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Development and validation of an RP-HPLC method for simultaneous determination of sitagliptin and valsartan

Muhammad Waqas[1], Muhammad Zaman[1]*, Huma Hameed[1], Waqar Siddique[2], Ahmad Salawi[3], Yosif Almoshari[3] and Jawaher Abdullah Alamoudi[4]

[1]Faculty of Pharmaceutical Sciences, University of Central Punjab, Lahore, Pakistan
[2]Riphah Institute of Pharmaceutical Sciences (RIPS), Riphah International University (RIU), Lahore Campus, Lahore, Pakistan.
[3]Department of Pharmaceutics, College of Pharmacy, Jazan University, Jazan, Saudi Arabia
[4]Department of Pharmaceutical Sciences, College of Pharmacy, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh, Saudi Arabia

Abstract: **Background**: Diabetes mellitus and hypertension are commonly coexisting diseases and combination therapies are required to improve therapeutic outcomes and patient compliance. **Objectives**: Current research aims to develop and validate a precise reverse-phase High-Performance Liquid Chromatography (RP-HPLC) method for the concurrent determination of sitagliptin and valsartan. **Methods**: For analysis, an Agilent 1260 Infinity II HPLC system provided with a C-18 column was used. The method was validated as per the ICH guidelines. **Results**: Calibration curves confirmed excellent linearity for sitagliptin and valsartan with R^2 values exceeding 0.99. The limits of detection (LOD) and quantification (LOQ) were calculated as 8.5 ppm and 25.7 ppm for sitagliptin and 7.8 ppm and 23.5 ppm for valsartan, respectively. Recovery studies and robustness confirmed method accuracy and indicated no significant impact from small variations in chromatographic parameters. **Conclusion**: In conclusion, this validated RP-HPLC method is consistent and efficient for compliance with pharmaceutical standards.

Keywords: Calibration; Diabetes; Hypertension; RP-HPLC; Validation

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INTRODUCTION

Hypertension (HT) and diabetes mellitus (DM) are two conditions that frequently coexist. In 2000, an estimated 171 million people had diabetes and by 2030, this number is expected to exceed 366 million. HT is also a leading cause of death worldwide. It is marked by a consistent increase in blood pressure (BP) beyond the normal range of 120/80 mmHg (Tannor *et al*., 2022; Yan *et al*., 2017; Tayefi *et al*., 2017). For individuals with DM, HT raises the risk of developing or worsening various health issues (Song *et al*., 2020; Drummond *et al*., 2019).

Sitagliptin is an orally administered potent anti-hyperglycemic drug that belongs to the gliptin group. Initially, the optimization of the β-amino acid class was discovered, which has a molecular weight of 407.31 g/mol. Sitagliptin can be utilized to treat type 2 diabetes as an individual drug or in conjunction with other hypoglycemic drugs (Bakkar *et al*., 2021).

Valsartan binds to the AT1 receptor and affects the renin-angiotensin-aldosterone system (RAAS) by opposing the action of angiotensin II. Clinical studies have shown that valsartan may be used alone or in combination with other anti-hypertensive drugs to improve morbidity and mortality rates. It is completely absorbed after oral administration. Valsartan has a molecular weight of 435.5 g/mol (Abdullah and Rusli, 2020).

Combination therapy has some benefits over monotherapy, like reducing the likelihood of dose-dependent undesirable effects. The inclusion of a single agent could moderate certain negative consequences of another and the use of minimal amounts of two different agents decreases the therapeutic and metabolic complications that take place with the highest dosages of individual drugs of the entire tablet (Delou *et al*., 2019). The oral route is still considered a flexible and patent-friendly route of administration. Bi layer tablet (BLT) is the most advantageous dosage form for delivering two drugs simultaneously, in which one layer acts as immediate while the other behaves as sustained release (Panda, Mishra *et al*., 2021). Antibiotics, antihypertensives, diabetics and analgesics were considered to be the most favorable entities to be formulated as BLT, as these drugs are mostly effective in combination therapy (Nikita *et al*., 2022). The ultimate rationale of formulating BLT is controlling the release rate of one or more drugs, to give the patient a synergistic effect and modified release can be achieved either by erodible or swellable barriers (Panda *et al*., 2021).

One of the widely used techniques for the identification, quantification and separation of complex compounds is HPLC. In analytical processing, the most critical step is the determination of selectivity, accuracy and sensitivity of the

**Corresponding author:* e-mail: m.zaman2157@gmail.com

analysis. The optimization of parameters, which includes column, wavelength, mobile phase and stationary phase, plays a crucial role in selecting a suitable method for determination. A developed method must have low detection limits, reproducibility and high resolution. The most common challenges in method development are the selection of the stationary phase, degradation of the column and analysis of chiral compounds (Bhalerao *et al*., 2023).

In the current study, sitagliptin was combined with valsartan to enhance patient compliance. The major goal of this research was to develop and validate an HPLC method by incorporating valsartan and sitagliptin simultaneously.

To the best of the authors' knowledge, no such simultaneous method for estimation of valsartan and sitagliptin is available. The aim of current research work was to design a sensitive, simple and rapid RP-HPLC method for determination of both drugs.

MATERIALS AND METHODS

Method

Sitagliptin and valsartan were received as a gift sample from CCL Pharmaceuticals, Lahore, Pakistan, acetonitrile (HPLC grade) (Merck, Darmstadt, Germany), dicalcium phosphate (2411, Lorong Perusahaan Satu, Industrial Complex, Penang, Malaysia), HPLC water (Merck, Germany), methanol HPLC grade (Merck, Darmstadt, Germany), phosphate buffer 6.8 (Fischer Chemicals, New Hampshire, United States), column, C-18 agilent (250mm x 4.6mm; 5µm) (Santa Clara, California, United States). Tablets Sitagliptin (50 mg) and Valstartan (80 mg) were used.

Chromatographic conditions

For effective analysis of both drugs, a C-18 column (250 mm x 4.6 mm; 5 µm) and a mobile phase composed of 0.5% phosphoric acid buffer and acetonitrile in a 45:55 ratio were used. Methanol served as the diluent. Membrane filters with a pore size of 0.45 µm were employed to filter the mobile phase before use. The flow rate was set at 1.5 mL/min, the run time was 15 minutes, the injection volume was 10 µL and the column temperature was maintained at 35 ± 0.2°C (Chaudhari *et al*., 2020).

Preparation of 0.5% phosphoric acid buffer for mobile phase

Measure 5 mL of ortho-phosphoric acid and dilute it with HPLC-grade water to bring the total volume to 1000 mL in a volumetric flask (Nyarko *et al., 2024*).

Procedure

Separate chromatograms of standard and sample preparations were obtained by injecting an equal volume of about 10 µL into the HPLC system. The major peaks, area and percentage content of sitagliptin and valsartan were calculated accordingly.

Instrumentation

All the parameters were determined using an HPLC made of an Agilent 1260 series Infinity II equipped with an auto sampler, column oven, pump and DAD sensor for calculating peak purity.

Preparation of standard solution

With the help of an analytical balance, sitagliptin 5 mg and valsartan, 80 mg were precisely weighed for the working standard and then transferred into a volumetric flask (100 mL). The final volume was made up using methanol and then the solution was sonicated for 10 minutes.

Preparation of sample solution

Twenty tablets were accurately weighed and powdered using a mortar and pestle. Precisely weigh the exact amount of powder equal to 50 mg of sitagliptin and 80 mg of valsartan. Transfer the powder to a volumetric flask (100 mL), make up the volume with methanol, and sonicate for 30 minutes.

Wavelength selection for both drugs

The mixture of both drugs was scanned within the range of 200-400 nm (Hamid *et al*., 2025) and 255 nm wavelength was detected as the maximum wavelength.

System suitability

It is considered an integral part of method development and validation. System suitability includes sensitivity, reproducibility and resolution in order to ensure that the chromatographic conditions are suitable for analysis**.** Some of the factors were calculated to ensure the suitability of the method used, which includes determination of peak, theoretical plates and tailing capacity (Bhardwaj *et al*., 2015).

Validation of the established method

In accordance with the guidelines of the International Council for Harmonization (ICH), the method was validated for linearity, LOD, precision, LOQ, accuracy, specificity and robustness (Mehmood *et al*., 2022).

Linearity

Linearity means the test results are directly related to the analyte concentrations. It is usually calculated as the confidence level on the slope of the regression line (Riaz *et al*., 2025). Linear regression data indicate a good relationship between concentration and peak area. The value of the coefficient of variation (R^2) was determined. The value of R^2 must be greater than 0.99 for an accurate and acceptable test result. The efficacy of the method is directly related to the value of R^2 (Attwa *et al*., 2024).

Accuracy

Recovery studies at three levels of 70, 100 and 130 % were determined for the marketed formulation. The percentage recovery of both drugs was calculated. As per ICH

guidelines, the % recovery must fall within the acceptance criteria of 98-102 % (Patel *et al*., 2021: Guideline IH., 2022).

Precision

Precision was estimated as repeatability precision. Six replicates of the sample (70,100 and 130 µg/mL solution of both drugs were prepared and 100 µg/mL sample is considered as 100%) were investigated on the same day for repeatability studies and their Relative standard deviation (RSD) was determined (Arten *et al.,* 2025).

Robustness

The robustness of the method was assessed to determine the impact of small variations on the analytical method's outcomes. These changes include pH, flow rate, column temperature and composition variation of the mobile phase. The pH of the buffer was adjusted by ±0.2, the flow rate by ±15 %, the temperature by ±5 °C and the variation of the mobile phase by ±3 % (Varma *et al*., 2021).

LOD

It represents the lowest concentration of the analyte that can be detected and serves as a key indicator of the method's sensitivity, calculated using Equation 1 (Varma *et al*., 2021).

$$LOD = 3.3 * SD/S \quad (1)$$

S.D represents the standard deviation and S represents the calibration curve's slope

LOQ

It refers to the minimum concentration of the analyte present in the sample that can be determined quantitatively with precision and accuracy by using equation 2 (Patel *et al*., 2021).

$$LOQ = 10 * SD/S \quad (2)$$

S.D represents the standard deviation and S represents the calibration curve's slope

Specificity

Achieving specificity is crucial for meeting regulatory standards and guaranteeing the quality and safety of products across various sectors, including pharmaceuticals, food and environmental monitoring. Specificity plays a vital role in analyzing and precisely detecting excipients, impurities, or degraded products. It specifically determines the analyte without the interference of other added materials. The correlation coefficient (R^2) of the calibration curve should be not less than 0.999, which indicates excellent linearity of the analytical method (Geetha *et al*., 2012, Shrivastava and Gupta, 2012). Samples of the drug product were exposed to an oxidizing agent, base, acid and light to produce degradation of the active. The samples were then analyzed for any degradation caused.

Stability studies

Stability studies of the samples and standards were done under normal conditions (temperature was maintained at 25℃ (Gholve *et al.,* 2021). to ensure that neither specific storage nor light-sensitive conditions were required (Varma *et al*., 2021).

Assay

Initially, twenty tablets were taken, weighed and crushed with the help of a mortar and pestle. Accurately weighs the exact quantity of ground powder equal to 50 mg of sitagliptin and 80 mg of valsartan. Transfer the grounded tablets to a volumetric flask (100 mL). Add methanol until a clear solution is prepared and perform sonication for 15 min. Use the diluent to make up the final volume. Filter the solution by using 0.22 µm syringe filters. The prepared solution was then investigated with the help of HPLC, having the same chromatographic conditions as those of linearity (Patel *et al*., 2021).

RESULTS

System suitability

Six standard injections were injected and their theoretical plates, symmetry and %RSD were calculated as given in table 1. The results depicts an excellent system suitability as %RSD values of sitagliptin and valsartan are <0.5, <0.4 respectively as shown in Table 1.

Linearity

The linear regression equation for the sitagliptin range was y = 18.117x - 36.857 and the regression coefficient was 0.9994. The linear regression for valsartan was y = 9.6313x - 21.141 and the regression coefficient was 0.9996.

Accuracy

Accuracy was determined by preparing three concentration levels (70%, 100% and 130%) for sitagliptin and valsartan. If the values of accuracy fall between 98% and 102 %, it was deemed acceptable (Iqbal *et al*., 2020, Nawaz *et al*., 2024). The results concluded that the method is accurate as recovery values falls within the range of 99.0-100.4% as shown in Table 2. The value of %RSD and recovery falls within the acceptance criteria of ≤2 (Ermer and Nethercote, 2014).

Precision

Formulation was used for precision analysis. Six injections for determination of repeatability and intermediate precision were estimated results of which were tabulated in Table 3. The suggested method was found to be precise for the determination of sitagliptin and valsartan, as the values were following USP criteria, i.e., RSD ≤ 2 % (Mehmood *et al*., 2022). By varying the analyst, equipment and day, the results have an absolute difference of <1.0% which falls within acceptance criteria, which shows that the method was precise as represented in Table 4.

Repeatability

Six independent replicates of samples and standards were analyzed. The mean recovery of sitagliptin was observed to be 99.7 - 100.1% with a %RSD of <0.6, while in case of valsartan mean recovery was observed to be 99.5 - 102.0% with a %RSD of <0.7% as represented in Table no 3.

Intermediate precision

Robustness

Robustness was assessed by varying flow rate (±15%), column temperature (±5°C) and mobile phase composition (±3%) as shown in table 5. The mean recovery in case of flow rate was observed to be 100.5-101.3% with a %RSD of <0.6, similarly mean recovery for column temperature was 100.1-101.2% with a %RSD of <0.5 and 99.8-101.0% recovery with a %RSD of <0.4 was observed in case of mobile phase composition. Hence, it was suggested that the developed method was found to be robust.

LOQ and LOD

LOQ signifies the signal-to-noise ratio and LOD represents the minimum concentration of the analyte. The LOD and LOQ of sitagliptin were 8.5 ppm and 25.7 ppm and those of valsartan were 7.8 ppm and 23.5 ppm, respectively.

Specificity

A precise, accurate and simple method for simultaneous determination of sitagliptin and valsartan was developed and validated. Meanwhile, injecting injections of mobile phase, drug and placebo, no interfering peak was observed. A validated method for specificity was shown in Fig. 1.

DISCUSSION

After studying the literature in detail and evaluating the physicochemical properties and molecular structures of sitagliptin and valsartan, it was assessed that sitagliptin belongs to the dipeptidyl peptidase-4 (DPP-4) inhibitor and is a highly polar drug; meanwhile, valsartan, an angiotensin II receptor blocker (ARB), and relatively lipophilic, exhibits distinct chromatographic behaviors. These features required careful optimization of the pH, composition of the mobile phase, and selection of the stationary phase in order to achieve acceptable retention time, resolution, and peak symmetry for both analytes within a single chromatographic run. The current method was developed and validated in accordance with the guidelines provided by ICH (Q2 (R1)). Following these guidelines, parameters such as specificity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ) were determined to ensure that they met the standards. The calibration curves showed excellent linearity, with correlation coefficients (R^2) of 0.9994 for sitagliptin and 0.9996 for valsartan. These values confirmed strong proportionality between analyte concentration and peak area, indicating that the method is reliable for quantitative analysis at varying levels.

It was found that no interfering peaks of drug, placebo and mobile phase were observed. Hence it was concluded that the formulation and the excipients do not interfere with the determination of both drugs simultaneously (Ali *et al.*, 2024). The main advantage of developing the current method is the significant reduction in retention time as compared to previously published HPLC methods. In 2013 a method was developed on sitagliptin that reports a retention time of 21.06 ± 0.40 min (Peraman *et al*., 2013) Following this, in 2018, Another method on sitagliptin was developed by using HPLC, and a retention time of 7.42 min was observed (Adsul *et al*., 2018). Furthermore, in a study conducted on method development of valsartan a retention time of 7.6 min was reported (Swamy *et al*., 2020). Similarly, a high retention time of valsartan, 10.177 min was reported by using HPLC (Kumar *et al*.). Meanwhile, the current developed method achieved short retention times of 4.596 min for sitagliptin and 5.623 min for valsartan. This noticeable reduction in retention time reflects an effective optimization of chromatographic conditions. Shorter retention time ensures a reduction in solvent consumption, increases batch monitoring, and prolongs the life of the column used (Abdelgawad *et al.,* 2022).

Significantly, the reduction in retention time did not compromise the parameters of system suitability. The obtained peaks showed adequate resolution between sitagliptin and valsartan, which ensures the absence of interference and co-elution. Theoretical plate count and symmetry of peak met the acceptable criteria. To be considered an analytical method suitable, it must be precise. %RSD indicates the precision of a method, the lower the values the higher is the precision. In current research work precision was assessed at two levels i.e. intermediate precision and repeatability. The results of precision obtained as shown in table 3, 4 showed that the method is precise as the values for accuracy ranged from 98-102% and deviation of %RSD is not more than ±2%. This suggests that the method is precise and suitable for analytical application (Shabbir *et al.,* 2026; Jabar *et al.,* 2026).

The current developed method is advantageous for routine quality control of tablets containing sitagliptin and valsartan. Overall, the HPLC method was optimized, and demonstrated a strong equilibrium between operational efficiency and analytical performance. By achieving shorter retention times while maintaining accuracy, precision, sensitivity, and resolution, the method offers a cost-effective, time-efficient, and regulatory-compliant solution for simultaneous estimation of sitagliptin and valsartan in pharmaceutical dosage forms.

CONCLUSION

Conclusively, the developed RP-HPLC method provides a simple, rapid and validated analytical approach for the

Table 1: Depicts the summarized results of system suitability

Component	Theoreticalp Plates	Symmetry	RSD (%)
Sitagliptin	>2000	<1.5	<0.5
Valsartan	>1500	<1.5	<0.4

Table 2: Summarized results of accuracy profiling

Concentration level (%)	Theoretical content (µg/mL)	Mean recovery (µg/mL)	Recovery (%)	RSD (%)
70	Adjusted as per assay method	Accurate values	99.9 - 100.4	<0.3
100	Adjusted	Accurate values	99.7 - 100.0	<0.2
130	Adjusted	Accurate values	99.0 - 99.8	<0.2

Table 3: Recovery analysis of sitagliptin and valsartan

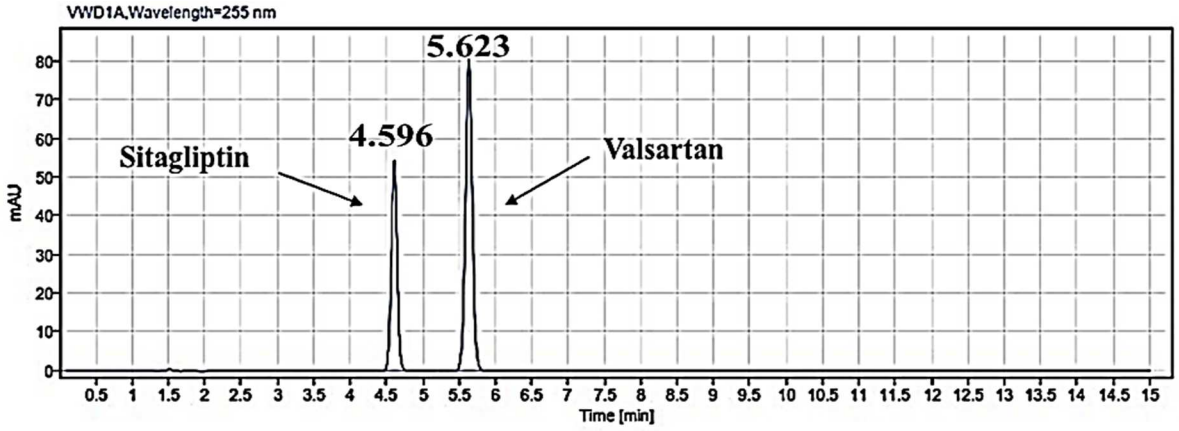
Component	Mean recovery (%)	RSD (%)
Sitagliptin	99.7 - 100.1	<0.6
Valsartan	99.5 - 102.0	<0.7

Table 4: Precision studies after changing analyst and day

Analyst/Day	Absolute difference (%)	Acceptance
Analyst 1	<1.0	Pass
Analyst 2	<1.0	Pass

Table 5: Robustness was assayed over varying R-HPLC conditions

Parameter changed	Mean recovery (%)	RSD (%)
Flow rate	100.5 - 101.3	<0.6
Temperature (°C)	100.1 - 101.2	<0.5
Mobile phase	99.8 - 101.0	<0.4



Signal: VWD1A,Wavelength=255 nm

Compound Name	RT [min]	Area	Height	Resolution	Symmetry	Theoratical Plates
Sita	4.596	308.15	52.82		0.95864	15313.04600
Valsartan	5.623	537.06	78.13	6.32276	0.97059	16283.92426
	Sum	845.21				

Fig. 1: HPLC chromatogram of sitagliptin and valsartan

Simultaneous determination of sitagliptin and valsartan. Unlike previously reported methods that employed long run times and complex chromatographic conditions, the present method achieves shorter retention times without compromising sensitivity or specificity. This improvement significantly reduces solvent consumption and analysis cost, making the method highly suitable for routine quality control and batch release testing in pharmaceutical industries. Furthermore, the robustness and reproducibility of the method ensure its applicability in future studies, including stability assessments and regulatory submissions.

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Authors' contributions
Data curation and formal analysis by M.W., W.S., Supervision by M.Z., H.H. Validation and visualization by A.S. Manuscript writing and finalization by Y.A., J.A.

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Data availability statement
All data generated or analyzed during this study are included in this published article.

Ethical approval
Not applicable, as no experiments involving animals or humans were performed in this study.

Conflict of interest
There is no conflict of interest.

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