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Development and Validation of UV Spectrophotometric and RP-HPLC Methods for the Quantitative Estimation of Sitagliptin in Bulk Drug and Pharmaceutical Dosage Forms

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Abstract

Two simple, sensitive, precise, and economical analytical methods were developed and validated for the quantitative estimation of Sitagliptin in bulk drug and pharmaceutical dosage forms. Method A involved UV spectrophotometric analysis, where Sitagliptin exhibited maximum absorbance at 264 nm in methanol and obeyed Beer–Lambert's law in the concentration range of 4–20 µg/mL. The regression equation was found to be $Y = 0.020X - 0.029$ with correlation coefficient (r^2) 0.997. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.559 µg/mL and 1.677 µg/mL, respectively. Method B involved reverse phase high performance liquid chromatography (RP-HPLC) using a C18 column with a mobile phase consisting of phosphate buffer and acetonitrile (40:60 v/v), flow rate 1 mL/min, and detection at 264 nm. The retention time was 5.93 min and linearity was observed in the range 20–120 µg/mL ($r^2 = 0.998$). The developed methods were validated according to ICH guidelines for accuracy, precision, linearity, LOD, LOQ, ruggedness, and robustness. Both methods were found suitable for routine pharmaceutical analysis.

Introduction

Pharmaceutical analysis plays a crucial role in ensuring the identity, purity, safety, and efficacy of drug substances and finished dosage forms. Analytical method development involves systematic selection, optimization, and validation of procedures for reliable quantification of active pharmaceutical ingredients (APIs). Sharma (2000) described instrumental methods as essential tools in pharmaceutical quality control. Beckett and Stenlake (1997) emphasized the importance of UV–Visible spectroscopy and chromatographic techniques in routine drug analysis.

Sitagliptin is an oral antihyperglycemic agent belonging to the class of dipeptidyl peptidase-4 (DPP-4) inhibitors, used in the management of type 2 diabetes mellitus. Literature survey indicates that only a limited number of analytical methods such as HPLC, HPTLC, UV/ Vis spectrophotometry, MEKC, and GLC have been reported for its estimation. Tarkase et al. (2013) developed a UV spectrophotometric method for estimation of Sitagliptin in tablet dosage form with RSD < 2%. Ravisankar et al. (2014) reported a validated UV method at 267 nm with correlation coefficient 0.9995. Kashid et al. (2012) developed an RP-HPLC method for plasma estimation. Deepthi et al. reported a stability-indicating RP-HPLC method with high sensitivity. In view of limited simple and economical analytical procedures, the present work aimed to develop and validate UV spectrophotometric and RP-HPLC methods for quantitative estimation of Sitagliptin in bulk and pharmaceutical formulations in accordance with ICH guidelines.

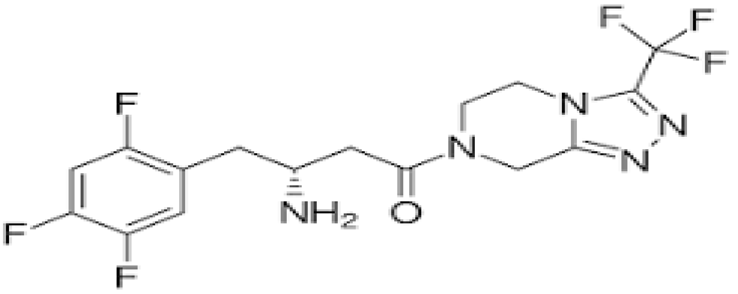

Fig.1: Sitagliptin

Nomenclature : (R)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-a]pyrazin-7(8H)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.
Molecular formula : $C_{16}H_{15}F_6N_5O$
Molecular weight : 407.314g / mol.
Characteristics : Odourless, White Crystalline Powder.
Category : Anti Diabetic.
Solubility: Soluble in N,N-dimethylformamide, slightly soluble in ethanol and methanol.

Materials and Methods

Materials

Reference standard Sitagliptin was obtained as gift sample. Methanol, acetonitrile, orthophosphoric acid, and phosphate buffer salts were of analytical/HPLC grade

Method A: UV Spectrophotometric Method

Instrumentation

UV–Visible double beam spectrophotometer.

Preparation of Standard Solution

100 mg of Sitagliptin was dissolved in methanol to obtain 1000 µg/mL stock solution. Further dilutions were prepared to obtain working solutions

Selection of Wavelength

The drug solution was scanned between 200–400 nm and showed maximum absorbance at 264 nm

Linearity

Calibration curve constructed for 4–20 µg/mL concentration range. Regression equation: **$Y = 0.020X - 0.029$ ($r^2 = 0.997$)**

Validation Parameters

- Accuracy (Recovery 100.50–101.27%)
- Precision (%RSD < 2%)
- LOD = 0.559 µg/mL
- LOQ = 1.677 µg/mL

Method B: RP-HPLC Method

Instrumentation

Shimadzu LC-20AT HPLC with SPD-20A UV detector; Phenomenex Gemini C18 column (250 × 4.6 mm, 5 µm)

Mobile Phase

Phosphate buffer: Acetonitrile (40:60 v/v), pH adjusted to 3 with orthophosphoric acid

Chromatographic Conditions

- Flow rate: 1 mL/min
- Detection wavelength: 264 nm
- Retention time: 5.93 min

Linearity

Concentration range: 20–120 µg/mL

Correlation coefficient: 0.998

Validation

- Accuracy: 99.98–100.73% recovery
- Precision: %RSD < 2%
- LOD: 1.0 µg/mL
- LOQ: 3.2 µg/mL

RESULTS & DISCUSSION

METHOD A: UV SPECTROSCOPY

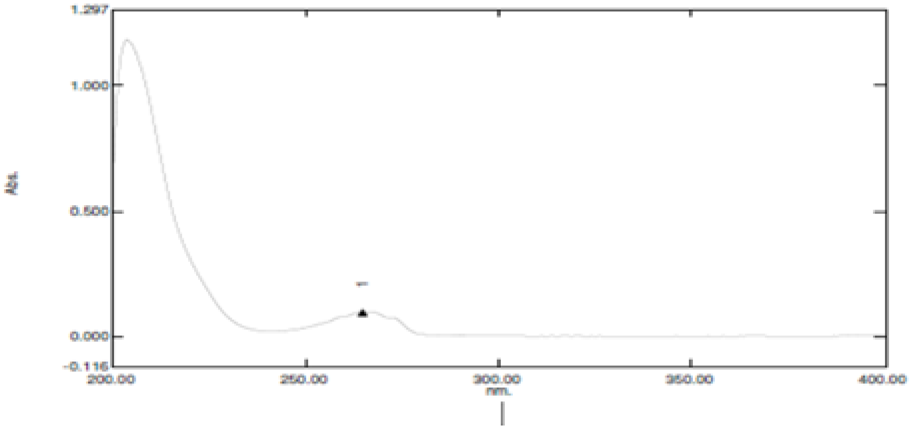

Fig no: 2. Spectra of Sitagliptin at 264nm

Table no: 1. Results of Calibration curve at 264 nm for Sitagliptin by Spectroscopy

Sl. no.	Conc.(µg/ml)	Absorbance at 264 nm
1	4	0.061
2	8	0.132
3	12	0.218
4	16	0.299
5	20	0.396

Table no: 2 Determination of Accuracy results for Sitagliptin by Spectroscopy

Amt. of sample (µg/ml)	Amt. of drug added (µg/ml)	Amt. Recovered (µg/ml)	% Recovery ± SD**
Tablet 1			
10	8.0	18.23	101.27 ± 0.03
10	10	20.10	100.50 ± 0.12
Tablet 2			
10	8.0	18.20	101.11 ± 0.14
10	10	20.15	100.75 ± 0.11

Table no: 3 Determination of Precision results for Sitagliptin at 264 nm by Spectroscopy

Conc. µg/ml	Inter-day Absorbance ± SD**	% CV	Intra-day Absorbance ± SD**	% CV
4	0.061 ± 0.000577	0.940	0.064 ± 0.000577	0.896
8	0.132 ± 0.000577	0.436	0.134 ± 0.000577	0.429
12	0.218 ± 0.001	0.458	0.221 ± 0.001	0.452
16	0.299 ± 0.001	0.335	0.232 ± 0.001528	0.657
20	0.396 ± 0.001	0.251	0.398 ± 0.000577	0.144

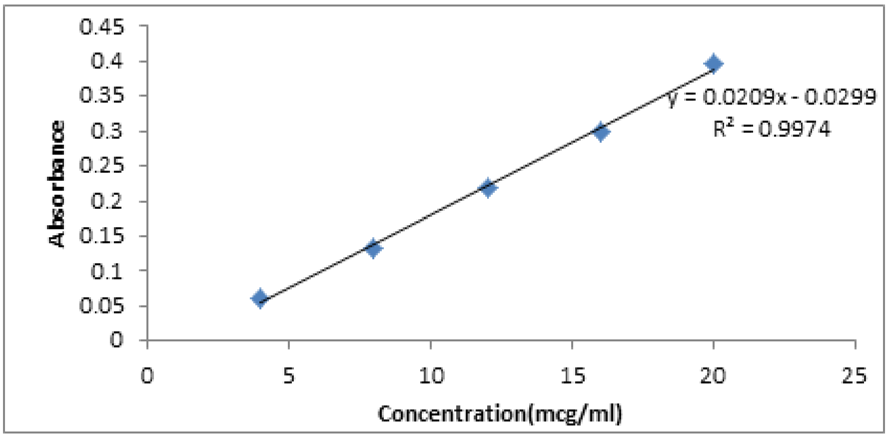

Fig no: 3. Linearity curve for Sitagliptin at 264 nm by Spectroscopy

Table no: 4 Ruggedness results for Sitagliptin at 264 nm by Spectroscopy

Sample	Label claim (mg)	Analyst I		Analyst II	
		Amount found (mg)	Recovery ± SD** (%)	Amount found (mg)	Recovery ± SD** (%)
Tab 1	30	29.8	99.3 ± 0.6	30.4	101.3 ± 0.9
Tab 2	30	30.2	100.6 ± 0.7	30.2	100.6 ± 0.8

METHOD B: HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

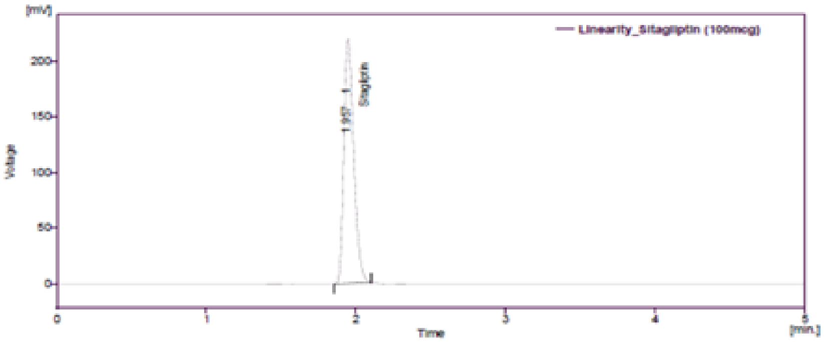

Fig no: 4. Chromatogram of Sitagliptin at 264 nm

ACCURACY:

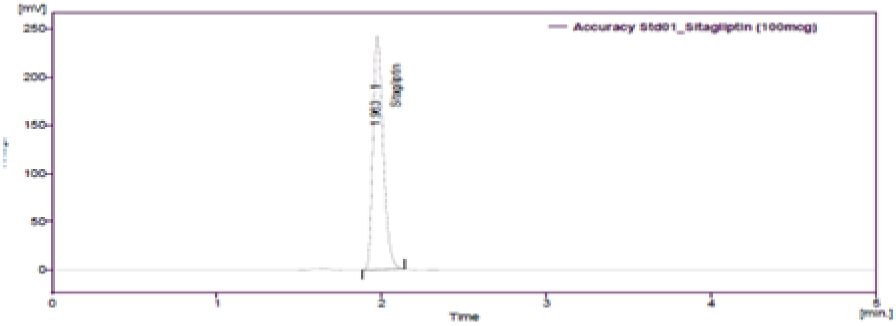

Fig.No: 5. Chromatogram of Sitagliptin (for pure drug)

Table: 5. Accuracy results for Sitagliptin

Sample	Percentage Recovery	Mean**
S1-90%	100.73	100.01
S2-90%	98.75	
S3-90%	99.76	
S1-110%	100.41	100.44
S2-110%	100.59	
S3-110%	99.12	
S1-130%	100.78	100.06
S2-130%	99.34	
S3-130%	99.98	

PRECISION

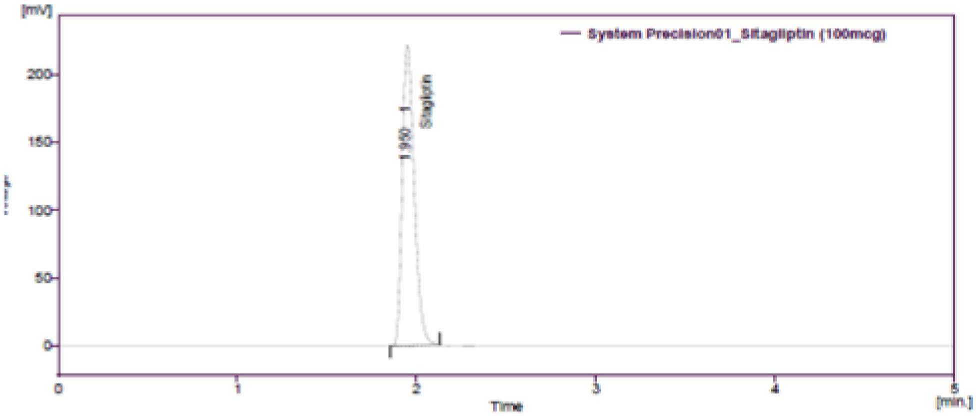

Fig no: 6. Chromatogram of System Precision

Table no: 6. System precision results for Sitagliptin

Sl. NO.	R.T**	Peak area**
1	1.953	1026.095
SD	0.00248	2.7584
% RSD	0.127	0.268

LINEARITY

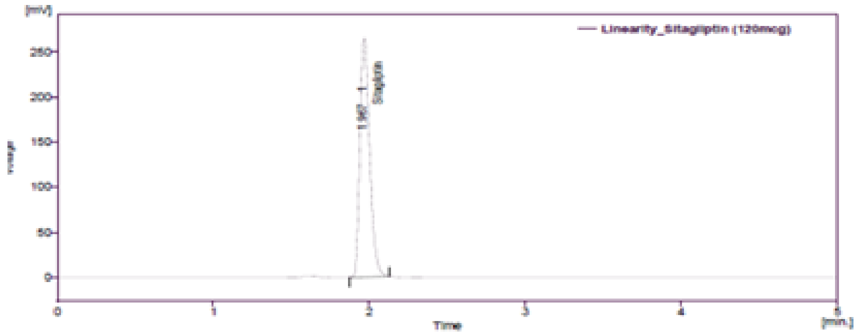

Fig no:7 Chromatogram of Linearity

Table no: 7 Linearity results for Sitagliptin

Conc. (µg/ml)	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml	120µg/ml
Peak Area**	190.339	378.896	580.421	750.749	980.574	1141.848
Correlation		0.998				

LIMIT OF DETECTION (LOD)

Table no: 8. LOD results for Sitagliptin

Sl. No.	Peak Area	Conc(µg/ml)
1	- 980.574	0.02
2	969.347	
3	970.261	

LIMIT OF QUANTITATION (LOQ)

Table no: 9. LOQ results for Sitagliptin

Sl. No.	Peak Area	Conc (µg/ml)
1	1030.057	0.06
2	1041.128	
3	1032.651	

RUGGEDNESS

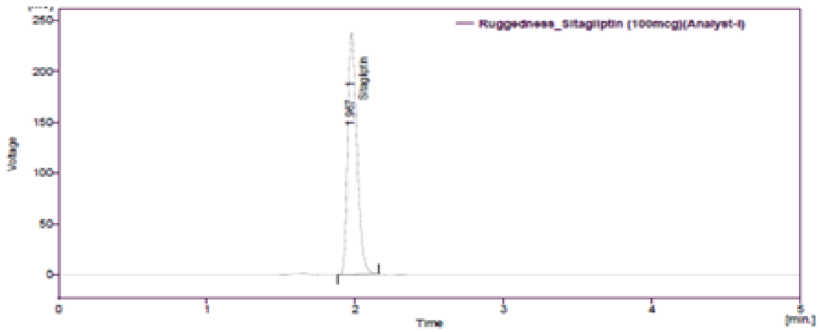

Fig no: 8. Chromatogram of Ruggedness

Table no: 10. Ruggedness results for Sitagliptin: (Day-2, Analyst-2)

Sl. No.	**Peak Area**	**% Assay**	**R.T**
1	1027.598	100.16	1.967
2	1027.561	100.22	1.967
%RSD	0.18	0.60	0.02

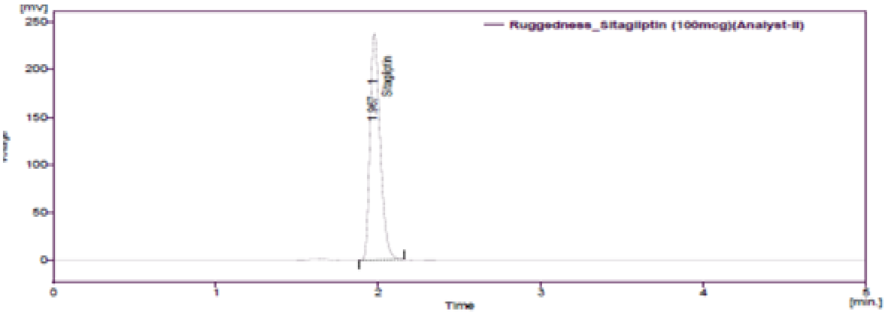

Fig no: 9. Chromatogram of Ruggedness

Robustness

Table no: 11. Robustness results for Sitagliptin: (266nm)

Sl. No.	**Area**	**% of Assay**	**R.T**
1	972.897	99.60	1.967
2	972.861	99.55	1.967
%RSD	0.38	0.27	0.02

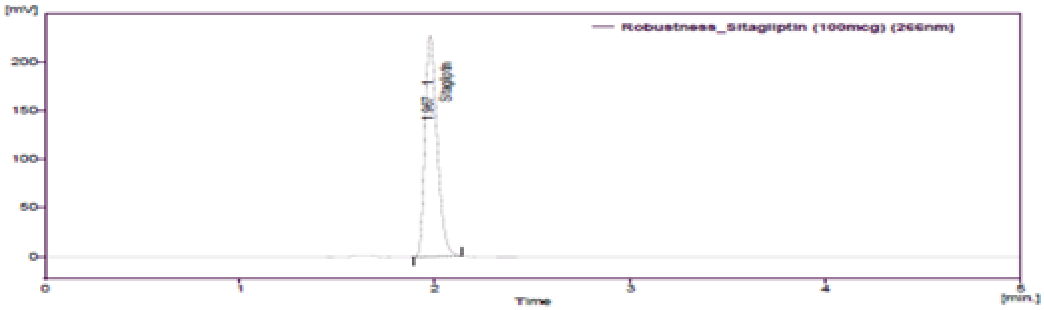

Fig no: 10. Chromatogram of Robustness (Sitagliptin-266nm)

Specificity

Table no: 5.26. Specificity results for Sitagliptin (Heat)

Sl. No.	**Area**	**% of Assay**	**R.T**
1	2107.642	100.05	1.967
2	2107.684	100.26	1.964
%RSD	0.68	0.61	0.03

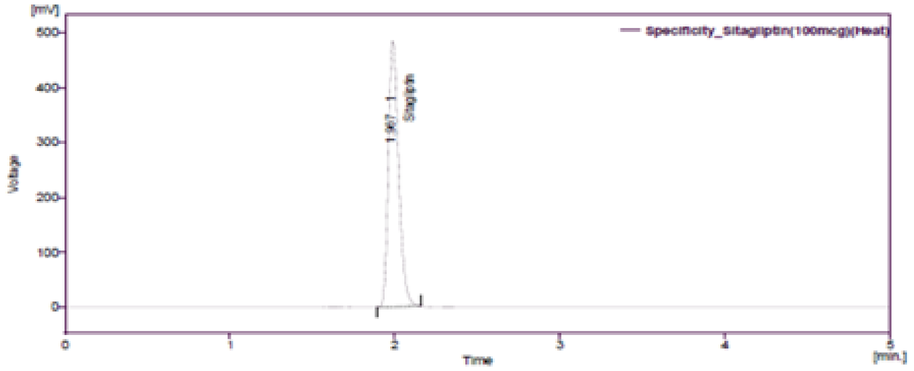

Fig no: 10.Chromatogram of Specificity (Heat)

DISCUSSION

The UV spectrophotometric method showed excellent linearity within Beer's law limits (4–20 µg/mL) and demonstrated acceptable accuracy and precision. Recovery studies confirmed absence of interference from excipients. The low %RSD values indicated method reproducibility.

In RP-HPLC, optimization of mobile phase composition ensured symmetrical peak with acceptable theoretical plates and minimal tailing. The retention time of 5.93 min allowed rapid analysis. The developed chromatographic method showed higher sensitivity compared to UV method, with improved specificity and resolution. Both methods comply with ICH validation parameters and are suitable for routine quality control analysis.

CONCLUSION

The developed UV spectrophotometric and RP-HPLC methods for estimation of Sitagliptin were found to be simple, accurate, precise, sensitive, and economical. The UV method is suitable for routine analysis due to its simplicity and low cost, whereas the RP-HPLC method provides higher sensitivity and specificity. Both methods were validated according to USP and ICH guidelines and can be effectively applied for quantitative determination of Sitagliptin in bulk drug and pharmaceutical dosage forms

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CONFLICT OF INTERESTS

The authors declare no conflict-of-interest

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