Journal of Advanced Zoology
ISSN: 0253-7214
Volume 45 Issue 2 Year 2024 Page 1711:1722

Development And Validation Of Uv Spectrophotometric Method For The Determination Of Sitagliptin In Bulk Material And In Tablets

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Article History

Received: 14 January 2024
Revised: 02 February 2024
Accepted:15 February 2024

Abstract

This study developed and validated novel UV spectrophotometric methods according to ICH Q2 (R1) guidelines for the quantitative analysis of tolvaptan and carvedilol in pharmaceutical dosage forms. The zero-order derivative method was found to be economical and reproducible for both drugs. Validation parameters such as accuracy, precision, specificity, and linearity were thoroughly evaluated, demonstrating compliance with ICH and USP requirements. The methods exhibited simplicity, accuracy, and precision, making them suitable for routine laboratory analysis with high levels of accuracy and precision.

The precision of the methods, measured in terms of repeatability, was determined by analysing a sufficient number of aliquots from homogeneous samples, showing satisfactory results. Application of the developed methods for analysing tolvaptan tablet dosage forms revealed no interference from excipients, highlighting their applicability in pharmaceutical analysis. These methods offer advantages over existing ones regarding sensitivity, simplicity, cost-effectiveness, and experimental conditions.

Furthermore, the developed UV-spectrophotometric methods do not entail tedious procedural steps, additional reagents, or prolonged analysis times, requiring only simple instrumentation. Their cost-effectiveness and minimal maintenance make them suitable for application in smallscale industries, ensuring therapeutic efficacy in pharmaceutical formulations.

Efforts were primarily directed towards method development and optimization to enhance final method performance. A well-developed method should be easy to validate and capable of rapidly analyzing preclinical samples, formulations, and commercial samples. Reviewing existing literature on tolvaptan and carvedilol analysis indicated a gap in methods for determination and validation in bulk and pharmaceutical dosage forms. Thus, this study addresses this gap by presenting improved UV spectrophotometric methods for the quantitative analysis of tolvaptan and carvedilol in pharmaceutical formulations. The developed methods demonstrate specificity, linearity, accuracy, precision, and compliance with regulatory requirements, making them suitable for routine analysis in pharmaceutical quality control.

Keywords: UV spectrophotometry, Method development, Validation, ICH guidelines, Analytical method, Quality control

INTRODUCTION:

Sitagliptin is a dipeptidyl-peptidase inhibitor (DPP-4 inhibitor) that has recently been approved for the therapy of type 2 diabetes. Like other DPP-4 inhibitors, its action is mediated by increasing levels of the incretin hormones glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP). Sitagliptin is effective in lowering HbA1c, and fasting as well as postprandial glucose in monotherapy and in combination with other oral antidiabetic agents. It stimulates insulin secretion when hyperglycemia is present and inhibits glucagon secretion. In clinical studies, it is weightneutral. This article gives an overview of the mechanism of action, the pharmacology, and the clinical efficacy and safety of sitagliptin in type 2 diabetes therapy[1].

Utilizing the therapeutic potential of GLP-1 in type 2 diabetes

Since glucagon-like peptide-1 (GLP-1) itself is not feasible for type 2 diabetes therapy due to its very short biological half-life, two major strategies have been developed to utilize the beneficial effects of GLP-1. On the one hand, long-acting, dipeptidyl-peptidase inhibitor (DPP-4 inhibitor)resistant peptides with high similarity to the native GLP-1 can be used as injectable therapeutic agents (incretin mimetics or GLP-1 analogs). Exendin-4, or exenatide in the recombinant form, is such a peptide originally found in the saliva of the Gila monster. Exenatide has a very high amino acid sequence similarity with GLP-1 and is a GLP-1 receptor agonist. It has been approved for type 2 diabetes therapy for patients having insufficient glucose control under therapy with metformin, sulfonylureas, or a combination of both under the trade name Byetta® (Eli Lilly Pharmaceuticals, Indianapolis, IN, USA, and Amylin Pharmaceuticals, San Diego, CA, USA). Liraglutide is a GLP1 analog under development by Novo Nordisk Pharmaceuticals (Copenhagen, Denmark) and is being evaluated for efficacy and safety in type 2 diabetes in clinical studies in phase III.

The other way to utilize GLP-1 effects in type 2 diabetes is the direct inhibition of DPP-4 by orally active substances. Sitagliptin is a highly selective DPP-4 inhibitor that has been approved for type 2 diabetes therapy. Other DPP-4 inhibitors are also in development or close to approval, such as vildagliptin[2].

The pharmacological profile of sitagliptin

Sitagliptin (MK-0431), chemically (2R)-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 has a very high selectivity towards DPP-4, with an $IC_{(50)}$ of 18 nm. There is no affinity towards other DDP enzymes (DPP-8 and DPP-9). It has been approved for the treatment of type 2 diabetes in the USA and Europe. In healthy

volunteers and in patients with type 2 diabetes of different ethnic background, the tolerability of different doses given once or twice daily is good. The main pharmacokinetic parameters (T_{max}, C_{max} and $t_{1/2}$) measured in studies were similar at baseline and in the steady state after longer administration. Steady-state plasma concentrations of sitagliptin are reached after 3 days. Given once daily, the accumulation rate is modest (AUC accumulation ratio [day 10/day 1] range, 1.05–1.29). The elimination and excretion is mainly renal (75% of an oral dose is found in the urine as unchanged drug), and the rest is metabolized via the cytochromes CYP 3A4 and CYP 2C8. Drug–drug interactions were not observed under sitagliptin therapy in clinical studies, and especially no such interactions were found with other antihyperglycemic agents in type 2 diabetic patients. The elimination half-time is 12–14 hours. Doses of 50–200 mg/d sitagliptin administered once daily lead to a ≥80% inhibition of DPP-4 over 24 hours and sitagliptin plasma levels of >100 nm. As a result, the concentrations of biologically active, intact GLP-1 are increased 2–3-fold in the postprandial state. In all the studies performed the safety data are very good and hypoglycaemic episodes or other adverse events did not differ significantly from those observed in the control groups. In monotherapy or in studies investigating a combination with metformin or thiazolidinediones (TZDs), sitagliptin did not cause hypoglycemia[3,4].

Sitagliptin in clinical studies in type 2 diabetes

Sitagliptin improved the glycaemic parameters HbA1c, fasting glucose, and postprandial glucose in clinical studies in patients with type 2 diabetes in monotherapy in doses of 100 mg and 200 mg given once daily in a 24-week study. HbA1c was dose-dependently reduced by 0.79% (100 mg/d) and 0.94% (200 mg/d) as well as fasting glucose (17.1 mg/dL and 21.3 mg/dL, respectively). The postprandial glucose was significantly reduced in a standardized meal-tolerance test (2 h postprandial glucose 46.7 mg/dL and 54.1 mg/dL, respectively). Beta-cell function as determined by HOMA-B, the postprandial insulin- and C-peptide responses, as well as the proinsulin/insulin ratio also improved in type 2 diabetic patients. In other monotherapy studies with durations of 12 or 18 weeks, glycaemic parameters were also improved comparably (Figure 4). In all monotherapy studies, sitagliptin was weight neutral, the 200 mg/d doses even led to a weight reduction of 1.1 kg in the study patients. While the 200 mg dose was more potent than the 100 mg dose in the 24-week study by Ashner, this slight difference in the potencies of 100 mg and 200 mg were not observed in the shorter 18-week study by Raz. This might be due to the differences in study durations as well as the different study populations. The maximal approved dose for sitagliptin is 100 mg daily. The sitagliptin therapy was well tolerated, and the incidence of hypoglycemia or other adverse events was not increased[5,6].

Analytical method validation

Method validation is an integral part of the method development. It is the process by which a method is tested by the developer or user for reliability, accuracy and preciseness of its intended purpose and demonstrating that analytical procedures are suitable for their intended use that they support the identity, quality, purity, and potency of the drug substances and drug products Data thus generated become part of the methods validation package submitted to Center for Drug Evaluation and Research (CDER). Simply, method validation is the process of proving that an analytical method is acceptable for its intended purpose. Analytical monitoring of a pharmaceutical product or of specific ingredients within the product is necessary to ensure its safety efficacy throughout all phases of its shelf life. Such monitoring is in accordance with the specifications elaborated during product development. Analytical method validation is the corner stone of process validation without a proven measurement system it is impossible to confirm whether the manufacturing process has done what it purports to do. All new analytical methods developed are validated[7,8].

Steps followed for validation procedures[9,10]

Proposed protocols or parameters for validations are established

1. Experimental studies are conducted
2. Analytical results are evaluated

3. Statistical evaluation is carried out
4. Report is prepared documenting all the results

The International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use has developed a consensus text on the validation of analytical procedures. The document includes definitions for eight validation characteristics. The parameters as defined by the ICH and by other organizations[11,12]

- ➢ Specificity
- ➢ Selectivity
- ➢ Precision
- ➢ Repeatability
- ➢ Intermediate precision
- ➢ Reproducibili1ty
- ➢ Accuracy
- ➢ Linearity
- ➢ Range
- ➢ Limit of detection
- ➢ Limit of quantitation
- ➢ Robustness
- ➢ Ruggedness

MATERIALS & METHODS[13,14]:

Pure drug samples:
The drug sample of Sitagliptin was received as a gift sample from Aurobindo pharma, Hyderabad, Telangana, India.
Marketed product:
The formulation was purchased from local Apollo pharmacy, Hyderabad, Telangana, India.
Table 01: Chemicals and solvents used

S. No.	Chemicals and solvents	Manufacturer
1	HPLC grade Methanol	Merck
2	HPLC grade Water	Merck
3	HPLC grade Acetonitrile	Merck

METHODOLOGY[15,16] Selection of solvent

A number of trails were done to find out the ideal solvent for dissolving the drug. The solvents such as double distilled water, methanol and acetonitrile were tried based on the solubility of the drug. Sitagliptin was found to be freely soluble in Methanol And Double distilled water, insoluble in Acetonitrile. Methanol was selected as optimized solvent in this spectrophotometric method.
Preparation of standard stock solution
An accurately weighed quantity of Sitagliptin 10 mg was transferred to 50 ml of clean and dried volumetric flask, dissolved in 20 ml, the final volume was made with Methanol, to obtain standard solution having concentration of 1000 µg/ml. 1 ml of this solution was transferred to 10 ml volumetric flask, volume was made with Methanol, it gives 100 µg/ml. These stock solutions were used to prepare further dilutions throughout the experiment.
Selection of wavelength (λ_{max})
Appropriate volume 1 ml of standard stock solution of Sitagliptin was transferred into a 10 ml volumetric flask, diluted to a mark with methanol to give concentration of 10 µg/ml. The resulting solution was scanned in the UV range (200-400 nm).

Analytical method validation developed

The aim of method validation was to confirm that the present method was suitable for its intended purpose as prescribed in ICH guidelines. The method was validated in order to determine the linearity, precision, accuracy, repeatability, ruggedness, LOD and LOQ of the method.

Linearity

Linearity and range different concentrations of Sitagliptin solutions were prepared. The range of the solutions varies from 10% to 60% of standard concentration (µg/ml) of 1 mg. The absorbance of these solutions is noted. The absorbance of the lower-level linearity solution (10%) and the higherlevel linearity solution (60%) in 6 replicates were recorded. The graph of concentration vs absorbance of linearity solutions was plotted.

Repeatability

Repeatability was determined by preparing six replicates of 1 µg/ml of Sitagliptin and the absorbance was measured at 280 nm.

Precision

Precision studies were carried out to ascertain the reproducibility of the proposed method. Intraday precision study was carried out by preparing drug solution of six different concentrations (10, 20, 30, 40, 50, 60 µg/ml of Sitagliptin) and analyzing it at five different times in a day. Interday precision study was carried out by preparing drug solution of six different concentrations (10, 20, 30, 40, 50, 60 µg/ml of Sitagliptin) and analyzing it at three different days.

Accuracy

Accuracy of the proposed method was determined using recovery studies. The recovery studies were carried out by adding different amounts (50%, 100%, and 150%) of the pure drug to the preanalysed formulation. The solutions were prepared in triplicates and the %recovery was calculated. **Limit of Detection and Limit of Quantitation**[17,18,19]

The parameters LOD and LOQ were determined based on the response and slope of the regression equation. The limit of detection (LOD) and the limit of quantitation (LOQ) of the drug was derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$$LOD = 3.3 \times \sigma/S \quad \& \quad LOQ = 10 \times \sigma/S$$

Where,

σ = Standard deviation of the response, and S = Slope of the calibration curve.

Ruggedness studies

Ruggedness studies were performed by preparing three replicates of 1 µg/ml of Sitagliptin, analysed by two different analysts and on two different instruments and the results are reported as %RSD.

Assay for pharmaceutical formulation[20]

The solution was filtered through Whatman filter paper No. 41. 0.5 ml this solution was transferred to 10 ml volumetric flask and the final volume was made with methanol. It gives 0.5 µg/ml. It was scanned on a spectrophotometer in the UV range 200-400 nm. The spectrum was recorded at 269 nm against blank solution of methanol. Determine the amount of % Sitagliptin in tablet according to the following formula:

$$\% \text{ Assay} = \frac{\text{WS X AT X Sample D. F. X Avg. Wt.}}{\text{AS X Standard D. F. X WT X LC}} \text{ X PS}$$

Where,

WS = weight of standard;

WT = weight of sample

AT = Absorbance of tolvaptan in the test solution,

AS = Absorbance of tolvaptan in the standard solution,

Standard D.F. = Standard dilution factor,

Sample D.F. = Sample dilution factor, PS = Purity of working standard [%], LC = Label claim of tolvaptan.

RESULTS AND DISCUSSION ZERO ORDER DERIVATIVE

Validated analytical methods are aimed for the estimation of Sitagliptin in API and its formulation. Simple, precise, rapid, accurate methods were developed for the estimation of Sitagliptin in formulation by UV-spectroscopic method. The validated method was applied for the analysis of tablet containing 250 mg to Sitagliptin drug as the label claim. The method developed was simple. In case of UV-spectroscopic method solubility is the important parameter. Solubility parameter was studied and methanol was selected as the solvent, since it gave a maximum absorbance and a good spectral pattern when compared with other solvents. The marketed formulation was extracted and diluted to get the concentration in the linearity range. The solution was scanned and measured at 267nm. Percentage recovery, linearity, stability studies were also carried out. The above method gave a satisfactory recovery value and found to be stable, linear, hence it can be used for routine analysis of the drug formulation. Solutions of Sitagliptin and its marketed product were prepared by using methanol and UV spectrum of each was recorded by scanning between 200-400nm.

Selection of solvent

An overlain spectrum of Sitagliptin and marketed product was prepared in solvent like methanol. Better absorbance was observed for both the API and formulation when methanol is used as a solvent as shown in the Figure 6. Hence, methanol was selected as solvent for present study.

Absorbance maxima (λ_{max})

The absorbance maximum of sitagliptin was found to be 267 nm. The UV spectrum for sitagliptin is depicted in Figure 1.

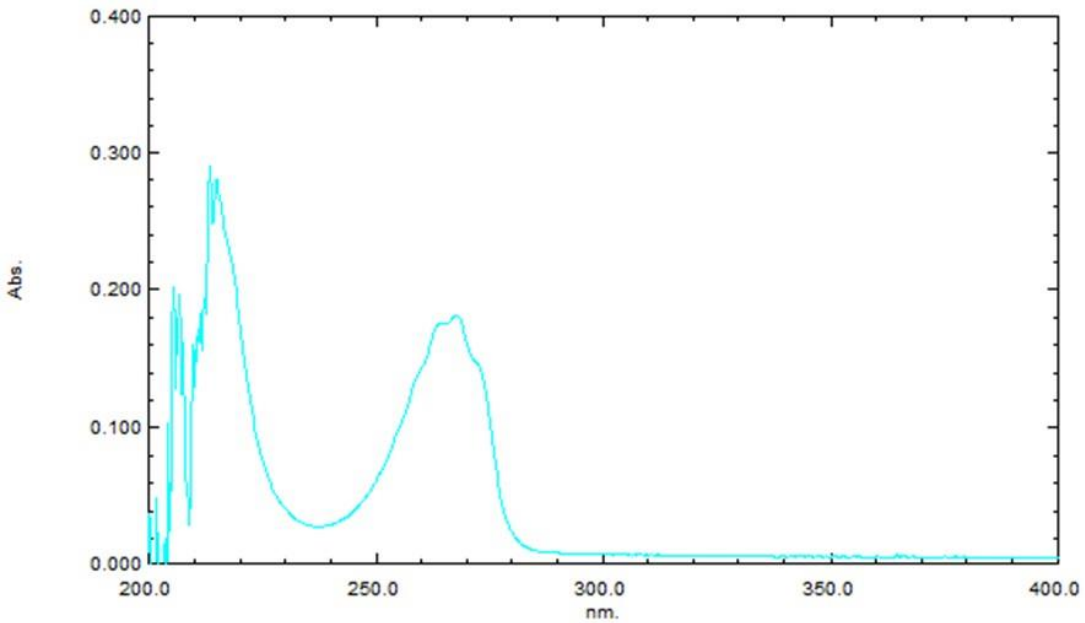


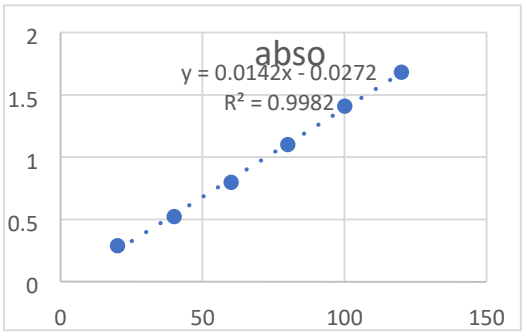
Figure-01: Absorption spectrum of Sitagliptin in methanol (zero order derivative) Method validation

The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experimental work.

Linearity

Standard solutions of sitagliptin in the concentration range of 10 to 60% were observed in UV Spectroscopy.

A graph of absorbance (on Y-axis) versus concentration (on X-axis) was plotted and calibration graph was shown in Figure 2. The regression equation was found to be Y=0.148x+0.075, Correlation coefficient was 0.9998.



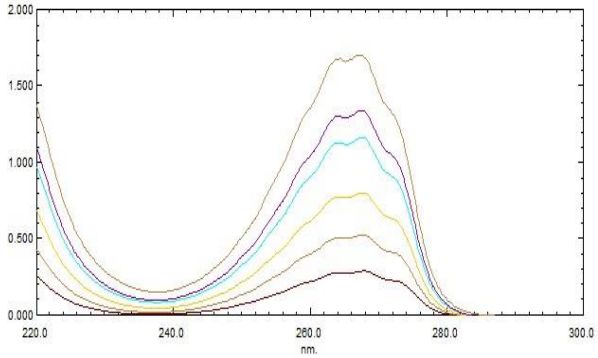


Figure-02: The graph of concentration vs absorbance of linearity solutions Linearity overlay Repeatability

Repeatability was determined by analyzing 1 µg/ml concentration of sitagliptin. for six times with %RSD < 2 which is illustrated in Table 2.

Table 2: Repeatability studies

Conc. in µg/ml	Absorbance at 267 nm	Absorbance Mean	SD	%RSD
10	0.171	0.172		
10	0.172			
10	0.171		0.001414	**0.008222**
10	O.172			
10	0.174			
10	0.172			

Precision

The precision of the developed method was expressed in terms of % relative standard deviation (%RSD). These results show reproducibility of the assay. The %RSD values found to be less than 2 that indicate this method precise for the determination of the pure form. The inter and intraday precision results were mentioned in Table 3 and 4 respectively.

Table 3: Intraday precision

Conc. in µg /ml	Inter day Precision S.D. ± Absorbance mean (n=3)	%RSD
10	0.001414 ± 0.172	0.008222
20	0.002±0.306	0.006536
30	0.002408±0.4546	0.005297
40	0.003493±1.0062	0.003471
50	0.016423±0.4962	0.033098
60	0.003647±1.0774	0.003385

Table 4: Inter day precision

Conc. in µg/ml	Inter day Precision S.D. ±Absorbance mean(n=3)	%RSD
10	0.007759±0.0552	0.140562
20	0.005891±0.1912	0.03081
30	0.005099±0.392	0.013007
40	0.005244±0.498	0.01053
50	0.009813±0.5896	0.016643
60	0.007021±0.6806	0.010315

Accuracy

Accuracy shall be determined by performing recovery studies at 3 levels in which a known amount of analyte shall be added and recovery shall be carried out in three replicates of each concentration

level and the % recovery was calculated. The mean recovery was found between 100-101% and %RSD between 0.7-1.0. The results are shown in Table 5.

Table 5: Recovery studies

Spiked level (%)	Formulation conc. (µg/ml)	Pure drug conc. (µg/ml)	Amount conc. recovered (µg/ml)	% Recovery	% Mean recovery SD	%RSD
50	10	5	14.98	99.86	99.88 ± 0.040415	**0.040**
	10	5	14.99	99.93		
	10	5	14.98	99.86		
100	10	10	99.98	99.98	99.98 ± 0.005774	**0.0057**
	10	10	99.99	99.99		
	10	10	99.99	99.99		
150	10	15	149.98	99.98	99.98 ± 0.005774	**0.0057**
	10	15	149.99	99.99		
	10	15	149.99	99.99		

Accuracy overlay

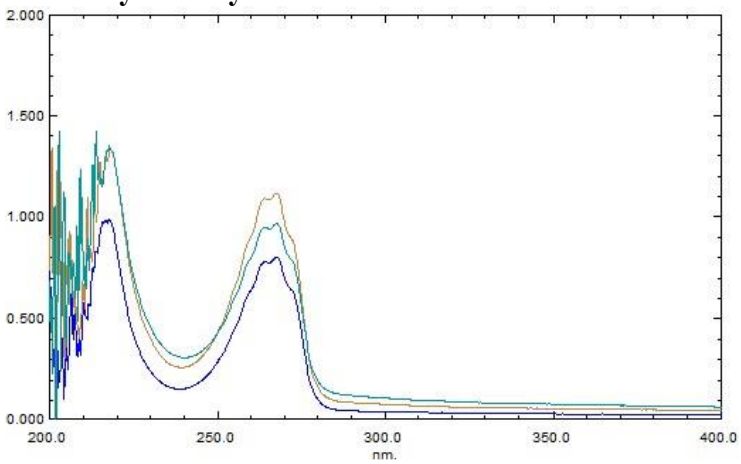


Figure-03: Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation. LOD and LOQ values are 0.361 and 1.00283 µg/ml respectively. The results are illustrated in Table 6.

Table 6: LOD and LOQ

Drug	LOD	LOQ
Sitagliptin	0.361 µg/ml	1.00283 µg/ml

Ruggedness studies

This study was performed by analysing 4 µg/ml of tolvaptan by two different analysts and on two instruments, results of the study were given in Table 7 and %RSD obtained was less than two which is within the acceptance limits.

Table 7: Ruggedness of Sitagliptin.

Parameter	Conc. (µg/ml)	Absorbance	Absorbance Mean ± S.D. (n=3)	%RSD
Different Analyst	10	0.115	0.116± 0.001528	**0.013**
		0.118		
		0.117		
Different instrument	10	0.114	0.118±0.0036	**0.030**
		0.119		
		0.121		

Assay for pharmaceutical formulation

The percentage recovery for istavel tablet formulation was found to be 99.6-101.06 % enlisted in Table 8. The results for assay are within acceptable limit.

Table 8: Assay of istavel tablets

Label claim (mg)	Amount found (mg)	% Purity	Mean % purity ± SD (n=3)	%RSD
50	50.14	100.02	100.22±0.177764	**0.177**
50	50.05	100.10		
50	50.18	100.36		

Table 9: Summary of validated parameters

Parameters	Method
λmax	267 nm
Beers law limit	10-60 µg/ml
Correlation coefficient (r^2)	0.999
Molar absorptivity	$3.41x10^4$ L mol^{-1} cm^{-2}
Regression equation (y=mx+c)	Y=0.0141x-0.0599
Slope (m)	0.0141
Intercept (c)	0.0599
Accuracy	99.24-100.24
Precision	0.339-1.015
LOD	0.361 µg/ml
LOQ	1.00283 µg/ml

The proposed UV analytical method for the quantification of tolvaptan in API and tablet formulation is simple, accurate, and rapid and can be employed for the routine analysis. Once the absorbance of the sample is determined, it requires only simple calculation. This method can be applied for the substances which obey Beer's law. The low standard deviation and good percentage recovery indicated the reproducibility and accuracy of the method.

B. FIRST ORDER DERIVATIVE

Validated analytical methods are aimed for the estimation of Sitagliptin in API and its formulation. Simple, precise, rapid, accurate methods were developed for the estimation of Sitagliptin in formulation by UV-spectroscopic method. The validated method was applied for the analysis of tablet containing 250 mg to Sitagliptin drug as the label claim. The method developed was simple. In case of UV-spectroscopic method solubility is the important parameter. Solubility parameter was studied and methanol was selected as the solvent, since it gave a maximum absorbance and a good spectral pattern when compared with other solvents. The marketed formulation was extracted and diluted to get the concentration in the linearity range. The solution was scanned and measured at 267nm. Percentage recovery, linearity, stability studies were also carried out. The above method gave a satisfactory recovery value and found to be stable, linear, hence it can be used for routine analysis of the drug formulation. Solutions of Sitagliptin and its marketed product were prepared by using methanol and UV spectrum of each was recorded by scanning between 200-400nm.

Selection of solvent

An overlain spectrum of Sitagliptin and marketed product was prepared in solvent like methanol. Better absorbance was observed for both the API and formulation when methanol is used as a solvent as shown in the Figure 6. Hence, methanol was selected as solvent for present study.

concentration	Absorbance
10	0.088
20	0.225
30	0.370
40	0.502
50	0.675
60	0.800

Absorbance maxima (λ_{max}) Table- 10: UV Spectrum

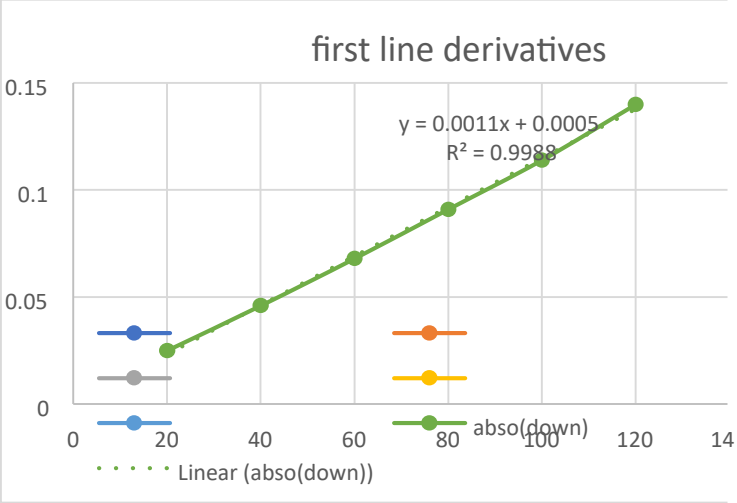


Figure-04: First line derivatives Table 11: Intraday precision

The absorbance maximum of sitagliptin was found to be 267 nm. The UV spectrum for sitagliptin.

Conc. in µg/ml	Intraday Precision S.D. ± Absorbance mean (n=3)	%RSD
10	0.001441 ± 0.178	0.00835
20	0.0025±0.312	0.00654
30	0.00250±0.4564	0.00547
40	0.00350±1.057	0.003574
50	0.0178±0.5014	0.03412
60	0.00399±1.0802	0.003487

Table 12: Inter day precision

Conc. in µg/ml	Inter day Precision S.D. ±Absorbance mean(n=3)	%RSD
10	0.007761±0.0555	0.140572
20	0.005122±0.1915	0.03092
30	0.005128±0.3931	0.013081
40	0.005248±0.501	0.01063
50	0.009817±0.590	0.016651
60	0.007050±0.6812	0.010321

Limit of Detection (LOD) and Limit of Quantitation (LOQ) Table 13: LOD and LOQ

Drug	LOD	LOQ
Sitagliptin	0.361 µg/ml	1.00283 µg/ml

Accuracy Table 13: Recovery studies

Spiked level (%)	Formulation conc. (µg/ml)	Pure drug conc. (µg/ml)	Amount conc. recovered (µg/ml)	% Recovery	% Mean recovery SD	%RSD
50	10	5	14.99	99.85	99.89 ±0.040418	**0.062**
	10	5	14.99	99.92		
	10	5	14.98	99.87		
100	10	10	99.96	99.96	99.98 ±0.005781	**0.0067**
	10	10	99.98	99.99		
	10	10	99.99	99.99		
150	10	15	149.98	99.98	99.98 ±0.005774	**0.0062**
	10	15	149.99	99.99		
	10	15	149.99	99.99		

SUMMARY AND CONCLUSION:

From present research work, it is concluded that the Zero order derivative and is economical and reproducible. The method was developed and validated as per ICH Q2 (R1) guidelines. The proposed methods can be employed for routine analysis of tolvaptan from pharmaceutical dosage form. The results obtained on the validation parameters met ICH and USP requirements. It is inferred that the methods were found to be simple, accurate, precise and linear. The methods were found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision. The precision was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogeneous sample. The results showed that the recovery of marketed product by the proposed method was satisfactory.

Application of this method for the analysis of tolvaptan tablet dosage forms showed that there was no interference of excipients in the determination. The method is advantageous over most of the reported methods in-terms of sensitivity, simplicity, cost-effectiveness and experimental conditions. The method does not involve any tedious procedural steps; do not require any extra reagents or longer analysis time and a very simple instrument are required. The method can be used to determine the purity of the drug available from various sources. Because of cost-effective and minimal maintenance, the present UV-Spectrophotometric methods can be preferred at small scale industries and successfully applied and suggested for the quantitative analysis of tolvaptan in pharmaceutical formulations for QC, where economy and time are essential and to assure therapeutic efficacy. To develop an effective method, most of the effect should be spent in method development and optimization as this will improve the final method performance. A well-developed method should be easy to validate. A method should be developed to analyse rapidly, the preclinical samples, formulations, and commercial samples. A review of the literature on drug strongly indicates that there are few methods available for the determination and validation of tolvaptan in bulk and pharmaceutical dosage forms. Keeping in this mind, we developed a method for the determination and validation of tolvaptan in bulk and pharmaceutical dosage forms by UV spectroscopic method with some improvements than the existing methods. The analytical procedure described for the assay was specific, linear, precise, accurate, and system-suitable for the determination of carvedilol in bulk and pharmaceutical dosage forms. The observations of the validation parameters such as accuracy, precision, specificity, and linearity, show that the developed methods can be employed for routine analysis of bulk and tablet forms of carvedilol. The result obtained from the validation parameters met the ICH and USP requirements as well as obeys BEER'S law.

REFERENCES:

1. Aqeela Raza et al, Pak. J. Pharm. Sci., Vol.35, No.1, January 2022, pp.015-021. doi.org/10.36721/PJPS.2022.35.1

2. Teragawa et al 2022 Effect of Anagliptin versus Sitagliptin on Renal Function: Subanalyzes from the REASON TrialDOI:10.2147/DMSO.S350518.
3. Pamu et al.; JPRI, 33(42B): 92-101, 2021; Article no. JPRI.72886
4. Journal of Innovation in Pharmaceutical Sciences (2019); 3(1)
5. Xia Wang et al (2018) Dipeptidyl peptidase-4 (DPP-4) inhibitors constitute a novel class of anti-diabetic agents. https://doi.org/10.1007/s10238-018-0519-0
6. Sudhir Adsul et al /International Journal of ChemTech Research, 2018
7. E., Sai & E, Sravya & Dodda, Sireesha & Bakshi, Vasudha. (2017). Development and Validation of RP-HPLC Method for the Estimation of Sitagliptin Phosphate in Tablet Dosage Form. International Journal of Applied Pharmaceutical Sciences and Research. 2.10.21477/ijapsr.v 2i3.8099.
8. Scott LJ. Sitagliptin: A Review in Type 2 Diabetes. Drugs. 2017 Feb;77(2):209-224. doi:10.1007/s40265-016-0686-9. PMID: 28078647.
9. Deacon CF, Lebovitz HE. Comparative review of dipeptidyl peptidase-4 inhibitors and sulphonylureas. Diabetes Obes Metab. 2016 Apr;18(4):333-47. doi: 10.1111/dom.12610. Epub 2016 Jan 8. PMID: 26597596
10. Plosker GL. Sitagliptin: a review of its use in patients with type 2 diabetes mellitus. Drugs. 2014 Feb;74(2):223-42. doi: 10.1007/s40265-013-0169-1. PMID: 24407560.
11. Vani, R. et al. "Analytical method development and validation for the determination of sitagliptin and metformin using reverse phase hplc method in bulk and tablet dosage form." (2014).
12. Prasad, P, Satyanarayana, K. and Krishnamohan, G. (2014) Development and Validation of a Method for Simultaneous Determination of Metformin Hydrochloride and Sitagliptin Phosphate in a Formulation by RP-HPLC. *American Journal of Analytical Chemistry*, 5, 737742. doi: 10.4236/ajac.2014.511082.
13. J App Pharm Vol. 6; Issue 3: 327-338; July, 2014 Vinit et al, 2014
14. Chwieduk CM. Sitagliptin/metformin fixed-dose combination: in patients with type 2 diabetes mellitus. Drugs. 2011 Feb 12;71(3):349-61. doi: 10.2165/11206060-00000000000000. PMID: 21319871.
15. Seyoum B. Sitagliptin and metformin--novel combination therapy. Expert Opin Pharmacother. 2011 Mar;12(4):641-6. doi: 10.1517/14656566.2011.556113. Epub 2011 Feb 4. PMID: 21291346.
16. Choy M, Lam S. Sitagliptin: a novel drug for the treatment of type 2 diabetes. Cardiol Rev. 2007 Sep-Oct;15(5):264-71. doi: 10.1097/CRD.0b013e318123f771. PMID:17700385
17. M Akiful Haque, Boggula Narender, Cherukuri Sravanthi, Burugu Rakshanda Goud, Bukka Sony, Routhu Deepshika, Vasudha Bakshi. Development and Validation of Zero and First Order Spectrophotometric Method for Determination of Levomilnacipran in Bulk and Formulation. J. Pharm. Sci. & Res. 2020; 12(3):443-447.
18. Martindale: The complete drug reference. 36th ed. Pharmaceutical press, Lambeth High Street, London. 2009; p. 773-774.
19. Skoog D.R., Hollar F.J., Nieman 2005 T.A. Principles of Instrumental Analysis. 5th edition. Thomson Brooks/Cole; 1-3,300,726.
20. Jeffery GH, Bassat J, Mendham J. Denny RC. 1989: Vogels Textbook of Qualitative Chemical Analysis. 5th edition. Atkin. ELB Swithlongman Publication. Harlow; 3.