Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423)

Volume 8 Issue 8 August 2024

Research Article

Method Development and Validation of Simultaneous Estimation of Sitagliptin Phosphate and Metformin Hydrochloride by RP-HPLC Method – A Comparative Study

Suba Geetha A*, Saravanan VS, Jambulingam M, Ravi Kumar R and Jayakarthi M

The Erode College of Pharmacy, Erode, Tamil Nadu, India

***Corresponding Author:** Suba Geetha A, The Erode College of Pharmacy, Erode, Tamil Nadu, India.

Received: May 10, 2024

Published: July 03, 2024

Abstract

The simple, rapid, accurate, economic and a gradient RP-HPLC method showed excellent sensitivity, reproducibility, accuracy, and repeatability for the simultaneous estimation of Sitagliptin and Metformin in bulk and tablet formulation was developed and validated as per the ICH Guidelines and comparative studies were performed with different brands on same combination with same dose.

Method: It was performed by using C18250×4.6mm 5µ at low- pressure gradient method with the detection on UV at 261nm. The mobile phase comprises of mixture of buffer (pH 4) and acetonitrile at the ratio of 60:40 at the flow rate of 1 ml/min by keeping the column oven temperature up to 30°C throughout the analysis.

Result and Discussion: The retention time for Metformin was found to be 2.4 mins and for Sitagliptin 7.0 mins. This method shows good linearity on the concentration 1-5µg/ml for Metformin and 5-25µg/ml for Sitagliptin with the correlation coefficient (r2) of 1.0 and 0.996 for Metformin Sitagliptin respectively. The Limit of Detection and Limit of Quantification of Metformin was found to be 0.011 and 0.033 and for Sitagliptin 0.002 and 0.007 respectively.

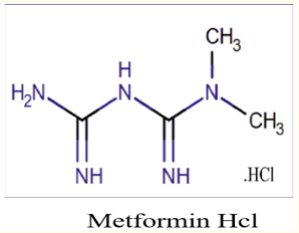
Keywords: RP-HPLC; Diabetes Mellitus

Introduction

Type 2 diabetes mellitus is a prevalent metabolic disorder stemming from inadequate insulin secretion by pancreatic β cells, altering carbohydrate, lipid, and protein metabolism [1,2]. Sitagliptin phosphate, chemically represented as (3R)-3-amino-1-[3-(trifluoromethyl)-6,8-dihydro-5H-[1,2,4]triazolo[4,3-a]pyrazin-7-yl]-4-(2,4,5-trifluorophenyl)Butane-1-one phosphoric acid [3] is an oral DPP-4 inhibitor, enhances glycemic control in type 2 diabetes by preserving incretin hormones, boosting insulin secretion, and reducing glucagon release, it's typically dosed between 25mg to 100mg [4,5]. Metformin hydrochloride, chemically represented as 3-(diamino methylidene)-1,1-dimethylguanidine hydrochloride [6], a biguanide, an oral hypoglycemic, reduces insulin resistance and increases tissue sensitivity to insulin. Commonly prescribed separately or with other antidiabetic drugs, it's typically dosed between 250mg to 2500mg daily, often in extended-release forms [7,8].

As per the literature, in this combination, the various techniques were performed which includes few HPLC Methods in simultaneous estimation [8-13], bioanalytical studies [14], UV spectroscopy [15], impurity studies [16] and UPLC method [17] which are performed alone or in combination on tablet formulation.

In Sitagliptin and Metformin combination, many literatures were reviewed and reported by comparing the standard with one tablet formulation or at individual. But there is no literature available on the simultaneous estimation with the comparative studies



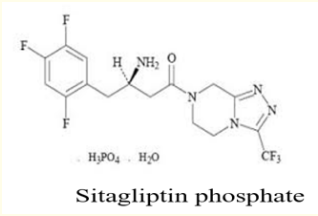


Figure 1

of sitagliptin and metformin tablets from different brands on same dose. The aim of this work is to develop and validate an analytical method for the simultaneous estimation of Sitagliptin and Metformin in tablet dosage form and to perform a comparative study on different brands of tablet dosage form. In this present study, different commercial brand tablets were compared with standard and evaluated the validation parameters using ICH Q2(R1) guidelines [18].

Chemicals

Sitagliptin phosphate and Metformin hydrochloride were purchased from Dhamtec Pharma and Consultants (Maharashtra). HPLC Grade Acetonitrile were procured by Thermo Fisher Scientific India Pvt. Ltd. HPLC Acetic acid 99.9% and Triethyl Amine 99.5% were procured from Sisco Research Laboratories Pvt. Ltd and HPLC grade Water was procured by Sisco Research Laboratories Pvt. Ltd By comparing each brand of tablets Sitabright-M 50/500, Sitacip-M 50/500, Alsita- M 50/500 brands were purchased from the local pharmacy.

Selection of wavelength

The standard stock solutions (1000 µg mL^{-1}) of Sitagliptin and Metformin were separately and suitably diluted with water to obtain a final concentration of 10µg mL^{-1}. This solution of Sitagliptin and Metformin in water was scanned between 200 to 400 nm in the UV double beam spectrophotometer using water as blank to determine the λ max of the drugs. The UV spectra of both the drugs Sitagliptin and Metformin were overlaid to determine the iso- absorptive or isobestic point. Hence 261 nm were selected as detection wavelength for estimation of both the drugs by RP-HPLC method with Gradient elution technique.

Chromatographic conditions

Separationswere performed under Reverse phase C_{18} column under manual injection system taken up to the volume of 20µl, column oven temperature at 30°C should be maintained throughout the analysis. The optimized method was performed under Triethyl ammonium acetate buffer at pH 4 and acetonitrile at the ratio of 60:40.A flow rate of 1.0 ml/min was used for the separation and internal standard with the detection at wavelength of 261nm.

Mobile phase preparation

7ml of Triethyl amine and 2.9ml of Acetic acid was pipetted out and mixed with water. The volume was made up to 1000ml with water and the pH was adjusted to 4 with Acetic acid. It was filtered by Nylon filter 0.45µ and allowed to degas the solution in sonicator for 20min.

Standard stock solution preparation

The stock solutions of Sitagliptin phosphate and Metformin Hydrochloride were prepared by using water as diluent. Accurately weighed 100mg of Metformin Hydrochloride and 10 mg of Sitagliptin Phosphate were transferred into clean, dry 100ml volumetric flask. Sufficient volume of water was added to dissolve and makeup the volume till the mark. Resulting solution is at the concentration of 10µg/ml.

Preparation of sample solution for comparative studies

Comparative studies on the tablets of different brands with same dose were done by the optimized method. The label claim of each brand comprises of 50 mg of sitagliptin and 500mg of metformin. 10 tablets of each brand were weighed and the average weight was calculated. The tablets were crushed and powdered. The powder was weighed equivalent to the label claim of 50 mg of sitagliptin and transferred to 50ml volumetric flask. Then sufficient amount of water was added to dissolve with continuous shaking and allowed to sonicate for 30 mins. The solution was made up to the volume with water. The solution was filtered with filter assembly at the filter size of 0.45µ Nylon filter. From the clear solution further dilution were made to 100µg/ml. 2.5 ml of the resulting solution was diluted to 10 ml with water to obtain 25µg/ml of sitagliptin. 20µL of the obtained solution is injected for a run time of 10 mins.

Result and Discussion

Method development

The chromatographic condition for the comparison study of combined drugs on Sitagliptin phosphate and Metformin Hydrochloride were analyzed by RP-HPLC method by experimenting the several factors such as suitable mobile phase system, pH of the single mobile phase solution. Initial separations were done using combination of Acidified water, Acetonitrile, Methanol, and Triethyl Ammonium Acetate buffer in different ratios. At final the systemic experiment was performed by Triethyl Ammonium Acetate at pH 4 by adjusting with Acetic Acid and Acetonitrile at the ratio of 60:40at a run time of 10 mins. The optimized chromatogram shows the retention time at 2.4 mins for Metformin and 7.0 mins for Sitagliptin the chromatogram is shown below.

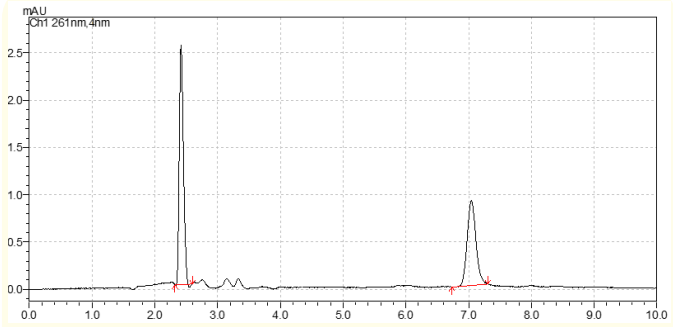


Figure 2: Optimized chromatogram for metformin and sitagliptin.

Method validation

The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) provides guidelines for the validation of analytical procedures, commonly known as the ICH Q2(R1) guideline, which is used for validation of the optimized method [18].

System suitability parameter

To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase were allowed to flow through the column at a flow rate of 1.0 ml/min to equilibrate the column at 30°C. Chromatographic separation were achieved by injecting a volume of 20 µL of standard into C_{18} Column (250mm X 4.6 mm i.d.,5µ), the mobile phase of composition triethyl ammonium acetate buffer at pH (4.0) : Acetonitrile (60:40) were allowed to flow through the column at a flow rate of 1.0 ml per minute at a run time of 10 mins.

Parameters	Sitagliptin	Metformin
Area of peak	15346	132938
%RSD	0.029	0.025
Retention time	7.0 mins	2.42 mins
Theoretical plate	5675	3808
Tailing Factor	1.219	1.183

Table 1: System suitability parameter.

Linearity

In linearity parameter, the standard solutions of Sitagliptin phosphate and Metformin hydrochloride were prepared each by five different concentrations. For Sitagliptin phosphate 5-25µg mL^{-1} and for Metformin hydrochloride 1-5µg mL^{-1}. The regression equation for Sitagliptin phosphate were found to be y = 5850x+2639 and Metformin hydrochloride were found to be y = 22864x+26890 and the correlation coefficient (r^2) were found to be 0.996 and 1.0 for Sitagliptin phosphate and Metformin hydrochloride respectively, where 'x' indicates the concentration of solution and 'y' indicates the peak area obtained. The linearity charts were prepared by Microsoft Office Excel 2007.

Parameters	Sitagliptin	Metformin
Beer's law limit	5-25µg/ml	1-5µg/ml
Correlation coefficient	r^2 = 0.996	r^2 = 1
Regression equation	Y = 5850x+2639	Y = 22864x+26890
Slope(m)	5850	22864
Intercept(c)	2639	26890
LOD(µg/ml)	0.002	0.011
LOQ(µg/ml)	0.007	0.033

Table 2: Linearity chart.

Accuracy and precision

For accuracy studies, each drug should be analyzed at the concentration of 80%, 100%, and 120%. The data are enlisted as given below.

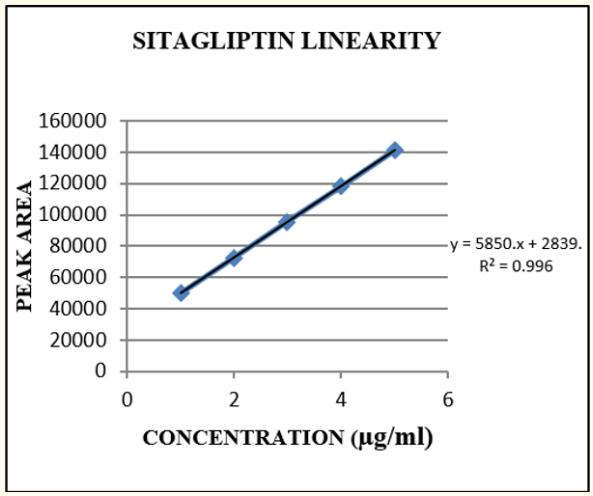


Figure 3: Sitagliptin linearity.

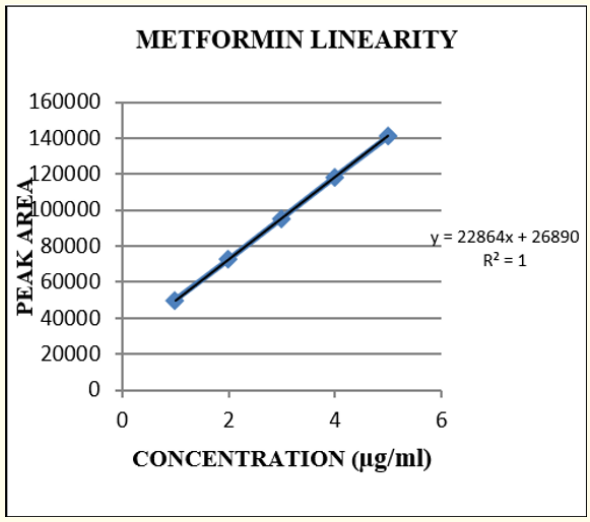


Figure 4: Metformin linearity.

S.NO	AMOUNT ADDED	AMOUNT PRESENT	%Recovery	Average	SD	%RSD
1.	20mg	19.8	99.00%	98.33%	0.763	0.776
		19.7	98.50%			
		19.6	98%			
2.	25mg	24.5	98%	99%	1.404	1.414
		25.2	101%			
		24.8	99.20%			
3.	30mg	29.2	100.60%	99.20%	1.311	1.322
		29.4	98%			
		29.7	99%			
Accuracy of Sitagliptin						
S.NO	AMOUNT ADDED	AMOUNT PRESENT	%Recovery	Average	SD	%RSD
1.	200mg	201	100.5%	99.8%	0.6245	0.6257
		199.2	99.6%			
		198.6	99.3%			
2.	250mg	249.2	99.6%	99.9%	0.4358	0.4363
		251	100.4%			
		249.3	99.7%			
3.	300mg	300.4	100.1%	99.87%	0.2081	0.2084
		299.4	99.8%			
		299.1	99.7%			
Accuracy of metformin						

Table 3: Accuracy of sitagliptin and metformin.

For precision studies, each concentration of two samples were prepared and analyzed by HPLC method at inter-day and intra-day method.

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection and Limit of Quantification were calculated and were found to be 0.002 µg mL^{-1} and 0.007 µg mL^{-1} for Sitagliptin and 0.011 µg mL^{-1} and 0.033 µg mL^{-1} for Metformin.

Parameter	Intraday precision		Interday precision	
Individual concentration	Mean of peak area ± SD	%RSD	Mean of peak area ± SD	%RSD
Sitagliptin 25µg mL^{-1}	155044 ± 1687	1.088	154027 ± 1716	1.114
Metformin 5µg mL^{-1}	1315137 ± 13104	0.996	1281994 ± 11555	0.9013

Table 4: Intraday and Interday precision.

Robustness

In this study, the standard solution of Sitagliptin and Metformin were prepared. Robustness was performed by altering the mobile phase ratio, flow rate, the detection wavelength etc., In this study, it is performed by altering the flow rate at the difference of ± 0.2ml/min and by increasing the temperature at ± 3°C, the % RSD of the tailing factor which does not exceed 2.5% which is shown below table.

Peak name	Parameter	Peak area	USP Plate Count	USP Tailing Factor
Sitagliptin	Flow rate (± 0.2ml/min)			
	Actual = 1.0ml/min			
	Flow rate = 0.8ml/min	142,930	6,960	1.633
	Flow rate = 1.2ml/min	189,411	7,431	1.72
	Column temperature	126,240	7,052	1.592
	(± 3°C)			
	Actual at 30°C	142,930	6,960	1.633
	At 33°C	155,108	6,781	1.653
	At 27°C	149,019	6,845	1.616
Metformin	Flow rate (± 0.2ml/min)			
	Actual = 1.0ml/min			
	Flow rate = 0.8ml/min	1,301,315	4,716	1.266
	Flow rate = 1.2ml/min	1,640,264	4,505	1.305
	Column temperature(± 3°C)	1,087,072	4,226	1.286
	Actual at 30°C	1,301,315	4,716	1.266
	At 33°C	1,314,188	4,600	1.283
	At 27°C	1,307,717	4,383	1.287

Table 5: Data for robustness.

Conclusion

A RP-HPLC method for the simultaneous determination of Sitagliptin phosphate and Metformin Hydrochloride in Pharmaceutical formulation were developed and validated according to currently accepted ICH guidelines of analytical method validation. The results of all methods were very close to each other as well as to the Label value of commercial pharmaceutical dosage form. Therefore, there is no significant difference in the results achieved by the proposed method. The standard run time analysis time is about 10 minutes. This approach is distinguished by its simplicity, rapidity, and effectiveness. Validation data provide great precision and accuracy, confirming the suggested method's reliability. Given its consistency, this technique shows potential for adapting to other

formulations under consideration. Comparative study on different brand formulation helped in evaluating the consistency and quality of the brand. It helps in evaluating the analytical parameters employed in simultaneous estimation across the various brands showing its accuracy, precision, linearity, and robustness.

Future Perspective

The outcome of the study reveals that the method developed and validated by RP-HPLC for the proposed drug combination is simple, accurate, precise and economical. In future, the work can be extended by adopting the proposed method to analyze other formulation containing the same combinations of different strength. Further, the study can be used as a lead to perform Bio-equivalent and stability studies.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgement

The authors would like to thank Dr. R. Sambathkumar, M. Pharm., Ph.D., Principal, The Erode College of Pharmacy, Erode, for his support to our project.

Bibliography

1. Padmaja Udaykumar. "Medical Pharmacology". Fifth edition; Delhi; CBS Publishers and Distributors (2015): 641-655.

2. Afroj A Shaikh., *et al*. "Review on: Diabetes Mellitus is a Disease". *International Journal of Research in Pharmaceutical Sciences* 13.1 (2022): 102-109.

3. Magdy G., *et al*. "Analytical quality-by-design approach for development and validation of HPLC method for the simultaneous estimation ofomarigliptin, metformin, and ezetimibe: application to human plasma and dosage forms". *BMC Chemistry* 17.45 (2023): 1-10.

4. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. PubChem Compound Summary for CID 4369359, Sitagliptin (2004).

5. Zaid AN., *et al*. "Stability of extemporaneously prepared sitagliptin phosphate solution". *Plos One* 17.3 (2022): e0262068.

6. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004-. PubChem Compound Summary for CID 14219, Metformin Hydrochloride.

7. Corcoran C and Jacobs TF. "Metformin". In: StatPearls. Treasure Island (FL): StatPearls Publishing (2024).

8. Rao GVP., *et al*. "Stability Indicating Assay Method Development and Validation of Sitagliptin and Metformin Combination Tablets by Reverse Phase – HPLC". *International Journal of Pharmaceutical Quality Assurance* 14.3 (2023): 573-578.

9. Balamurugan Krishnan and Kirtimaya Mishra. "Quality by Design based Development and Validation of RP-HPLC Method for Simultaneous Estimation of Sitagliptin and Metformin in Bulk and Pharmaceutical Dosage Forms". *International Journal of Pharmaceutical Investigation* 10.4 (2020): 512-518.

10. Deepak M., *et al*. "Development And Validation Of RP-HPLC Method For Simultaneous Estimation Of Metformin Hcl And Sitagliptin Phosphate In Tablet Dosage Form". *European Chemical Bulletin* 12.1 (2023): 2713-2729.

11. SD Mankar., *et al*. "Development and validation of RP-HPLC method for simultaneous estimation of Ertugliflozin and Sitagliptin in bulk drug and tablet dosage form". *International Journal of Experimental Research and Review* 32 (2023): 288-296.

12. Sunitha Gurrala., *et al*. "Chromatographic study of sitagliptin and ertugliflozin under quality-by-design paradigm". *Brazilian Journal of Pharmaceutical Sciences* 59 (2023): e21328;1-18.

13. Shyam Rangari and Makarandpatil. "RP- HPLC Method Development for Simultaneous Estimation of Sitagliptin Phosphate and Metformin Hydrochloride". In Pharmaceutical Dosage Form International Journal of Research in Pharmacy And Chemistry 11.3 (2021): 64-70.

14. Dibya Das., *et al*. "Determination of Metformin and Sitagliptin In Healthy Human Volunteers' Blood Plasma And Its Bioequivalence Study Under Fasting Condition". *International Journal of Applied Pharmaceutics* 14.6 (2022): 42-50.

15. "Development And Validation of UV Spectrophotometric Method for Simultaneous Estimation Of Sitagliptin And Metformin In Bulk And Combined Pharmaceutical Formulation". *International Journal of Current Pharmaceutical Research* 14.2 (2022): 65-68.

16. Chittireddy HNPR., *et al*. "Development and Validation for Quantification of 7-Nitroso Impurity in Sitagliptin by Ultraperformance Liquid Chromatography with Triple Quadrupole Mass Spectrometry". *Molecules* 27.23 (2022): 8581.

17. Ashritha Narikimalli and Rajitha Galla. "A stability indicating UPLC method development and validation for the simultaneous estimation ofnateglinide and metformin hydrochloride in bulk and tablet dosage form". *Future Journal of Pharmaceutical Sciences* 9.55 (2023): 1-12.

18. ICH Harmonized Tripartite Guideline Validation of Analytical Procedures: Text And Methodology Q2 (R1) Current Step 4 Version Parent Guideline Dated 27 October (1994).