Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 7 (2025)
https://www.jneonatalsurg.com

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Simultaneous Method Development And Validation Of Vildagliptin And Metformin In Bulk By Rp-Hplc

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Cite this paper as: Saniya, Dillip Kumar Brahma , Gunosindhu Chakraborthy , Jayendra Kumar , R. Suresh , Abhimanyu Kumar Jha , Shivani Gupta , Ashwini Rakhamaji Dhavade (2025). Simultaneous Method Development And Validation Of Vildagliptin And Metformin In Bulk By Rp-Hplc *Journal of Neonatal Surgery*, 14 (7), 203-211.

ABSTRACT

The research goal is to provide a quick, easy, accurate, and economical RP-HPLC technology for simultaneously analyzing the amounts of Vildagliptin and Metformin in bulk samples and pharmaceutical products. The separation of Vildagliptin (VLD) and Metformin (MTF) has been accomplished successfully with this approach. An Xterra C18 column (250 mmL×4.6 mm) analytical column operating at 210 nm was used to carry out the separation. The mobile phase was made up of a 40:40:20 mixture of phosphate buffer, acetonitrile, and water, with the buffer's pH set to 5.5. At a flow rate of 1.0 ml/min, the separation was performed in isocratic elution mode. Using a linear calibration curve and PDA detection at 210 nm, metformin and vildagliptin were quantitatively analyzed. For accurate quantification, the concentration ranges of 10–50 µg/ml (correlation coefficient of 0.9998) for MTF and 1–5 µg/ml (correlation coefficient of 0.9998) for VLD were employed. Vildagliptin had a limit of detection (LOD) of 0.332 µg/ml, while metformin had a LOD of 1.662 µg/ml. Whether employed alone or in combination, the suggested approach is ideal for use in quality-control labs for pharmaceutical quantitative analysis and bulk analysis. This method is distinguished by its efficiency and simplicity while maintaining a high degree of precision and accuracy

Keywords: *Metformin, Vildagliptin and RP-HPLC.*

1. INTRODUCTION

treatments for acute gout (5). The best course of action for treating chronic gout is to use diuretic acid medicines in conjunction with allopurinol, an XOD inhibitor (6).

Celecoxib has a weakly acidic (pKa = 11.1) and hydrophobic (log P = 3.5) nature. It is a white, crystalline powder that is

Type 2 diabetes (T2DM) is a chronic condition that requires a variety of anti-diabetic medications with distinct modes of action in order to achieve glycaemic objectives[1]. When dual therapy with metformin and a sulphonylurea (SU) is ineffective in improving glycaemic control, a third anti-hyperglycaemic medication must be added [2]. A novel oral anti-diabetic medication called Vildagliptin (VGT) [(S)-1-[N-(3-hydroxy-1-adamantyl) glycyl] pyrrolidine-2-carbonitrile] is a member of the dipeptidyl peptidase-4 inhibitor class, which lowers glucose-induced glucagon-like peptide and gastric inhibitory polypeptide secretion. It is used as monotherapy for adults with type 2 diabetes mellitus, particularly in those whose condition is not adequately managed with diet and exercise alone [3, 4].

When metformin monotherapy is not enough to manage blood sugar levels, vildagliptin can be used as a dual oral treatment in conjunction with metformin[5, 6]. When compared to sulphonylurea, it is just as effective when administered with metformin and lowers the risk of hypoglycemia without causing weight gain. As a pharmaceutical supplement to metformin, vildagliptin increases glycaemic control, weight control, and hypoglycemia by inhibiting glucagon release and improving glucose-dependent insulin secretion.[7].

Metformin (MTF), an oral anti-diabetic medication belonging to the biguanide class, is chemically known as [1-carbamimidamido-N, N-dimethylmethanimidamide]. It serves as the first-line medication for the treatment of noninsulin-dependent diabetic mellitus [8]. By reducing hepatic glucose synthesis, reducing glucose absorption, and boosting insulin-mediated glucose uptake, it improves glycemic control. For adults with type 2 diabetes mellitus, especially those who are overweight and cannot attain adequate glycaemic control with oral metformin alone at their highest tolerated dose, metformin competent is recommended as a second-line treatment [9].

By activating the enzyme AMP-activated protein kinase (AMK) and the Peutz-Jeghers protein, LKB1, to control AMPK [10], metformin HCl lowers blood glucose and lipid contents. Therefore, reducing hepatic glucose production was used as a supplementary mode of action to treat patients with type 2 diabetes. Rat plasma [11] was used to investigate the potential relationship between metformin and sugar. MTF has a molar mass of 129.16 g/mol and the general formula is C4H11N5. HPLC has been utilized in numerous research to create a simple, quick, accurate, and precise approach for evaluating chemicals in various medicinal dosage forms [12-14].

Numerous techniques, including UV-Vis spectroscopies, HPLC, and LCMS/MS approaches, were developed for the analysis of both vildagliptin and metformin in combination. The current study focused on chromatographic analysis of vildagliptin and metformin in a less time-consuming simultaneous analysis of these compounds' inactive ingredient (API) and pharmaceutical dosage form, which are found in the pharmaceutical market [15–18]. Instantaneous estimation of these compounds by RP-HPLC methods were exhibiting more time of analysis and complicated procedures.

Materials and Methods

Weighing was done using a Shimadzu electronic balance (AX 200). The HPLC system (Shimadzu SPD-20A, Tokyo, Japan) used analytical-grade orthophosphoric acid (S.D. Fine Chemicals, Mumbai, India) for mobile phase preparation. Acetonitrile, methanol, water, potassium dihydrogen orthophosphate, and orthophosphoric acid (HPLC grade). The pH metre (Eutech and ultrasonicator (Unichrome, UCA701).

Instrumentation

Tokyo, Japan's Shimadzu SPD-20A HPLC system was used for the HPLC investigations. A photodiode array detector and a separation module were part of the system, and an autosampler was used to conduct the experiments in isocratic mode. LC solution software was used to gather and process the data. The eluents were measured at 210 nm, and the separation was carried out using an Xterra C18 column (250 mmL×4.6 mm) analytical column.

Preparation of mobile phase

Mobile phase was prepared by mixing phosphate buffer, acetonitrile and water in the ratio of 40:40:20 and the pH of the buffer was adjusted to 5.5 and was filtered through 0.45µ membrane.

Preparation of standard stock solution

Through the use of a digital microbalance, 100 mg of Metformin and 10 mg of Vildagliptin were weighed into a volumetric flask of 10 millilitres. After adding few millilitres of diluent, it was sonicated to dissolve it. After that, the solution was diluted to volume with the diluent, and lastly, it was diluted to a final volume by adding more diluent.

Chromatographic conditions

High Performance Liquid Chromatography equipped with PDA detector.

For Metformin and Vildagliptin (isocratic)

Column	:	Xterra C18 column (250 mmL×4.6 mm)analytical column
Wavelength	:	210 nm
Injection Volume	:	20 µl
Column Temperature	:	Ambient
Flow rate	:	1.0 ml/min

At 3.352 minutes, the MTF peak was found to have an area of 785698, with a tailing factor of 1.12. As shown in Fig. 1 and Table 1, the VLD peak was seen at 5.587 min with a peak area of 160139, a tailing factor of 1.11, and a resolution of 4.25. This experiment was deemed optimal due to its positive outcomes and shorter retention duration. MET has a retention time of around 3.352 minutes and VLD has a retention time of 5.587 minutes.

Table 1: System suitability parameters

S.No.	Name of the Peak	Retention Time (Mins)	Peak Area	Tailing Factor	Resolution	Plate Count
01	Metformin	3.352	785698	1.12	---	5007
02	Vildagliptin	5.587	160139	1.11	4.25	5432

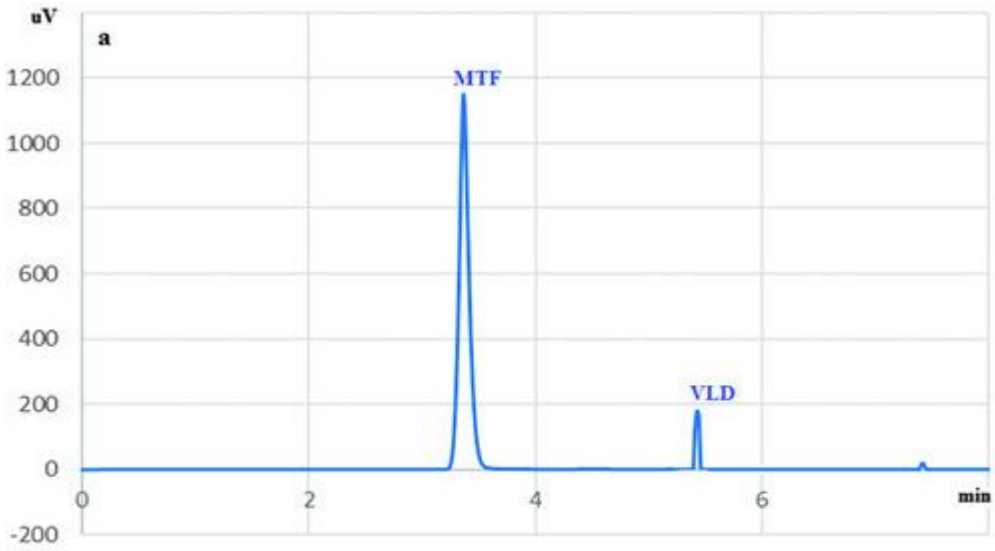

Fig.No. 01 : Typical Chromatogram of Metformin and Vildagliptin

Preparation of sample solution

About 10 mg of sample was weighed into a 10 ml volumetric flask, and then 7 ml of diluent was added. The mixture was then sonicated to dissolve the material, and then diluted to volume with diluent. Further diluted to 10 ml with the diluent and filtered through 0.45µ Nylon syringe filter.

Procedure

Five injections of 20 µl each of active MTF and VLD standard solutions were performed. Chromatograms were obtained and peak responses were evaluated. The system's suitability was calculated by evaluating its parameters. The quantification of MTF and VLD in the sample was achieved by the analysis of the peak responses.

Method Validation

The present study examined many parameters to establish the validity of the HPLC methodology for quantifying MTF and VLD in accordance with the specified procedure, hence demonstrating its suitability for the intended use. The implementation of all validation criteria was done in compliance with the standards set by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH).

Linearity and Range

The concentrations of MTF and VLD that showed a linear relationship with peak area were (10 - 50 µg/ml), (1 - 5 µg/ml). Results are shown in (Fig.2 & 3), (Table 2 & 3), and the linearity of the calibration curve is confirmed by the high value of the correlation coefficient of the regression equation.

Table 2: Linearity data of MTF

S.No.	Concentration (µg/ml)	Peak Area
1	0	0
2	10	261892
3	20	526745
4	30	785698

5	40	1047985
6	50	1326785
Slope		26510
Intercept		-5486.8
Regression		0.9998

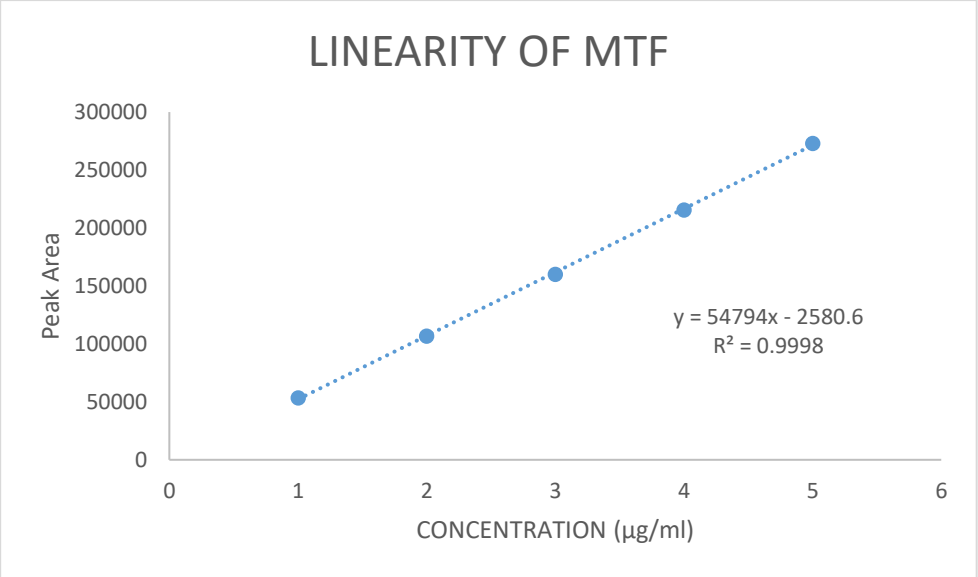

Fig.No. 02: Linearity of Metformin

Table 3: Linearity data of VLD

S.No.	Concentration (µg/ml)	Peak Area
1	0	0
2	1	53388
3	2	106854
4	3	160139
5	4	215678
6	5	272945
Slope		54794
Intercept		-2580.6
Regression		0.9998

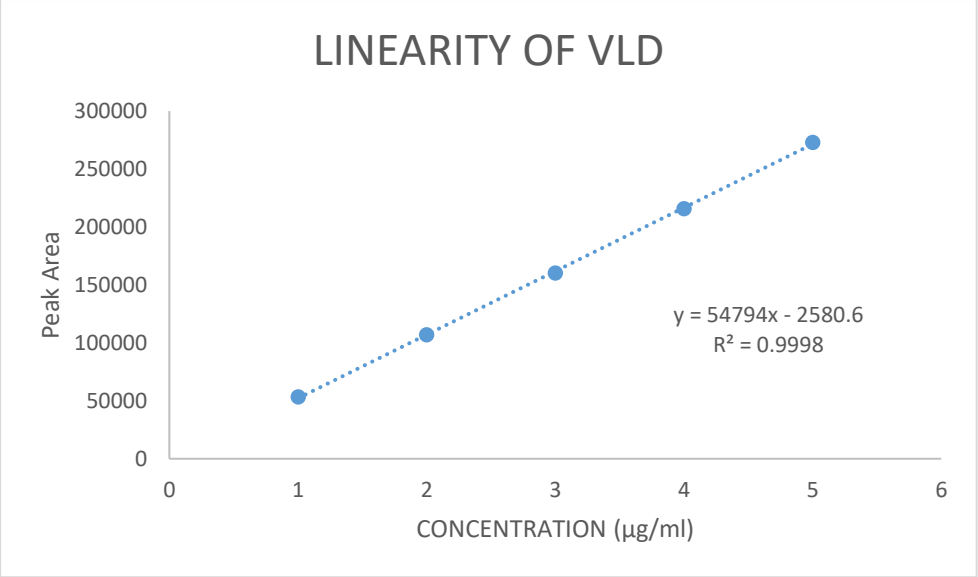

Fig.No. 03 : Linearity of Vildagliptin

Accuracy and Precision:

Accuracy as recovery was examined by spiking previously analyzed test solution with extra Standard drug at three different concentration levels. With a relative standard deviation (RSD) of less than 2%, we observed that the suggested technique is accurate for the simultaneous estimate of both MTF and VLD, with a recovery of 99.97 % for MTF and 100.24% for VLD, respectively. The high reproducibility and low RSD values show that the Method is reliable. (table-4 & 5).

Table 4: Precision data of MTF and VLD

Injection Number	MTF				VLD			
	Retention Time	Peak Area	Plate Count	Peak Symmetry	Retention Time	Peak Area	Plate Count	Peak Symmetry
1	3.352	785698	5213	1.12	5.587	160139	5567	1.22
2	3.351	785796	5245	1.23	5.578	159210	5597	1.22
3	3.349	785887	5256	1.34	5.565	161222	5667	1.34
4	3.353	784698	5200	1.25	5.589	161345	5521	1.56
5	3.352	783598	5113	1.34	5.542	160543	5715	1.1
6	3.351	777698	5123	1.45	5.598	160675	5675	1.11
Average	3.351	1403270			5.577	160522		
Standard Deviation	0.001	3161.28			0.020	782.20		
% RSD	0.0408	0.23			0.36	0.49		

Table 5: Accuracy data of MTF and VLD

Sample Preparation No.	MTF Assay (%)	VLD Assay (%)
1	99.89	99.78
2	100.23	100.12
3	101.34	99.89
4	100.23	100.55
5	100.83	99.25
6	99.13	100.25
Mean	**100.28**	**99.97**
SD	**0.7623**	**0.4468**
RSD (%)	**0.7602**	**0.4469**

Robustness:

The results of the robustness analysis are shown in Table no.6. Both components exhibited comparable tailing factors, elution orders, resolutions, relative standard deviations, and recoveries. The analysis revealed that the relative standard deviation (RSD) of the peak sites was much below 2.0%.

Table 6: Robustness data of MTF and VLD

Condition	Metformin			Vildagliptin		
	% RSD	Tailing Factor	% Recovery	% RSD	Tailing Factor	% Recovery
1) Change in Flow rate						
Normal Condition (1.0 ml per minute)	0.56	1.42	99.23	0.12	1.21	99.21
Flow rate (0.8 ml per minute)	0.57	1.34	99.45	0.35	1.23	100.67
Flow rate (1.2 ml per minute)	0.87	1.22	100.23	0.53	1.22	100.89
2) Change in minor component in the mobile phase						
Normal Condition (Phosphate buffer, acetonitrile, and water in a ratio of 40:40:20)	0.23	1.45	99.34	0.56	1.22	100.89
(Phosphate buffer, acetonitrile, and water in a ratio of 50:30:20)	0.43	1.34	99.45	0.57	1.34	100.41
(Phosphate buffer, acetonitrile, and water in a ratio of 30:50:20)	0.76	1.37	100.42	0.87	1.25	101.21
3) Change in Wave Length						
Normal: Wave Length 210 nm	0.23	1.45	99.89	0.56	1.23	100.45

Wave Length 205 nm	0.43	1.32	98.97	0.57	1.20	100.49
Wave Length 215 nm	0.76	1.41	98.83	0.87	1.19	100.93
4) Change in Ph						
Normal: pH 5.5	0.45	1.22	99.89	0.34	1.45	100.45
pH 5.0	0.54	1.34	99.97	0.67	1.29	99.49
pH 6.0	0.76	1.25	98.93	0.78	1.41	101.93

Ruggudness:

Metformin and Vildagliptin had respective mean peak areas of 785777 and 160142 with an RSD of 0.35 and 0.28%, respectively.

SUMMARY

A novel and validated RP-HPLC method has been created to evaluate MTF and VLD in pharmaceuticals and bulk. Given the results of the literature review, which showed that there are few techniques for estimating MTF and VLD in large numbers, a straightforward, economical, and accurate solution to this problem is desperately needed. A combination of phosphate buffer, acetonitrile, and water (40:40:20) with a pH of 5.5 was injected onto an Xterra C18 column (250 mmL×4.6 mm) to measure the concentrations of MTF and VLD. The injection volume was 20 µl, and the flow rate was set at 1.0 ml/min. The VLD peak eluted after 5.587 minutes, while the MTF peak had a retention time of 3.352 minutes.

Following improvement, the method was verified for linearity, sensitivity parameters, precision, accuracy, resilience, and system compatibility in accordance with ICH requirements. Every validation parameter produced results that fell within reasonable bounds. The tests' relative standard deviations (RSDs) were below two. The range of recoveries was 98% to 102%.

2. CONCLUSION

The suggested RP-HPLC technology provides a quick and easy method that is nevertheless straightforward, quick, accurate, precise, resilient, and economical. As a result, it is a preferred technique for determining Vildagliptin and Metformin simultaneously. Every part of the implemented method was carefully checked to ensure it complied with ICH rules.

REFERENCES

1. Inzucchi SE, Bergenstal RM, Buse JB, Peter AL and Winder R: Management of hyperglycemia in type 2 diabetes: a patient- centered approach: position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetes Care.2012; 35: 1364-1379.
2. Cook MN, Girman CJ, Stein PP, Alexander CM and Holman RR: Glycemic control continues to deteriorate after sulfonylureas are added to metformin among patients with type 2 diabetes. Diabetes Care. 2005; 28: 995-1000.
3. Vishvakrama P, Sharma S. Liposomes: an overview. Journal of Drug Delivery and Therapeutics. 2014 Jun 24:47-55.
4. Vishvakarma P. Design and development of montelukast sodium fast dissolving films for better therapeutic efficacy. Journal of the Chilean Chemical Society. 2018 Jun;63(2):3988-93.
5. Vishvakarma P, Mandal S, Verma A. A review on current aspects of nutraceuticals and dietary supplements. International Journal of Pharma Professional's Research (IJPPR). 2023;14(1):78-91.
6. Sergei H, Anja S, Biljana M, James F and Sylvie D: Combination treatment in the management of type 2 diabetes: focus on vildagliptin and metformin as a single tablet. Vascular Health Risk Management. 2008; 4: 481- 492.
7. Ahren B, Foley JE and Bosi E: Clinical evidence and mechanistic basis for vildagliptin's action when added to metformin. Diabetes Obesity and Metabolism. 2011; 13: 193-203.
8. National Collaborating Centre for Chronic Conditions. NCCCC. Type 2 diabetes: National clinical guideline for

management in primary and secondary care (update) London (UK): Royal College of Physicians; 2008. http://www.nice.org.uk/nicemedia/pdf/CG66diabetesfullgu ideline.pdf.

9. Canadian Diabetes Association (CDA). Clinical practice guidelines for the prevention and management of diabetes in Canada. Can J Diabetes. 2008; 32(S-1): 1-201.
10. Wild S, Roglic G, Green A, Sicree R and King H: Global prevalence of diabetes. Estimates for the year 2000 and projections for 2030.Diabetes Care, 2004; 27: 1047-1053.
11. Awad R, Mallah E, AL-Ani I, Abu Dayyih W, Zakarya Z and Arafat T: Investigation of possible pharmacokinetic interaction of metformin with sugar replacement sweeteners in rats. Journal of Applied Pharmaceutical Science. 2016; 6: 210-215.
12. Abu Dayyih W, AL-Fayez A, Tamimi L, Mallah E and Arafat T: Simultaneous determination of atorvastatin, glimepiride and amlodipine in solution and plasma matrix using HPLC/UV method. Journal of Chemical and Pharmaceutical Research. 2014; 6: 515-522.
13.. Abu Dayyih W, Tamimi L, Mallah E, Mansour K, Arafat T and Bustami M: Saxagliptin levels and its pharmacokinetic application in presence of sucralose in animal's serum by HPLC method. International Journal of Pharmacy and Pharmaceutical Science.2015; 7: 243-250.
14.. Hamad M, AL-Sharqawi A, Abu Dayyih W, Mallah E and Arafat T:Simultaneous estimation of esomeprazole and tadalafil in pharmaceutical formulations using High Performance Liquid Chromatography. Journal of Applied Pharmaceutical Science.2016; 6: 52-59.
15. Murthy TGK and Geethanjali J: Development of a validated RP-HPLC method for simultaneous estimation of metformin hydrochloride and rosuvastatin calcium in bulk and in-house formulation. Journal of Chromatography and Separation Techniques. 2014; 5: 252-260.
16.. Prabhakar Vishvakarma, Jaspreet Kaur, Gunosindhu Chakraborthy, Dhruv Kishor Vishwakarma, Boi Basanta Kumar Reddy, Pampayya Thanthati, Shaik Aleesha, Yasmin Khatoon. Nephroprotective Potential of Terminalia Arjuna Against Cadmium-Induced Renal Toxicity by In-Vitro Study. J. Exp. Zool. India Vol. 28, No. 1, pp. 939-944, 2025
17.. Shrikrishna B, Mulgund SV and Ranpise NS: Simultaneous spectrophotometric estimation of vildagliptin and metformin in bulk and tablet dosage form. Der Pharma Chemica. 2013; 5: 24-27.
18. Kar M and Choudhury PK: HPLC method for estimation of metformin hydrochloride in formulated microspheres and tablet dosage form. Indian Journal of Pharmaceutical Sciences. 2009; 71: 318-320