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Rp-Hplc Method for Simultaneous Estimation of Vildagliptin and Metformin in Bulk and Pharmaceutical Formulations

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ABSTRACT

Background: Combination therapy of vildagliptin and metformin provides a comprehensive effect on cardiovascular risk factors in the form of preventing hyperinsulinemia and reducing insulin resistance.

Objective: To develop a simple, fast, precise, accurate, sensitive RP-HPLC method for simultaneous estimation of metformin and vildagliptin in pure and tablet dosage forms.

Method: The mobile phase, a mixture of acetonitrile, methanol and water (15:60:25v/v) pumped at a flow rate of 1.0 ml/min through the column (C18; 5µ, 4.6 X 250 mm, Hypersil) at 35°C temperature. The mobile phase was degassed before use under vacuum by filtration through a 0.2µ nylon membrane. Concentrations were measured at 278 nm by a UV detector at a sensitivity of 20 µl.

Results: The linearity lies between 1-5 µg/ml for metformin and 1-5 µg/ml for vildagliptin in the method. The correlation coefficient (r^2) was found to be 0.982 and 0.998 for Metformin and vildagliptin, the limit of detection and limit of quantification were found to be 0.617and 1.87 µg/ml for metformin and 0.154 and 0.468 µg/ml for vildagliptin, respectively. The results of the analysis have been validated statistically by recovery studies as per International Conference on Harmonization guidelines.

Conclusion: The method showed good reproducibility and recovery with %RSD <2. Hence, the method was found to be rapid, specific, precise, and accurate and can be successfully applied for the routine analysis of metformin and vildagliptin in the pure and combined dosage form.

Key Words: Vildagliptin, Metformin hydrochloride, Antidiabetic drugs, RP-HPLC method, Simultaneous Estimation, Method development and validation

INTRODUCTION

Chemically known as 3-[diaminomethylidene]-1, 1-dimethylguanidine HCl, metformin HCl is an oral pharmaceutical product used to treat type 2 diabetes mellitus. Metformin is considered an antihyperglycemic drug because, without inducing hypoglycaemia, it reduces blood glucose concentrations in type II diabetes. Metformin is widely known as an insulin sensitizer that leads to a decrease in insulin resistance and a clinically important decrease in insulin levels from plasma fasting. Modest weight loss is another well-known advantage of this drug. For obese type II diabetes patients, Metformin is the medication of choice.[1,2]

Chemically, Vildagliptin is known as pyrrolidine-2-carbonitrile, (2S)-1-{2-[(3-hydroxyadamantan-1-yl) amino] acetyl}. Vildagliptin, previously known as LAF237, is a novel oral antidiabetic drug in the drug class of the new dipeptidyl peptidase-4 (DPP-4) inhibitor. Vildagliptin subsequently works by inhibiting glucagon-like peptide-1 (GLP-1) and gastric polypeptide inhibitor (GIP) inactivation by DPP-4.[3,4]

Literature review reveals that for estimation of metformin and vildagliptin in combination and individual dosage form[5-9] and validated with parameters,[10-16] various analytical methods such as UV-Vis spectroscopy, HPLC and LCMS / MS methods are available. Without using any buffers, the procedure with new composition was developed in the estimation of these compounds using RP-HPLC methods and also the development and validation of a simple, precise, fast and specific method for the determination of metformin and vildagliptin in pure form and its pharmaceutical dosage form were considered of interest.

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MATERIALS AND METHODS

Chromatographic conditions

Column: ODS (4.6×250mm, 5µm, Hypersil)

Mobile phase: ACN: Methanol: Water (15:60:25)

Flow rate: 1ml/min

Column temperature: 35°C

The volume of injection: 20µl

λmax: 278nm

Degradation studies

The forced degradation was carried under acidic, basic, oxidative conditions. Both the drugs were separately exposed to stress conditions. After exposing stress conditions, drugs were diluted to the standard concentration of Metformin (30µg/ml & 160µg/ml) and Vildagliptin (30µg/ml & 160µg/ml). Equal volume of both drugs were mixed and analyzed in the chromatographic conditions. The acid stress condition was carried out using 1N HCl, when mixed with the drug solution and kept for 48hours and then injected into the chromatographic column, degradants evaluated using chromatograms. The alkali hydrolysis was carried out using 1N NaOH, when mixed with the drug solution and kept for 48hours and then injected into the chromatographic column to evaluate the degradedness. Hydrogen peroxide is a strong oxidant when 3% H_2O_2 was added to the 1000µg/ml drug and kept for 48hours and injected into the chromatographic column to check the degradation**.**

RESULTS

Validation by Method

The method defined has been validated, including parameters such as suitability of the system, linearity, accuracy, precision, robustness, LOD and LOQ.

System suitability

Results of the system suitability study are summarized in the above table 1. Six consecutive injections of the standard solution showed uniform retention time, theoretical plate count, tailing factor and resolution for both the drugs which indicate a good system for analysis.

Specificity

The analytical peak was evaluated as per the methodology and observed the interference of blank, placebo with the analyte peak was there or not. Metformin and vildagliptin peaks were observed at their respective retention times of analyte peaks in figure 3 and 4.When the blank solution was injected, no peak was found in figure 2. The forced degradation study showed the method was highly specific; the chromatographic peak does not interfere with any other impurities. This proves that excipients do not affect the analytical method. On the other hand, the blank peak did not overlap the drug peak. So the method is highly selective.

Accuracy

Validated the accuracy in this method, accurately quantify metformin and vildagliptin tablets content at 50%, 100%, 150% and performed assay in triplicate. The mean per cent recovery of metformin and vildagliptin at each spike level should be not less than 98% and not more than 102%. Results of the accuracy study are presented in table 2. The measured value was obtained by the recovery test. The spiked amount of both the drugs were compared against the recovery amount.

Precision

The precision of the test method by preparing six test preparations using the product blend by mixing the active ingredient with excipients as per the manufacturing formula was determined. Repeatability of Standard metformin and vildagliptin solution was injected six times and peak areas were measured and metformin &vildagliptin per cent RSD was found to be 0.566 & 1.903. Results are given in table 3.

Linearity

A linear relationship between peak areas versus concentrations was observed for metformin and vildagliptin in the range of 50% to 150% of normal concentration. The correlation coefficient was 0.982 for metformin and0.988 for vildagliptin. This proves that the method is linear in the range of 50% to 150 %. Results are given in Table 4.

Robustness

The results of the robustness of the present method had shown by changes in the flow rate and wavelength did not produce significant changes in analytical results which were presented in table 5. As the changes are not significant we can say that the method is robust.

The Detection Limit

LOD for Hydrochloride of metformin = 0.6177

LOD = 0.1544 for vildagliptin

Limit of Quantification

LOQ for Hydrochloride of metformin = 1.8711

LOQ = 0.4688 for vildagliptin

The LOD and LOQ values indicate that the method developed was sensitive, precise and reliable.

Studies of stability

Comparative studies were performed on drugs called metformin and vildagliptin before and after degradation, and acid, base and oxidative degradation results were reported in Table 6.

Research on Stress Degradation

The properties of stress degradation were analysed using a validated chromatographic method for metformin and vildagliptin. Studies of forced degradation are listed in the table. Reports show that the validated approach effectively isolated and separately classified the degradation products. From the reports, it is very clear that drugs were responsive to acidic environments where there was less degradation.

DISCUSSION

The proposed method for the simultaneous determination of metformin and vildagliptin in pharmaceutical dosage form was found to be precise, selective, rapid and economical.[5] The interaction study between the two drugs in the standard solution was carried out by comparing peaks of each drug individually with peaks obtained in drug mixture indicating that the analytes did not interact with each other and data were within the acceptance level of ±2.0%.[1] The linearity for detector response was observed in the concentration range of 50 to 150% of test concentration and the correlation coefficient (r) for the calibration curve was found to be 1.0. Per cent recovery was found to be within the range of 98.0 % to 102.0% indicating the accuracy of the method.[6] The per cent RSD for the tablet analysis and recovery studied is less than 2 which is indicating a high degree of precision.[5] The results of recovery studies were found to be linear in 50 % to 150 % of the final assay concentration range indicating linearity and range of the proposed method. The results of the robustness study indicate that the method is robust and is unaffected by small variations in the wavelength and flow rate trails.[7] Hence, it can be concluded that the developed RP-HPLC method is accurate, precise, rapid and selective and can be employed successfully for the estimation of metformin and vildagliptin in bulk and pharmaceutical dosage forms.[8] Degradation was not observed in metformin and vildagliptin stressed samples that were subjected to acid hydrolysis and oxidative conditions. However, degradation was observed under base hydrolysis. This indicates that the method is specific and stability-indicating.[1]

CONCLUSION

In pharmaceutical formulations, the author sheds light on the enhancement of HPLC methods for estimating metformin hydrochloride and vildagliptin. It is usually important to design methods in which a very large number of samples are to be analysed with appropriate precision and accuracy in a very short period. It is possible to obtain qualitative results via the HPLC process and can thus be used in analytical analysis. This is an effective technique that provides good results for validation parameters. This approach explicitly performs well on Metformin hydrochloride and Vildagliptin.

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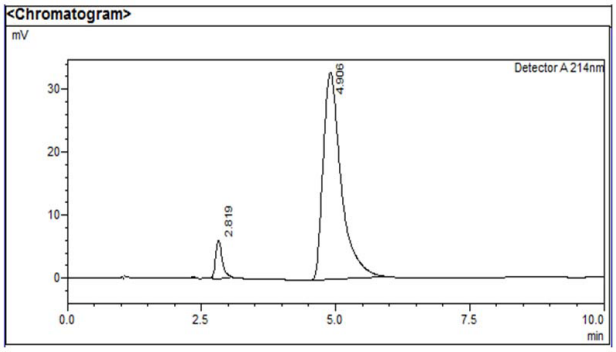

Figure 1: Chromatogram for the optimized method.

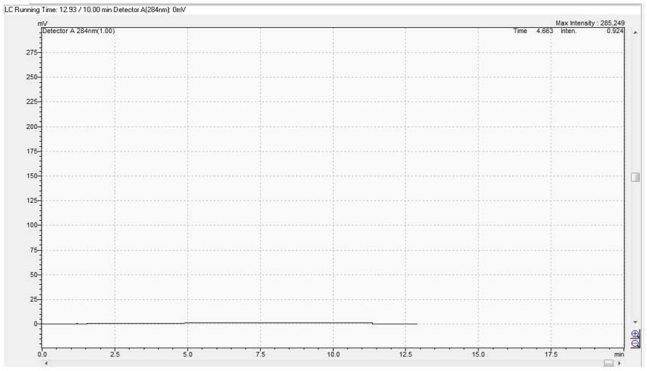

Figure 2: Typical chromatogram of the blank solution.

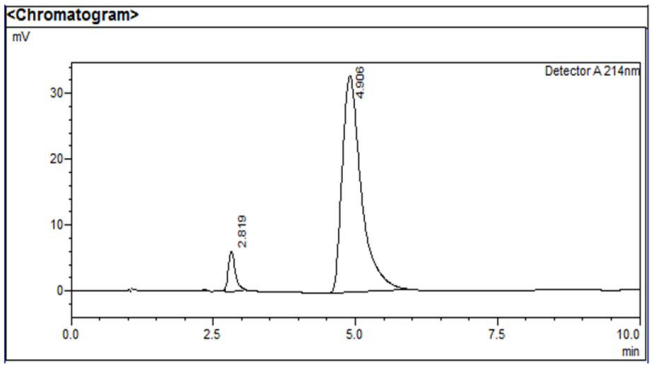

Figure 3: Typical chromatogram of the standard solution.

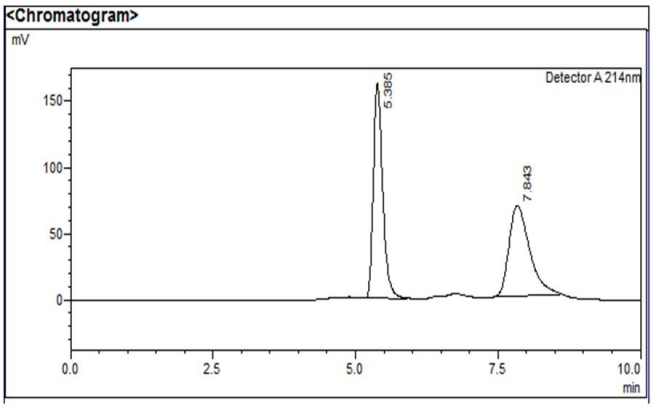

Figure 4: Typical chromatogram of the sample solution.

Table 1: System suitability for Metformin and Vildagliptin

Sample	Injection	RT (Min)	Peak Area	USP Plate count	USP Tailing
Metformin	1	4.960	76492	7242	1.653
	2	4.906	76816	7665	1.709
	3	4.856	78590	7876	1.732
	4	4.823	76262	8260	1.760
	5	4.776	77416	8205	1.773
	Mean		77115.2		
	SD		931.579		
	% RSD		1.208		
Vildagliptin	1	2.823	49597	16529	-
	2	2.819	48742	17463	1.420
	3	2.834	47396	17549	-
	4	2.831	48223	18867	1.193
	5	2.838	47359	17408	1.443
	Mean		48263.4		
	SD		945.978		
	% RSD		1.96		

Table 2: Accuracy data for Metformin and Vildagliptin

Sample	Accuracy level	Sample name	Sample weight	µg/ml added	µg/ml found	% recovery	% Mean
		1	50	50	48.674	96.34%	
	50%	2	50	50	49.232	99.36%	99.34%
		3	50	50	51.168	102.33%	
		1	100	100	101.171	101.17%	
Metformin	100%	2	100	100	100.863	100.86%	101.33%
		3	100	100	101.96	101.96%	
		1	150	150	146.623	97.748%	
	150%	2	150	150	152.912	101.94%	100.06%
		3	150	150	150.753	100.50%	
		1	50	50	49.21	98.42%	
	50%	2	50	50	50.479	100.95%	50%
		3	50	50	48.302	96.64%	
		1	100	100	107.78	107.78%	
Vildagliptin	100%	2	100	100	95.98	95.98%	100%
		3	100	100	100.138	100.138%	
		1	150	150	145.71	97.14%	
	150%	2	150	150	150.674	100.44%	150%
		3	150	150	153.711	102.474%	

Table 3: Precision data for Metformin and Vildagliptin

Sample	Injection No	R_t (mins)	Area	Tailing Factor	Plate count
	1	4.776	774164	1.773	8250
	2	4.823	762622	1.760	8260
	3	4.856	765900	1.732	7876
	4	4.906	767672	1.680	7642
Metformin	5	4.960	764921	1.653	7242
	6	4.783	771735	1.685	7158
	Mean		767835.66		
	Std. Dev.		4352.098		
	%RSD		0.566		
	1	2.838	47359	1.443	17408
	2	2.831	47223	1.193	18867
	3	2.835	45396	-	17549
	4	2.819	47582	-	16856
Vildagliptin	5	2.823	47597	-	16529
	6	2.890	47872	1.433	18695
	Mean		47171.5		
	Std. Dev.		897.827		
	% RSD		1.903		

Table 4: Calibration curve data for Metformin and Vildagliptin

Sample	Concentration (µg/ml)	Peak Area	Correlation co-efficient
Metformin	1	472645	0.982
	2	487296	
	3	494104	
	4	501819	
	5	512770	
Vildagliptin	1	124157	0.998
	2	142770	
	3	163685	
	4	185745	
	5	203685	

Table 5: Robustness data for Metformin and Vildagliptin

Sample	Parameter	R_t (min)	Peak Area	Tailing factor	Theoretical plates
Metformin	Limits	>2 min		NMT 2.0	NLT 2000
	Flow rate(0.8mL/min)	7.544	980324	1.615	6434
	Flow rate(0.6mL/min)	10.347	1289313	1.337	4588
	Wave length (219nm)	4.792	748602	1.664	8170
	Wave length (209nm)	1.685	831735	1.685	7158
Vildagliptin	Limits	>2 min		NMT 2.0	NLT 2000
	Flow rate(0.8mL/min)	7.544	980324	1.615	6434
	Flow rate(0.6mL/min)	10.347	1289313	1.337	4588
	Wave length (219nm)	4.792	748602	1.664	8170
	Wave length (209nm)	1.685	831735	1.685	7158

Table 6: Stability data for Metformin and Vildagliptin

Sample	Degradation studies	Rt (min)	Area	Theoretical plates	Tailing factor
Metformin	Acid Degradation-30µg/ml	4.740	770848	842	1.635
	Acid Degradation-160µg/ml	5.337	235206	346	1.216
	Alkali Degradation - 30µg/ml	2.331	278667	890	1.297
	Alkali Degradation -160µg/ml	2.403	424477	1466	-
Vildagliptin	Oxidation -30µg/ml	4.770	30339	1413	2.462
	Oxidation -160µg/ml	4.806	68162	1309	2.467
	Acid Degradation-30µg/ml	2.035	37277	306	0.883
	Acid Degradation-160µg/ml	1.972	31169	237	1.231
	Alkali Degradation-30µg/ml	2.391	266639	1980	1.838
	Alkali Degradation-160µg/ml	2.352	445057	1459	-
	Oxidation -30µg/ml	2.889	224263	1171	-
	Oxidation -160µg/ml	3.712	163058	625	2.443