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Original Article

Development and validation of UPLC-MS/MS method for estimation of Saxagliptin in bulk and tablet dosage forms

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ABSTRACT

Background and Aims: Saxagliptin is an antidiabetic drug used to treat type 2 diabetes mellitus. During the manufacture of bulk and dosage forms, assay of the drugs is important in determining the percentage purity. Hence, this study aimed to develop and validate a simple, rapid, and selective method based on ultra-performance liquid chromatography-Tandem mass spectrometry (UPLC-MS/MS) analysis for the determination of saxagliptin in bulk and tablet dosage forms.

Methods: Chromatographic separation was performed using a Waters Acquity UPLC BEH C_{18} column (2.1 X 50 mm, 1.7 µm) with a mobile phase consisting of a mixture of acetonitrile: 0.1% formic acid (60:40, v/v) at a flow rate of 0.120 mL/min. Separation was performed in 3 min run time. The analyte was ionised and detected by tandem mass spectrometry, which is performed in positive ion and multiple reaction monitoring modes.

Results: Linearity was established in the 10–150 ng/mL with r^2=0.9980. The results were observed to be well within the limits when validation was performed as per the ICH guidelines.

Conclusion: The proposed method can be applied successfully for the analysis of saxagliptin in bulk and tablet formulations.

Keywords: Saxagliptin, UPLC-MS/MS, Method development, Validation.

INTRODUCTION

Saxagliptin, chemically known as 1S, 3S, 5S-2[(2S)-2-amino-2-(3-hydroxy-1-adamantyl) acetyl]-2-azabicyclo [3.10] hexane-3-carbonitrile, is an anti-diabetic drug (Figure 1). It is used by patients with type 2 diabetes as second-line therapy when first-line treatment (metformin) cannot control the disease (Mengistu, Ole & Alemayehu, 2021). It is a highly selective, reversible, and competitive dipeptidyl peptidase-4 inhibitor, increasing the secretion of insulin and decreasing the secretion of glucagon (Darshan, 2011). Inhibition of plasma DPP-4 activity can be achieved by administering saxagliptin to type 2 diabetes mellitus patients once daily before breakfast (Kulsa & Edelman, 2010; Anderson, Hayes &Stephens, 2016).

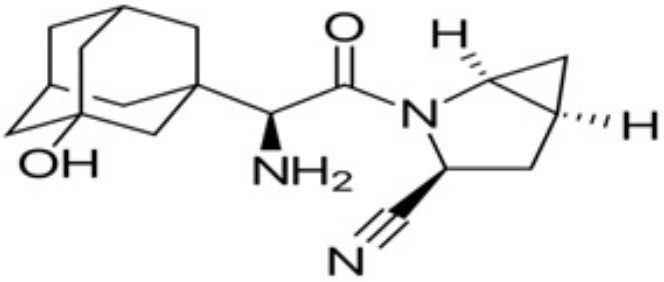

Figure 1. Chemical Structure of saxagliptin

As per the available literature, methods have been reported for the quantification of saxagliptin alone (Lais, Ana, Andrea & Clarice, 2015; Maha, Omar, Miriam & Mariam, 2015; Sridhar et al., 2014) or in combination with other anti-diabetic drugs (Saiful, Taleb, Sukalyan, Abdul & Rafiquzzaman, 2016; Faroqui & Kakde, 2016; Sanchay, Patel, Gaikwad & Jadhav, 2019; Singh, Bansal, Maithani & Chauhan, 2018; Mahnoor & Roshan, 2022; Rageeb et al., 2020; Ghawate & Chopade, 2019; Darshak, Ujashkumar, Jayvadan, Darshana & Pavan, 2021; Rahul & Ganesh, 2019; Amit & Bhuvnesh, 2021; Vijaya, Fainaz, Riya & Tejaswini, 2018; Padmaja, Sivagami, Chandrasekar & Niranja, 2018; Sarada, Narendra & Pragati, 2017; Rohini & Nagaraju, 2018; Vijaya & Narendra, 2020; Abdul-Azim, Ehab & Marwa, 2012; Narender & Shanmuga, 2021) using liquid chromatography techniques. The method reported by Lais et al. (2015) employed the RP-HPLC technique with a PDA detector. A run time of 12.5 min was required for regular sample anal-

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ysis. The method reported by Maha, Omar, Miriam & Mariam (2015) quantified saxagliptin using the RP-HPLC technique with a UV detector, followed by the elucidation of the structures of major degradation products using LC-MS. However, this method has less sensitivity (25-400 µg/mL) and a longer run time of 10 min. The RP-HPLC method reported by Sridhar et al., (2014) utilised the PDA detector for the assay of saxagliptin and tandem mass spectrometry for the characterisation of degradation products. This method has a run time of 30 min, which is not suitable for the routine analysis of saxagliptin. All methods reported for the assay of saxagliptin along with other drugs have longer run times and are therefore not appropriate for the regular analysis of saxagliptin samples.

The proposed work describes a UPLC-MS/MS technique for estimating saxagliptin in bulk and tablet formulations within a short run time of 3 min. This method is sensitive with a low LOQ value (10 ng/mL). The method was successfully applied for the quantification of saxagliptin in tablet form after its validation following the ICH guidelines.

MATERIALS AND METHODS

Chemicals and Reagents

The working standard for saxagliptin was procured from Vivan Life Sciences (Mumbai, India). Research Lab Fine Chem Industries (Mumbai, India) supplied HPLC-grade methanol, acetonitrile, and water. Analytical grade Formic acid was purchased from Merck India (Mumbai, India).

UPLC-MS/MS Instrument and Conditions

A Waters ACQUITY UPLC system (Waters, Milford, MA, USA) equipped with a Waters Acquity UPLC BEH C_{18} column (2.1 X 50 mm, 1.7 µm) was utilised for the present work. A mobile phase consisting of a mixture of acetonitrile:0.1 formic acid (60:40, v/v) was used in isocratic mode at a flow rate of 0.120 mL/min. A Quattro Premier XE™ triple quadrupole mass spectrometer (Waters, Milford, MA, USA) was used to detect the analyte. The data were acquired in the positive ion mode. The desolvation gas temperature was kept at 400 ºC, capillary voltage of 3 KV, source temperature was kept at 120 ºC, and cone gas flow rate was set at 102 L.h-1. The collision energy was maintained at 25 V. The multiple reaction monitoring (MRM) mode was used to monitor the ions by detecting the transition of the precursor ion to the product ion of saxagliptin from m/z 316.40 to 179.86. Mass Lynx software version 4.1 was employed for analysing the acquired data.

Preparation of Standards

A standard stock solution was prepared by dissolving the required amount of working standard saxagliptin with the diluent (HPLC grade methanol: water, 50:50, v/v) to obtain a concentration of 1000 µg/mL. The stock solution was diluted appropriately using the mobile-phase solvent to obtain the required concentrations of the standard solution.

Preparation of Samples

The tablet dosage form of saxagliptin (Brand name: Onglyza; label claim: 5 mg) was weighed and powdered, and a powder equivalent to 5 mg of saxagliptin was taken. It was solubilised in 50 mL of diluent. The solution was then filtered, and the filtrate was diluted to a concentration of 10 ng/mL with the mobile-phase solvent.

Method Validation

The developed method for estimating saxagliptin using UPLC-MS/MS was validated according to ICH guidelines (ICH Q2 (R1), 2005). The validation parameters were accuracy, precision, linearity, limit of detection, limit of quantification, robustness, and stability.

The linearity of the method was evaluated by measuring the peak area response of standard solutions at concentrations of 10, 25, 50, 75, 100, 125, and 150 ng/ml. A graph was plotted based on standard concentrations on the abscissa and peak areas on the ordinate. Recovery studies were conducted to assess the accuracy of the proposed method. Blank solutions were spiked with the working standards at three different levels (50%, 100%, and 150%, and triplicate measurements were performed at each level. A working standard solution of saxagliptin was injected into the instrument six times on the same day to assess intra-day precision and on different days for inter-day precision. The limits of detection and quantification of the method were assessed at S/N (signal to noise) ratio of 3:1 and 10:1, respectively. The flow rate of the mobile phase was altered from 0.12 mL/min to 0.14 mL/min, and the composition of the organic phase in the mobile phase was changed from 60% to 62% to evaluate its robustness. Three measurements were performed at a concentration of 100 ng/mL for each of the altered conditions, and the %RSD was calculated.

Assay of marketed tablet formulation:

The tablet formulation - was analysed, employing the proposed method to quantify the amount of saxagliptin. Three replicate injections of the sample solutions were analysed to calculate the %assay of tablet formulation.

Table 1. Literature survey

Parameter	Lais et al.	Maha et al.	Sridhar et al.	Present method
Technique	RP-HPLC-PDA	RP-HPLC-UV	RP-HPLC-PDA	LC-MS/MS
Column	Waters XBridge C_{18} (250x4.6 mm, 5 µm)	Symmetry C_{18} (150x4.6 mm, 5 µm)	Zorbax Eclipse plus (150x4.6 mm, 5 µm)	Waters Acquity UPLC BEH C_{18} (2.1 X 50 mm, 1.7 µm)
Mobile phase	0.1% phosphoric acid (pH 3):methanol (70:30, v/v)	Potassium dihydrogen phosphate buffer:acetonitrile:methanol (40:30:30, v/v/v)	10 mM ammonium formate:methanol (gradient elution)	Acetonitrile: 0.1% formic acid (60:40, v/v)
Flow rate (mL/min)	1.0	1.0	0.5	0.12
Retention time (min)	8	2.5	19	1
Run time (min)	12.5	10	30	3
Concentration range	15-100 µg/mL	25-400 µg/mL	50-150 µg/mL	10-150 ng/mL

RESULTS AND DISCUSSION

Method Development

As reported in the available literature, few methods for estimating saxagliptin in bulk and pharmaceutical dosage forms. All of these methods have longer run times and are not appropriate for routine analysis of saxagliptin (Table 1). Hence, the objective of this work was to develop a rapid and sensitive method for determining saxagliptin using UPLC-MS/MS.

During method development, the chromatographic and mass spectrometry conditions were optimised. An electrospraying-ion source was employed to record the mass spectrum of saxagliptin in both positive and negative ionisation modes. The presence of an amino group in the structure of saxagliptin imparts a basic nature, resulting in a higher response observed in the positive ion mode. During multiple reaction monitoring, the transition from the precursor ion to the product ion occurred from m/z of 316.40 to m/z 179.86 (Figure 2).

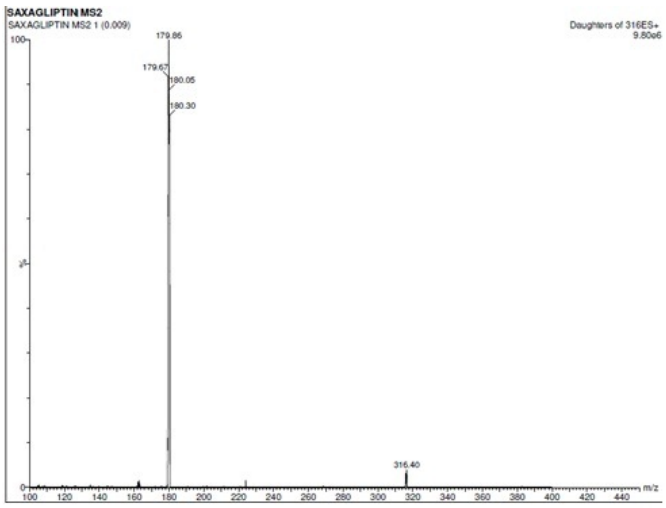

Figure 2. Product ion Mass Spectra

During chromatographic analysis, the column, mobile-phase composition, and flow rate were optimised to achieve good response and peak shape within a short run time. In combination with acetonitrile, various concentrations of acetic acid and formic acid were tested at altered ratios on Zorbax XDB-Phenyl (75 x 4.6 mm, 3.5 µm), Kromasil 100-C¬18 (100 x 4.6 mm, 5 µm), and Waters Acquity UPLC BEH C18 (2.1 X 50 mm, 1.7 µm) columns. Finally, a mixture of acetonitrile and 0.1% formic acid (60:40, v/v) as the mobile phase on a Waters Acquity UPLC BEH C18 column (2.1 X 50 mm, 1.7 µm) at a flow rate of 0.12 mL/min produced an acceptable response and satisfactory peak shape within 3 min of run time (Figure 3).

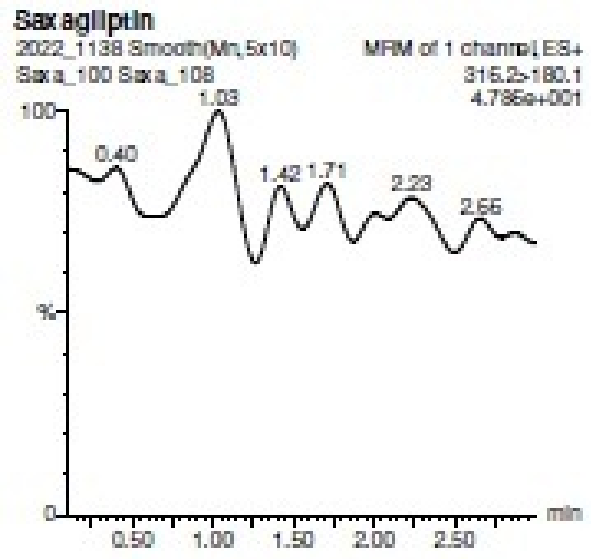

Figure 3. (a) Multiple Reaction Monitoring Chromatogram of blank

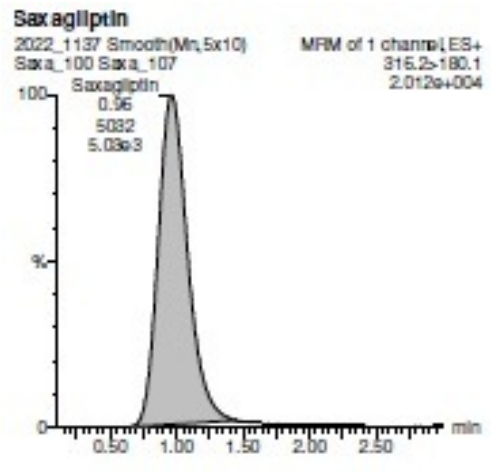

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Figure 3. (b) Multiple Reaction Monitoring Chromatogram of analyte at LOQ level-10ng/mL

Validation Results

The calibration curve showed good linearity over the concentration range of 10 ng/mL to 150 ng/mL with R2>0.99. The linear equation established is y=388.92x+652.71 (Table 2) (Figure 4) The percentage recovery of the analyte was found in the range of 98.20% to 98.55%, proving the accuracy of the proposed method (Table 3.)

Table 2. Linearity data

S.No	Concentration (ng/ml)	Mean Peak area (n=3)
1	10	3771
2	25	14612
3	50	21296
4	75	28842
5	100	40463
6	125	49113
7	150	58642
Range (ng/ml)		10-150
R^2		0.9980
Slope		396.588
Y-intercept		- 22.269

R^2 – Regression coefficient

The % RSD of the peak area and retention times were calculated and were found to be between 1.16% and 1.45% for the peak area and between 0% and 0.54% for the retention time (Results are shown in Table 4).

The limit of detection and limit of quantification were determined to be 3 and 10 ng/mL, respectively, proving the method to be highly sensitive.

The robustness of the proposed method was demonstrated by the %RSD values in the range of 0.07% to 1.5% for peak area and 0.1% for retention time.

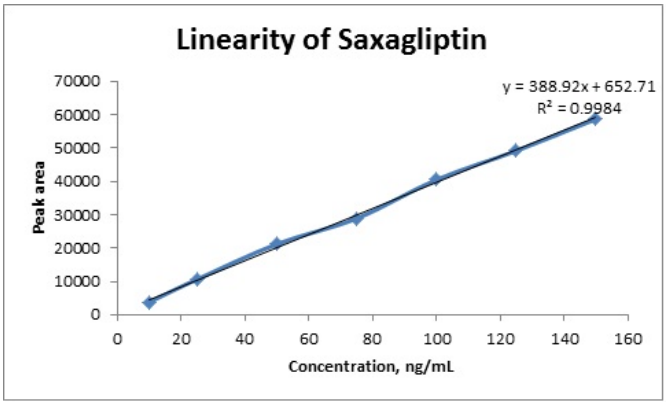

Figure 4. Linearity graph

The analyte present in the standard solution remained stable for 24 h at the auto-sampler temperature with a %stability value of 101.60%.

Assay of commercial formulation:

The percent purity of the tablet formulation was determined to be in the range of 98.60%–101.47% (Table 5).

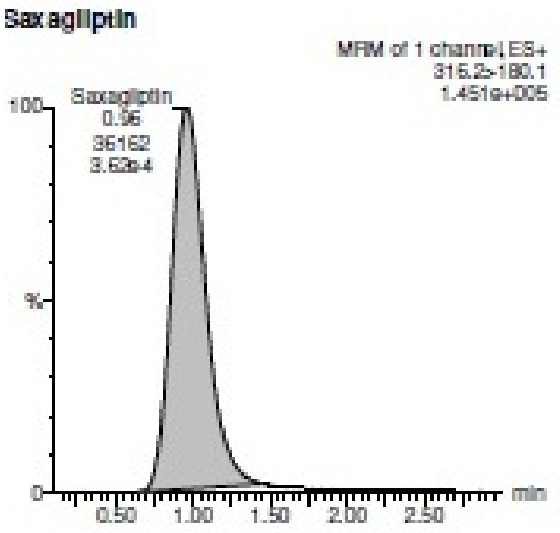

Figure 5. MRM chromatogram of a saxagliptin tablet formulation

Table 3. Accuracy data

Concentration (ng/mL)	Amount recovered	Mean % recovery	% RSD
50	49.10	98.20	0.78
100	98.30	98.30	1.15
150	147.83	98.55	0.55

RSD–Relative standard deviation

Table 4. Precision data

Concentration, ng/mL	Intra-day precision				Inter-day precision			
	Peak area	%RSD	RT	%RSD	Peak area	%RSD	RT	%RSD
50	22663	0.42	0.96	0	22585	0.48	0.96	0
	22637		0.96		22478		0.96	
	22489		0.96		22368		0.96	
100	40857	0.99	0.96	0	40568	0.55	0.96	0
	40675		0.96		40147		0.96	
	40092		0.96		40488		0.96	
150	61135	0.34	0.96	0	61536	0.59	0.96	0
	61008		0.96		61154		0.96	
	61410		0.96		61878		0.96	

RSD–Relative standard deviation

Table 5. Precision data

S.No.	Label claim	Amount found	% Assay	%RSD
1	5 mg	5.07	101.4	1.51
2		4.93	98.6	
3		5.05	101.4	

RSD–Relative standard deviation

CONCLUSION

An LCMS/MS method was developed and validated for quantifying saxagliptin in bulk ?drug and tablet formulations. It is a sensitive, accurate, precise, and linear quantification method in the concentration range of 10–150 ng/mL. Hence, the developed method is recommended for the regular analysis of saxagliptin in bulk? drug and tablet form. The shorter run time (3 min.) allows the analysis of more samples per day.

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