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Research article

Development and Validation of Simultaneously Estimation of Vildagliptin & Metformin Hydrochloride by RP-HPLC in Bulk and Oral Dosage Form

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ABSTRACT

A new, simple, accurate, precise and rapid reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and subsequently validated for the simultaneous estimation of Vildagliptin and Metformin HCl in pure and oral dosage form. The proposed method is based on the separation of the two drugs in reversed-phase mode using ZODIACcolumn (250×4.6 mm I.D., 5µm particle size). The optimized mobile phase was disodium hydrogen phosphate buffer (pH 3.5): methanol in the ratio 73.5:26.5 v/v. The flow rate was at 1.0 ml/min and UV detection at 200 nm. The retention times were 2.490 and 4.243 for Metformin HCl and Vildagliptin respectively. The method was validated according to ICH guidelines. It was found to be accurate and reproducible. Linearity was obtained in the concentration range of 75-175µg/ml for Metformin HCl and 7.5-17.5µg/ml for Vildagliptin. Mean percent recovery of samples at each level for both drugs were found to be 98.88% for Metformin HCl and 99.85% for Vildagliptin. The proposed method can be successfully applied in the quality control of bulk and pharmaceutical dosage forms.

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Key Words
Vildagliptin, Metformin HCl, Validation, RP-HPLC, Simultaneous estimation.

INTRODUCTION

Vildagliptin chemically (S)-1-[N-(3-hydroxy-1-adamantyl) glycyl] pyrrolidine-2-carbonitrile, is a potent di-peptidyl peptidase IV (dip-IV) inhibitor, a drug for the treatment of diabetes. DPP-IV inhibitors represent a new class of oral antihyperglycemic agents to treat patients with type 2 diabetes[1-4].

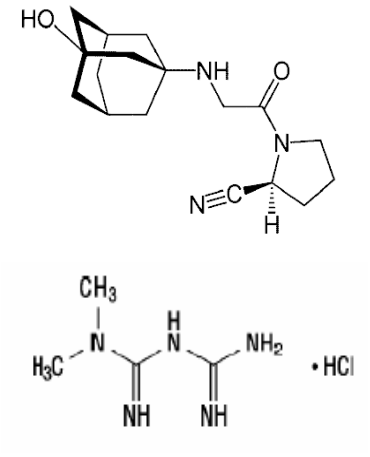

Fig 1. Chemical Structure of Vildagliptin and Metformin HCl

Metformin HCl (MET) chemically N, N-(dimethyl imidodicarbonimidicdiamide HCl) is an orally administered biguanide widely used in the treatment of type 2 (non-insulin dependent) diabetes mellitus[5-6]. MET has been reported to be determined by UV spectroscopy[9], HPLC [10], HPTLC[11], and LC/MS[12] from formulations and Vildagliptin has reported UV spectroscopic[13], RP-HPLC[14], RP-LC/MS[15], from different formulation and composition. Simultaneous determination of Vildagliptin and Metformin HCl in pharmaceutical dosage forms was reported by HPLC. However, there is no method reported for the simultaneous determination combination of these two drugs by RP-HPLC. Therefore, an attempt was made to develop a new, rapid, and sensitive method for the simultaneous estimation of Vildagliptin and Metformin HCl. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines.

MATERIALS AND METHOD

Materials and reagents

Vildagliptin, Metformin HCl, Methanol, Acetonitrile, Disodium hydrogen Phosphate and Ortho phosphoric acid were procured from Chandra Labs, Hyderabad, India. Commercial tablet (Galvusmet) containing 500 mg of Metformin HCl and 50 mg of Vildagliptin combination procured from a local Pharmacy in Hyderabad.

Chromatographic Conditions

The HPLC system used was Shimadzu with model SPD-10A equipped with UV detector source of deuterium lamp. The chromatogram was recorded and peaks quantified by means of PC based Spinchrome software.

The separation was achieved on ZODIAC C18 (250 x 4.6mm, 5µ) analytical column. The mobile phase consisted of disodium hydrogen phosphate buffer (pH 3.5): methanol in the ratio of 73.5:26.5 v/v (pH 3.5 was adjusted with orthophosphoric acid). The flow rate was 1.0 ml/min and UV detection performed at 200 nm. The mobile phase was shaken on an ultrasonic bath for 30 min. The resulting transparent mobile phase was filtered through a 0.45µ membrane filter. The injection volume was 20µl and all the experiments were performed at ambient temperature.

Preparation of Standard Stock Solution

Accurately weigh 125mg of Metformin HCl and 12.5mg of Vildagliptin and transfer into a clean and dry 100ml volumetric flask, dissolve with sufficient volume of mobile phase and make up to 100ml with mobile phase. 5ml of stock solution was further diluted in a 50ml volumetric flask with mobile phase to get a concentration of 125µg/ml of Metformin HCl and 12.5µg/ml of Vildagliptin.

Preparation of Sample Solution

Twenty tablets (Galvusmet) average weight was determined. The tablets were crushed into a fine powder. Accurately weigh and transfer the amount of powdered drugs equivalent to 125 mg of metformin HCl and 12.5mg of Vildagliptin into a 100 ml volumetric flask. Make up the volume with mobile phase. Sonicated for 15 min with intermittent shaking and filter through whatmann filter paper. 5ml of the sample stock solution was further diluted in a 50ml volumetric flask with mobile phase.

Method Validation

The method was validated in accordance with ICH guidelines[16-17]. The parameters assessed were System Suitability Parameters, Linearity, Accuracy, Precision, Specificity, Assay, Limit of Detection (LOD), Limit of Quantification (LOQ), Robustness, Ruggedness and Stability of the solution.

RESULTS AND DISCUSSION

Method Development

Lots of mobile phase and their different proportions were tried and finally were selected as disodium hydrogen phosphate buffer (pH 3.5): methanol in the ratio of 73.5:26.5 v/v (pH 3.5 was adjusted with orthophosphoric acid) and UV spectra of individual drugs were recorded at the wavelength from 190 to 400nm and the response for optimization was compared (Fig 2).The choice of

wavelength 200nm was considered satisfactory, permitting the detection of both drugs with adequate sensitivity (Fig. 3)

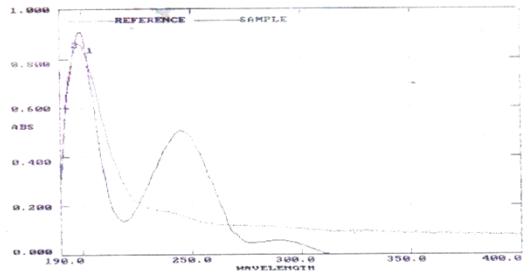

Fig 2. Comparison of UV Spectra of Vildagliptin and Metformin HCl

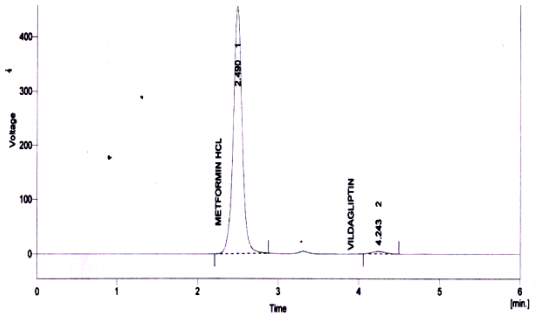

Fig 3. Typical Chromatogram for the simultaneous estimation of Vildagliptin and Metformin HCl

Method Validation

System Suitability

System performance parameters of developed RP-HPLC method were determined by injecting standard solutions. Parameters such as number of theoretical plates (N), tailing factor, resolution(R), retention time (RT) were determined. The results are shown in table 1. Asymmetry (tailing factor) should be Not More Than 2.0 and theoretical plates should be Not Less Than 2000

Linearity

Under the experimental conditions described above, linear calibration curves for the two drugs were obtained throughout the concentration ranges studied. The linear ranges of Metformin HCl and Vildagliptin are 75-175µg/ml and 7.5-17.5µg/ml respectively showed in table 2 & figure 4.

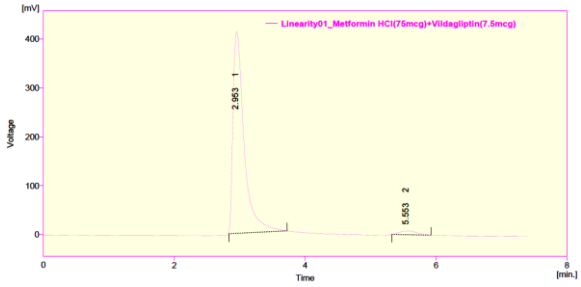

Fig 4. Chromatogram of Metformin HCl and Vildagliptin for 60% linearity range

Accuracy (recovery)

The % recovery at each level and Mean Recovery for Metformin HCl and Vildagliptin should be between 98.0% and 102.0%. The results were given in table 3.

Precision

Precision is the degree of closeness of agreement among individual test results when the method is applied to multiple samplings of a homogenous sample. The results were showed in table 4. % RSD for the areas of six

sample injection results should not be more than 2.0%.

Specificity

Specificity is the ability to measure accurately and specifically the analyte in the presence of components that may be expected to be present in the sample.

No interference from any of the excipients was found at retention times of the examined drugs. These results demonstrate the absence of interference from other materials in the pharmaceutical formulations and therefore confirm the specificity of the proposed method.

Tab 1. System Performance for Metformin HCl and Vildagliptin

Sample	**Retention time (min)**	**Area (mV.s)**	**Height (mV)**	**Resolution**	**Efficiency (T.P)**	**Asymmetry**
Metformin HCl	2.490	3474.188	455.77	6.729	2524	1.059
Vildagliptin	4.243	56.259	4.808		2763	1.149

Tab 2. Linearity – Regression analysis data

Parameters	**Metformin HCl**	**Vildagliptin**
Slope	1394.2	34.449
Y-Intercept	3490.9	111.54
Correlation Coefficient, R^2	0.998	0.9988

Sensitivity

LOD was found to be 0.09ppm for metformin and 0.38ppm for vildagliptin and LOQ was found to be 0.28ppm for metformin and 1.15ppm for vildagliptin indicating high sensitivity of the method. The results are showed in table 6.

Robustness

The robustness of the method was determined to check the reliability of an analysis with respect to deliberate variations in method parameters. The results are showed in table 7 & 8.

Tab 3. Accuracy-% Recovery of each analyte

Sample	Accuracy	Peak area	Mean % recovery
Metformin HCl	88%	6849.93	98.79
	88%	6915.29	
	88%	6898.71	
	108%	8016.46	98.41
	108%	8051	
	108%	8091.98	
	128%	9794.02	99.44
	128%	9741.53	
	128%	9763.34	
Vildagliptin	88%	202.267	99.52
	88%	193.962	
	88%	199.545	
	108%	221.565	98.59
	108%	224.381	
	108%	229.836	
	128%	259.164	101.45
	128%	265.604	
	128%	286.225	

Tab 4. Precision (Repeatability) data

Injection No	Metformin HCl		Vildagliptin	
	Retention time (min)	Peak area	Retention time (min)	Peak area
1	2.717	7566.372	5.143	203.192
2	2.757	7477.095	5.117	201.565
3	2.713	7522.690	5.150	205.089
4	2.693	7537.107	5.100	202.394
5	2.680	7501.017	5.080	202.984
6	2.653	7512.803	5.020	209.208
Mean	2.70	7519.51	5.10	204.07
SD	0.0356	30.665	0.048	2.774
%RSD	1.32	0.41	0.94	1.36

Tab 5. Assay Data of standard and sample chromatograms

	Metformin HCl		**Vildagliptin**	
	S.No	**Peak Area**	**S.No**	**Peak Area**
Standard Area	1	7501.548	1	217.163
	2	7613.004	2	206.306
	3	7590.766	3	226.597
	4	7515.666	4	155.488
	5	7313.392	5	184.248
	Average	7568.439	**Average**	197.96
Sample area	1	7404.109	1	155.488
	2	7226.054	2	184.248
	3	7314.196	3	177.385
	4	7600.216	4	217.163
	5	7590.766	5	230.389
	Average	7427.068	**Average**	192.934
%Assay		98.96		98.14

Tab 6. Linearity Data (LOD, LOQ)

Levels	**Metformin HCl**		**Vildagliptin**	
	Conc. (ppm)	**Peak area**	**Conc. (ppm)**	**Peak area**
Level 1 (60%)	75	4853.636	7.5	146.798
Level 2 (80%)	100	6338.593	10	181.417
Level 3 (100%)	125	7577.095	12.5	211.565
Level 4 (120%)	150	9207.908	15	249.807
Level 5 (140%)	175	10389.87	17.5	284.846
SD(σ)	39.5	2207	3.95	55
Slope(S)	1394.0		200.79	
Y-Intercept	2790.1		34.44	
LOD	0.09ppm		0.38ppm	
LOQ	0.28ppm		1.15ppm	

Tab 7. Robustness data for effect of flow rate variation

Flow rate (mL/min)	Metformin HCl				Vildagliptin		
	Injection No.	R_t (min)	Efficiency (T.P.)	Asymmetry	R_t (min)	Efficiency (T.P.)	Asymmetry
0.8	1.	3.040	2380	1.429	5.343	4706	1.244
1.2	1.	2.043	2033	1.400	3.593	3830	1.194

* R_t = Retention Time T.P. = Theoretical Plates

Tab 8. Robustness data for effect of wavelength variation

Wavelength (nm)	Metformin HCl				Vildagliptin		
	Injection No.	R_t (min)	Efficiency (T.P.)	Asymmetry	R_t (min)	Efficiency (T.P.)	Asymmetry
198	1.	2.450	2309	1.379	4.293	4343	1.270
202	1.	2.453	2192	1.448	4.300	4173	1.214

* R_t = Retention Time, T.P. = Theoretical Plates

Tab 9. Ruggedness Data

	Analyst -1		Analyst -2	
	Metformin HCl	Vildagliptin	Metformin HCl	Vildagliptin
Standard Area	7427.632	206.589	7655.620	214.299
Sample Area	7537.107	222.394	7597.595	214.817

Ruggedness

Ruggedness is the degree of reproducibility of the results obtained under a variety of conditions. It was checked that the results were reproducible under differences in conditions, analysts and instruments. The results are showed in table 9.

Stability of the Solution

Stability of the solution was studied by testing the drug solutions to various stress conditions like acidic

(adding 0.1ml of 0.1 N HCl to drug solution), alkaline(adding 0.1ml of 0.1 N NaOH to drug solution), oxidative(adding 0.1ml of 50% H_2O_2 to drug solution), thermal(exposing drug solution to heat by placing in a water bath) and photolytic(placing drug solution under UV lamp) conditions. Stability was checked for 24hrs at room temperature. On comparing the forced degradation conditions with the normal condition, the drugs were found to be stable.

CONCLUSION

The new HPLC method developed and validated for simultaneous estimation of Metformin HCl and Vildagliptin pharmaceutical dosage forms and assured the satisfactory Ruggedness and Robustness and also determining lower concentration of each drug in its solid combined dosage form by RP-HPLC method. The method was found to be simple, accurate, economical and rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation.

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