ORIGINAL ARTICLE

Development and validation of ultraviolet spectrophotometric method for the simultaneous estimation of sitagliptin phosphate and dapagliflozin in bulk and marketed formulation

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Abstract

Introduction: The necessity for trustworthy analytical techniques for combination therapy quality control has increased because of the increase in the incidence of type 2 diabetes mellitus. Sitagliptin phosphate and dapagliflozin, used in combination for glycemic control, require a validated method for their simultaneous quantification in pharmaceutical formulations. This work attempts to establish a simple, precise, and accurate ultraviolet (UV) spectrophotometric approach. **Materials and Methods:** UV spectrophotometric testing was done at 266 nm for Sita and 229 nm for Dapa. The technique was validated following the International Conference on Harmonisation Q2 (R1) guideline, and various parameters were checked, including linearity, precision, accuracy, limit of detection, limit of quantitation, ruggedness, and robustness. **Results:** Excellent linearity was demonstrated by the UV spectrophotometric technique with coefficients of correlation (R2) for >0.999 for both drugs. **Conclusion:** The newly developed UV method is simple, sensitive, and reliable for simultaneously estimating sitagliptin phosphate and dapagliflozin in bulk and commercial formulations.

Key words: Dapagliflozin, linearity, method validation, sitagliptin phosphate, ultraviolet spectrophotometry

INTRODUCTION

Diabetes mellitus, particularly type 2 diabetes mellitus (T2DM), is a serious worldwide health concern defined by chronic hyperglycemia brought on by a combination of resistance to insulin and impaired insulin secretion. The long-term consequences of uncontrolled diabetes include microvascular and macrovascular complications such as cardiovascular disorders, neuropathy, retinopathy, and nephropathy.[1] To address the multifactorial nature of T2DM, fixed-dose combination therapies have gained significant clinical importance, as they enhance therapeutic efficacy, reduce the pill burden, improve patient adherence, and target different physiological pathways. Sitagliptin phosphate and dapagliflozin are two such drugs commonly used in combination for the effective management of T2DM.[2]

Sitagliptin phosphate (Figure 1) is a strong, selective, and orally active inhibitor of the dipeptidyl peptidase-4 (DPP-4) enzyme. By preventing DPP-4, Sita increases the endogenous concentrations of incretin hormones, especially glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1.[3] In a glucose-dependent fashion, hormones increase the production of insulin and suppress the release of glucagon. Hence, regulating postprandial and fasting blood glucose levels. Sitagliptin exhibits a favorable pharmacokinetic profile with good oral bioavailability (~87%), minimal hepatic metabolism, and a last half-life of approximately 12.4 h, which supports daily dosage. It is primarily excreted unaltered through the kidneys, making dose adjustments necessary in those suffering from

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renal impairment. Clinically, sitagliptin is well tolerated with a low risk of hypoglycemia and is considered weight-neutral, making it suitable for a broad spectrum of diabetic patients. Sitagliptin is often included in fixed-dose combinations to enhance glycemic control and improve therapeutic outcomes when used alongside drugs with complementary mechanisms.[4]

Dapagliflozin (Figure 2), belonging to the class of inhibitors of sodium-glucose co-transporter 2 (SGLT2), offers a unique insulin-independent mode of action. It selectively prevents the proximal renal tubules from expressing SGLT2, thereby decreasing the filtered glucose's reabsorption and promoting its removal through urine (glycosuria).[5] This not only lowers blood glucose levels but also contributes to reducing body weight and a decrease in systolic blood pressure. Dapagliflozin has an oral bioavailability of approximately 78% and a half-life of 12–13 h, supporting once-daily administration.[6] It is metabolized primarily via glucuronidation and is excreted through both urine and feces. In addition to glycemic control, dapagliflozin has shown significant cardio-renal protective effects in clinical trials, leading to its use in heart patients as well as long-term renal dysfunction. Due to its complementary pharmacodynamic profile, it is frequently used alongside other antidiabetic medications, including DPP-4 inhibitors such as sitagliptin, to achieve comprehensive management of T2DM.[7]

Given the increasing use of sitagliptin and dapagliflozin in fixed-dose combination therapies, there is a pressing need for efficient and validated analytical techniques for their simultaneous estimation in dosage forms for pharmaceuticals. Accurate quantification of both drugs is essential during formulation development, regular testing for stability, and quality control.[8] Ultraviolet (UV) spectrophotometry is a commonly used analytical method due to precision, sensitivity, and repeatability.[9]

The present study focuses on the development and validation of a novel UV spectrophotometric technique for the simultaneous estimation of sitagliptin phosphate and Dapagliflozin in bulk drugs and marketed formulations. Method validation was carried out in accordance with International Conference on Harmonisation (ICH) guidelines, assessing critical parameters such as accuracy, linearity, precision, specificity, limit of detection (LOD), and limit of quantitation (LOQ). These validated analytical tools are expected to offer reliable and reproducible results for routine quality assurance, regulatory compliance, and stability testing of combination antidiabetic formulations.

MATERIALS AND METHODS

Sitagliptin phosphate and dapagliflozin (analytical grade) were obtained as gift samples from Wockhardt Research Centre. Chh. Sambhajinagar, Maharashtra, India. Marketed combination tablets containing sitagliptin (100 mg) and dapagliflozin (10 mg) were bought from a local pharmacy in Chh. Sambhajinagar, Maharashtra. High-performance liquid chromatography (HPLC)-grade MeOH, ACN were purchased from Merck Life Science Pvt. Ltd., whereas H_3PO_4, NaOH, HCl, and H_2O_2 (AR grade) were sourced from Standard Deviation (SD) Fine-Chem Ltd., Mumbai. A 0.45 µm nylon membrane filter (Millipore) was used for filtration.

Instrumentation included a Shimadzu UV-1,800 spectrophotometer, and analytical procedures were supported by a Shimadzu digital balance and an Eutech pH meter. Stock solutions were processed in methanol and stored under specified conditions for use in method development and validation.

UV spectroscopy and calibration

UV analysis was done using a Lasany LI-2702 spectrophotometer to determine λmax. Standard solutions of drugs were prepared in phosphate buffer (pH 5.8): Acetonitrile (60:40), sonicated, and filtered. Calibration curves were obtained for sitagliptin (20–100 µg/mL) and dapagliflozin (10–50 µg/mL). An Elder digital balance and Pramaultrasonicator were used.[10,11]

UV method development

Solubility testing was performed using various solvents, and phosphate buffer (pH 5.8): ACN (60:40) was selected as the solvent for two drugs due to good solubility and stability. Standard dilutions were prepared for sitagliptin phosphate (20–100 µg/mL) and dapagliflozin (10–50 µg/mL) using the selected solvent for spectral analysis.[12]

Method validation

As required by ICH criteria, the approach was validated. Linearity was confirmed using five concentrations for each drug. LOD and LOQ were calculated from the SD and slope of that calibration curve. Accuracy was checked at 80%, 100%, and 120% levels through recovery studies. Precision was assessed by intra- and inter-day analysis at three concentrations. Robustness was evaluated using different solvent systems, and ruggedness was tested by analyzing samples at varying temperatures to ensure reliability.[13,14]

UV analysis of marketed formulation (Janusmart D 100/10)

The marketed tablet containing 100 mg sitagliptin phosphate and 10 mg dapagliflozin was analyzed by UV and the method. The powdered tablet was dissolved in the selected solvent mixture (phosphate buffer pH 5.8: ACN 60:40), sonicated, and diluted to prepare stock solutions (1,000 µg/mL).

Table 1: Evaluation data for the accuracy test of sitagliptin phosphate and dapagliflozin

Conc. (%)	Sitagliptin phosphate			Mean % recovery	Percentage RSD
	Origin level (µg/mL)	Amount added (µg/mL)	Percentage recovery		
80	20	16	100.49	101.149	0.744
80	20	20	101.97		
80	20	24	100.99		
100	60	48	99.51	99.836	0.432
100	60	60	99.67		
100	60	72	100.33		
120	100	80	99.90	99.840	0.204
120	100	100	100.00		
120	100	120	99.61		
Concentration (%)	**Dapagliflozin**			**Mean % recovery**	**Percentage RSD**
	Origin level (µg/mL)	**Amount added (µg/mL)**	**Percentage recovery**		
80	10	8	98.78	99.390	1.623
80	10	10	98.17		
80	10	12	101.22		
100	30	24	100.18	99.582	0.748
100	30	30	99.82		
100	30	36	98.75		
120	50	40	100.53	**100.53**	**0.423**
120	50	50	100.11		
120	50	60	100.96		

Table 2: Evaluation data for the intra-day and inter-day study of sitagliptin phosphate

Intra-day	Morning			Afternoon			Evening		
Concentration range (µg/mL)	Mean	Percentage assay	Percentage RSD	Mean	Percentage assay	Percentage RSD	Mean	Percentage assay	Percentage RSD
20	0.208	98.557	1.442	0.211	99.052	0.947	0.208	103.333	1.542
60	0.618	99.460	0.494	0.617	100.81	0.706	0.618	100.054	1.053
100	1.025	99.382	0.587	1.018	99.967	0.442	1.019	99.673	0.408
Inter-day	**Day 1**			**Day 2**			**Day 3**		
Concentration range (µg/mL)	Mean	Percentage assay	Percentage RSD	Mean	Percentage assay	Percentage RSD	Mean	Percentage assay	Percentage RSD
20	0.211	100.158	1.194	0.209	98.086	1.725	0.214	100.00	1.401
60	0.613	100.871	0.771	0.613	100.816	0.815	0.632	100.633	1.525
100	1.016	99.873	0.492	1.018	100.393	0.450	1.007	99.337	0.606

Aliquots were further diluted to 100 µg/mL (sitagliptin) and 10 µg/mL (Dapa), and these absorbance values were compared with standards to calculate % assay.[15,16]

UV analysis

Determination of λmaxfor sitagliptin phosphate and dapagliflozin

The λmax of sitagliptin phosphate (266 nm) and dapagliflozin (229 nm) was determined using a UV-visible spectrophotometer. Standard solutions (10 µg/mL) were prepared in a phosphate buffer (pH 5.8):ACN (60:40) and scanned between 200 and 400 nm.[17,18] These λmax values can be used for RP-HPLC method development to ensure accurate drug quantification. Refer Figure 3 and Figure 4 respectively.

Development of a standard curve for the sitagliptin phosphate and dapagliflozin

Standard calibration curves were optimized for both sitagliptin phosphate and dapagliflozin by plotting concentration against

Table 3: Evaluation data for the intra-day and inter-day study of dapagliflozin

Intra-day	Morning			Afternoon			Evening		
Concentration range (µg/mL)	Mean	Percentage assay	Percentage RSD	Mean	Percentage assay	Percentage RSD	Mean	Percentage assay	Percentage RSD
10	0.167	99.00	1.249	0.164	100.610	1.613	0.165	101.212	1.603
30	0.559	99.761	0.273	0.559	100.06	0.273	0.562	98.932	0.990
50	0.947	99.437	0.644	0.950	99.298	0.643	0.957	99.547	0.612
Inter-day	**Day 1**			**Day 2**			**Day 3**		
Concentration range (µg/mL)	Mean	Percentage assay	Percentage RSD	Mean	Percentage assay	Percentage RSD	Mean	Percentage assay	Percentage RSD
10	0.159	101.674	1.579	0.157	101.489	1.606	0.162	101.027	0.940
30	0.562	100.059	0.802	0.556	99.100	1.258	0.559	98.985	1.319
50	0.950	100.386	0.425	0.958	100.661	0.629	0.948	99.437	0.717

Table 4: Evaluation data for robustness of sitagliptin phosphate and dapagliflozin

Sitagliptin phosphate			
Concentration (µg/mL)	Solvents	Absorbance	Percentage RSD
80	Phosphate buffer (pH 5.8):Methanol (50:50)	0.833	0.661
80	Phosphate buffer (pH 5.8):Acetonitrile (60:40)	0.826	0.370
80	Phosphate buffer (pH 5.8):Acetonitrile: Methanol (40:30:30)	0.841	0.182
Dapagliflozin			
Concentration (µg/mL)	Solvents	Absorbance	Percentage RSD
40	Phosphate buffer (pH 5.8):Methanol (50:50)	0.719	0.579
40	Phosphate buffer (pH 5.8):Acetonitrile (60:40)	0.731	0.285
40	Phosphate buffer (pH 5.8):Acetonitrile: Methanol (40:30:30)	0.736	0.549

Table 5: Evaluation data for ruggedness of sitagliptin phosphate and dapagliflozin

Sitagliptin phosphate			
Concentration (µg/mL)	Temp (°C)	Absorbance	Percentage RSD
80	25	0.837	0.431
80	37	0.841	0.299
80	60	0.828	0.320
Dapagliflozin			
Concentration (µg/mL)	Temp (°C)	Absorbance	Percentage RSD
40	25	0.726	0.860
40	37	0.739	0.406
40	60	0.766	0.494

absorbance. Sitagliptin Phosphate showed a linear with a regression equation y = 0.0103x − 0.0036 and R^2 = 0.9993. Similarly, Dapagliflozin exhibited equation of regression y = 0.0193x − 0.0282 with an R^2 = 0.9991, indicating acceptable linearity for both drugs.[19,20]

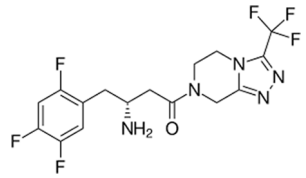

Figure 1: Structure of sitagliptin

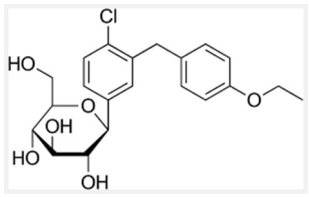

Figure 2: Structure of dapagliflozin

Method validation for UV method development

Linearity

Linearity was established for Sitagliptin Phosphate in the range of 20–100 µg/mL, with a regression equation y =

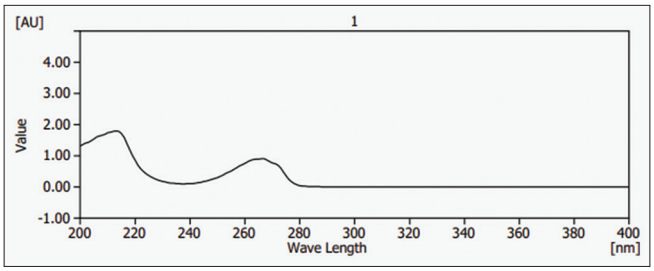

Figure 3: Maximum wavelength detection of sitagliptin phosphate

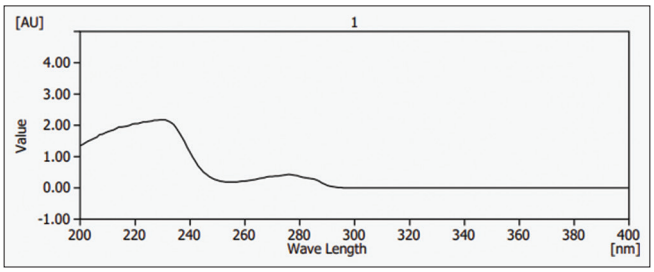

Figure 4: Maximum wavelength detection of dapagliflozin

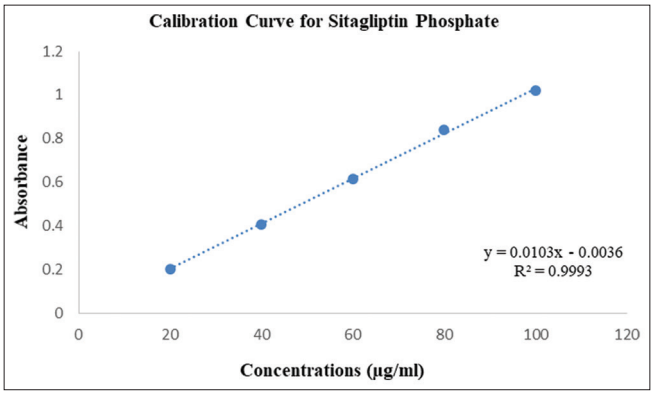

Figure 5: Standard curve for sitagliptin phosphate

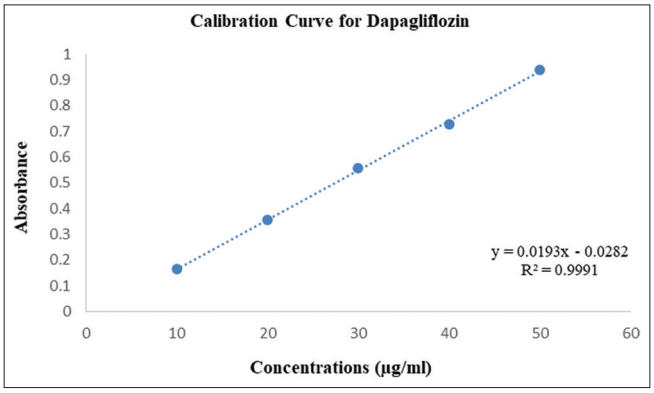

Figure 6: Standard curve for dapagliflozin

0.0103x − 0.0036 and a correlation coefficient of 0.9993. For Dapagliflozin, linearity were observed in the range of 10–50 µg/mL, with the equation y = 0.0193x − 0.0282 and an R^2 of 0.9991, indicating excellent linearity for both drugs. Refer Figure 5 and Figure 6 respectively.

Accuracy

The standard addition technique was used to measure accuracy at three different concentration levels. The percentage recovery for two drugs was close to 100%, confirming the high accuracy of the developed UV spectrophotometric technique refer Table 1.

Precision

Intra-day and inter-day precision were assessed at 3 concentration levels for individual drugs: 20, 60, and 100 µg/mL for sitagliptin phosphate and 10, 30, and 50 µg/mL for dapagliflozin. The mean absorbance, % assay, and % RSD were calculated, confirming the method's reproducibility and consistency refer Tables 2 and 3.

Robustness

The robustness research was assessed using three different solvents. The % RSD results are <2%, indicating that the approach was proven to be robust refer Table 4.

Ruggedness

The study of the drug's ruggedness was conducted at three distinct temperature ranges. The results showed that the approach was rugged, with a % RSD value <2% refer Table 5.

LOD and LOQ

The LOD and LOQ values for sitagliptin phosphate and dapagliflozin were discovered at the submicrogram level, indicating the high sensitivity of the developed UV method. For sitagliptin phosphate, the LOD was 0.211 µg/mL and LOQ was 1.389 µg/mL, whereas for dapagliflozin, the LOD was 0.457 µg/mL and LOQ was 1.354 µg/mL.

CONCLUSION

A UV spectrophotometric technique for the simultaneous estimation of dapagliflozin and sitagliptin phosphate in bulk and commercial formulations was successfully developed and validated. The technique showed rich linearity, accuracy, precision, sensitivity, and robustness. The techniques' applicability for regular quality control in pharmaceutical analysis was confirmed by their successful use on commercially available formulations.

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