three concentration levels for three replicates on three separate days. The percentage RSD and standard deviation were computed.

Accuracy

It was found by using the conventional addition approach to calculate the recovery of dapagliflozin and linagliptin. By spiking the standard, accuracy was measured by percentage recovery investigate at 80%, 100%, and 120%.

LOD and LOQ

In accordance with the ICH Guidelines, the signal to noise ratio was used to determine the LOD and LOQ for dapagliflozin and linagliptin. LOD and LOQ were calculated using a signal to noise ratio of 3:1 and 10:1, accordingly.

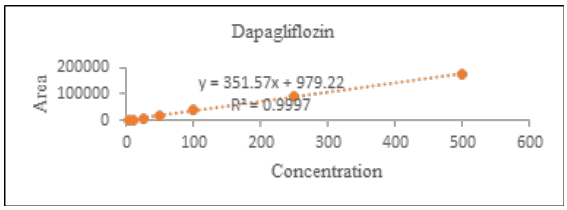
Robustness

Minor alterations to the optimized method parameters were made in order to assess the robustness of the established LC-MS/MS technique. factors including column temperature, mobile phase design, and modify flow pace.

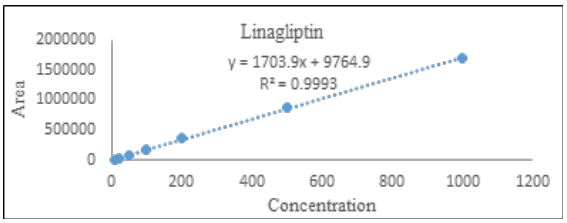
Results and Discussion

Linearity

Linearity of DAPA and LINA was calculated at six concentration ranging from of 5–500 ng/mL and 10–1000 ng/mL, respectively. The calibration curve in figure 5 (a), (b) shows correlation coefficient for DAPA 0.9996 and LINA 0.9993. Table 1 shows the linearity data for both the drugs.



(a)



(b)

Figure 5. Calibration curve of (a) Dapagliflozin and (b) Linagliptin

Table 1. Linearity data for Dapagliflozin and Linagliptin (n=6)**n=Average of determinations*

Dapagliflozin		Linagliptin	
Concentration (ng/ml)	Area	Concentration (ng/ml)	Area
5	2107	10	17387
10	3680	20	34318
25	9329	50	89851
50	18061	100	173650
100	36860	200	371599
250	90690	500	886768
500	175606	1000	1698129
Correlation	0.9996	Correlation	0.9993
Intercept	351.57	Intercept	9764.9
Slop	979.22	Slop	1703.9

Precision

Intraday precision and Inter-day precision for both drugs was found to be precise as table 2 shows % RSD value less than 2.

Table 2. Precision study for Dapagliflozin and Linagliptin

Drug	Sample	Intraday (n=3)		Inter day (n=3)	
		Peak area ± SD	% RSD	Peak area ± SD	% RSD
DAPA	80 %	31843.33 ± 294.80	0.93	32153.67 ± 460.29	1.43
	100 %	35877.67 ± 215.82	0.62	35215.67± 244.53	0.69
	120 %	44969.00 ± 79.67	0.18	45176.33 ± 492.25	1.09
LINA	80 %	316485.00 ± 2495.39	0.79	321825.00 ± 2893.73	0.90
	100 %	382012.33 ± 2421.18	0.63	390183.00± 3777.10	0.97
	120 %	475304.67 ± 8201.78	1.73	479712.00 ± 409.74	0.09

**n=Average of determinations*

Accuracy

Using the conventional addition approach, this study provided recovery analysis percentages at 80%, 100%, and 120%. Table 3 provides a summary of the recovery study's findings. The recovery percentage, which ranges from 99 to 102%, supports the established method's accuracy. The percentage recovery indicates that the additives are not interfering.

Table 3. Accuracy study for Dapagliflozin and Linagliptin (n=3)

Sample Name (level)	ID#1 Compound Name: Linagliptin m/z: 473.3000>420.3000			ID#2 Compound Name: Dapagliflozin1 m/z: 426.3000>135.1000		
	Area	Total Amount Found	% Recovery	Area	Total Amount Found	% Recovery
80%	656033	366.23	101.7	66482	182.05	101.7
	645712	360.47	100.1	66667	182.56	100.1
	654595	365.43	101.5	66586	182.34	101.5
100%	736405	406.09	101.5	74213	203.29	101.5
	717434	400.5	100.1	74238	203.36	100.1
	729769	407.38	101.8	73205	200.52	101.8
120%	793601	443.01	100.7	81821	224.19	100.7
	801036	447.16	101.6	81600	223.58	101.6
	787681	439.7	99.9	81741	223.97	99.9

**n=Average of determinations*

LOD and LOQ

The signal to noise ratio was utilized as the basis for calculating LOD and LOQ. For both medications, the LOD and LOQ values are displayed in Table 4.

Table 4. LOD and LOQ data for Dapagliflozin and Linagliptin (n=6)

Parameters	Dapagliflozin	Linagliptin
LOD	12.48 ng/ml	34.21 ng/ml
LOQ	38.41 ng/ml	103.67 ng/ml

**n=Average of determinations*

Robustness

The robustness result data is depicted in table 5. The established LC-MS/MS technique is robust, as evidenced by the %RSD value for all parameters being less than 2%.

Assay

The assay of DAPA and LINA was analyzed in the Dapavel-L tablet combined formulation. Table 6 illustrates the percentage of assay results, fall within the acceptance criteria of 98-102%. This shows that excipients are not

interfering, proving that the suggested method may be successfully used to analyze commercial formulations that contain DAPA and LINA.

Table 5. Robustness study for Dapagliflozin and Linagliptin (n=3)

Factor	Level	LINA			DAPA		
		Area Average	SD	%RSD	Area Average	SD	%RSD
Column Temperature	35 °C	330170	2833	0.86	39693	249	0.63
	45 °C	333514	2231	0.67	39619	231	0.58
Flow Rate	0.4 ml/min	335388	1158	0.35	40158	413	1.03
	0.6 ml/ min	331308	4690	1.42	37987	425	1.12
Mobile Phase Composition	85:15	353418	611.26	0.17	37847	574.53	1.52
	75:25	350807	1480.43	0.42	39263	316.04	0.80

**n=Average of determinations*

Table 6. Assay study in tablet formulation

Sr. No	Amount taken (ng/ml)		Drug Area		% Label Claim	
	DAPA	LINA	DAPA	LINA	DAPA	LINA
1	100	200	36630	376539	99.37602	101.63832
2	100	200	36642	372741	99.40857	100.3073
3	100	200	36245	369856	98.33152	99.53095
4	100	200	37324	367512	101.2588	98.90016
5	100	200	36985	371452	100.3391	99.96044
6	100	200	36745	372568	99.68801	100.2608
Average			36761.83	370111.3	99.73368	99.59966
SD			364.6847	2620.345	0.989378	0.705154
% RSD			0.99202	0.707988	0.99202	0.707988

Greenness assessment using AGREE tool

The suggested LC-MS/MS method's environmental sustainability was assessed using the AGREE tool, a piece of software created by Pena-Pereira et al. in 2020 (18). This tool displays results in a circular pictogram with twelve parts, and it is based on the 12 principles of Green Analytical Chemistry (GAC). An overall score between 0 and 1 is shown at the centre of each section, which is color-coded from red (low greenness) to green (high greenness).

Table 7. Greenness assessment by AGREE tool for proposed method

Parameters	Proposed method
Technique	LC-MS/MS
Elution	Isocratic
Mobile Phase	Methanol and 0.1% formic acid (80:20)
Column	50 mm × 4.6 mm, 5 µm packed column
Flow rate	0.5 ml/min
Run time	5 min
Waste	8 gm

AGREE Plot	

The suggested approach was evaluated using the AGREE software, which was downloaded on February, 2025. Each method's key parameters were assessed in relation to the twelve GAC principles. Table 7 presents the derived pictograms for direct comparison, summarizing the environmental performance. This quantitative and visual study supported the current method's compatibility with sustainable analytical procedures and validated its superior greenness.

0.76 AGREE score demonstrates that the method is highly aligned with green analytical chemistry principles delivering both excellent analytical performance and a strong environmental profile while indicating clear targets (e.g., solvent choice, energy optimization) for further greenness improvements.

Conclusion

A robust, sensitive, and environmentally friendly LC-MS/MS method was successfully developed and validated for the simultaneous estimation of Dapagliflozin and Linagliptin, in accordance with ICH Q2(R1) guidelines. The method showed excellent performance characteristics and proved suitable for application in pharmacokinetic studies.

Its high AGREE score further confirms its alignment with green analytical chemistry principles. This approach supports both scientific accuracy and environmental sustainability in pharmaceutical analysis. Future research should explore its application in metabolite profiling and therapeutic drug monitoring. Additionally, coupling with automated techniques could enhance its efficiency in clinical and regulatory settings.

Conflict of interest

The authors have no conflicts of interest regarding this investigation**.**

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