RESEARCH ARTICLE

Forced Degradation Method Development and Validation for Simultaneous Determination of Vildagliptin, Metformin Hydrochloride and Remogliflozin Etabonate in Bulk and its Formulation by RP-HPLC

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ABSTRACT

A simple and efficient stability-indicating method has been developed and validated for the simultaneous estimation of vildagliptin, metformin hydrochloride and remogliflozin etabonate in bulk and was applied on marketed formulations. KH2PO4 buffer (10 mM): Acetonitrile (70:30% v/v) at pH 5 was used as a mobile phase. Detection of drug peaks was at 215 nm by UV detector. The method was found to be linear in the concentration range of 2.5 to 7.5, 25 to 75 and 5 to 15 µg/mL for vildagliptin, metformin hydrochloride and remogliflozin etabonate, respectively. The limit of detection (LoD) and quantitation (LoQ) were found to be 0.137 and 0.415 µg/mL for vildagliptin, 3.737 and 11.326 µg/mL for metformin hydrochloride and 0.348 and 1.055 µg/mL for remogliflozin etabonate. Hydrolysis by HCl, NaOH, hydrogen peroxide, UV light and temperature were performed on a formulation which proves that the proposed method was specific.

Keywords: RP-HPLC, Metformin hydrochloride, Vildagliptin and Remogliflozin etabonate, Stability indicating, Validation**.**

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INTRODUCTION

Metformin hydrochloride is used in type-2 diabetes treatment as an oral hypoglycaemic agent. The chemical name of metformin hydrochloride (MET) is (3-(diamino methylidene)-1,1-dimethylaniline hydrochloride (shown in Figure 1). Its molecular formula is $C_4H_{12}ClN_5$.[1] Vildagliptin (VIL) is under the dipeptidyl peptidase class. Chemical name of it is (2S)-1-[2- [(3-hydroxy -1-admantyl) amino] acetyl]pyrolidene-2-carbonitrile[2] (shown in Figure 2). Remogliflozin etabonate (REM) is an example of sodium-glucose co-transporter 2 inhibitors class.[3] The chemical name of it is ethyl [(2R,3S,4S, 5R, 6S)-3,4,5-trihydroxy- 6-[5-methyl-1-propan-2-yl- 4-[(4-propan- 2-yloxyphenyl)methyl] pyrazol-3-yl] oxyoxan-2-yl] methyl carbonate (shown in Figure 3). Its molecular formula is $C_{26}H_{38}N_2O_9$.

The literature survey found that for this triple drug formulation, no forced degradation study was performed.[4-14] So, the present research aimed to develop a stability indicating reverse phase liquid chromatographic method for the simultaneous determination of MET, VIL and REM in bulk and drug formulation suitable for routine analysis.

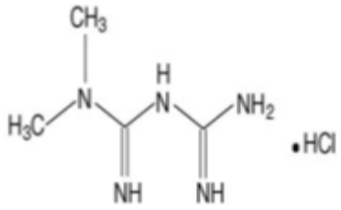

Figure 1: Chemical structure of metformin hydrochloride (MET)

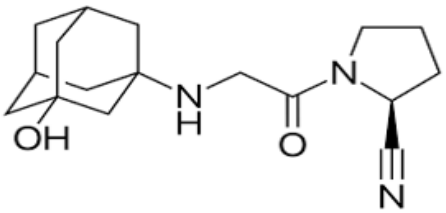

Figure 2: Vildagliptin chemical structure

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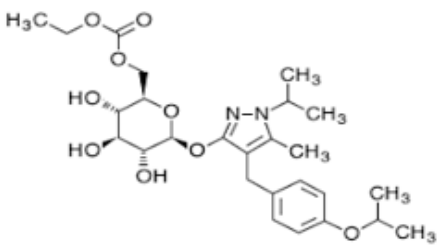

Figure 3: Remogliflozin etabonate (REM) chemical structure

MATERIALS AND METHODS

Materials

MET, VIL and REM were obtained as gift samples from Elikem Private Limited, Ahmedabad, Cadila Pharmaceuticals and Glenmark Pharmaceuticals Limited. Acetonitrile (ACN), water and methanol were of HPLC-grade (E. Merck. Chem. Limited, Mumbai). Other chemicals used were of analytical reagent grade (CYNOR Pharma Private Limited).

Instrument Used

Agilent 1200 infinity II LC model was used as a high-pressure liquid chromatograph equipped with 1260 Quat pump VL and a PDA detector. Column $C_{18,}$ having a particle size of 250 mm x 4.6 mL, 5 µm was used. EZ Chrom software was used for integration.

Selection of UV Wavelength

Drug peaks were detected at 215 nm using UV detector.

Optimization of Mobile Phase

From different trials, KH_2PO_4 buffer: Acetonitrile in the ratio of 70:30% v/v, pH 5 at 1-mL/min flow rate gave a better result than the other mixtures of solvents. Figure 4 indicates a chromatogram of selected combinations at optimized conditions.

Procedure

Standard stock solutions preparation

About 50, 5 and 10 mg of MET, VIL and REM were dissolved in 100 mL methanol to prepare 500, 50 and 100 µg/ mL for MET, VIL and REM stock solutions. One mL from these standard stock solutions was taken and transferred to different 10 mL volumetric flasks and 50, 5 and 10 µg/ mL of MET, VIL and REM standard solutions, respectively.

Calibration curve

The solutions for linearity assessment were prepared in the concentration range of 25 to 75, 2.5 to 7.5, 5 to 15 µg/mL of MET, VIL and REM, respectively.

Preparation of sample solution

Accurately weighed crushed 20 tablets of powder was taken in the ratio of 10 : 1: 2 for MET, VIL and REM, respectively to prepare sample stock solution containing 500, 50 and 100 µg/mL concentrations of MET, VIL and REM.

Force degradation study

Hydrolysis by acid (5 mL of 1N HCl, 2 hours at 60°C, neutralize with 2 N NaOH), hydrolysis by base (5 mL of 0.1N NaOH, 1-hour at 70°C, neutralize with 2 N HCl), degradation by hydrogen peroxide (5 mL of 3% H_2O_2, 30 minutes at 60°C), photo-degradation in UV chamber for 24 hours and thermal degradation at 70°C for 3 hours were performed on sample solution and injected for different degradation studies. Obtained chromatograms are shown in Figures 5-9.

Method validation

Accuracy, system suitability, precision, robustness, specificity, limit of detection and quantitation parameters were performed to check the validation of the method according to ICH guidelines.

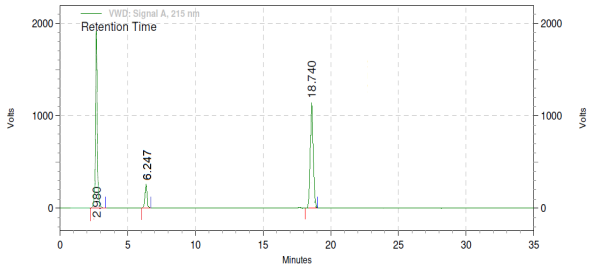

Figure 4: Chromatogram of MET, VIL and REM (50:5:10 µg/mL) using KH2PO4 Buffer (10 mM): Acetonitrile (70:30% v/v) at pH 5.

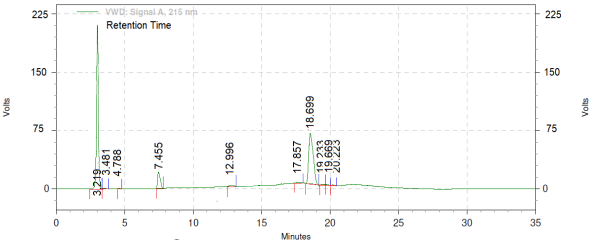

Figure 5: Degradation by acid (1NHCl, 2 hours)

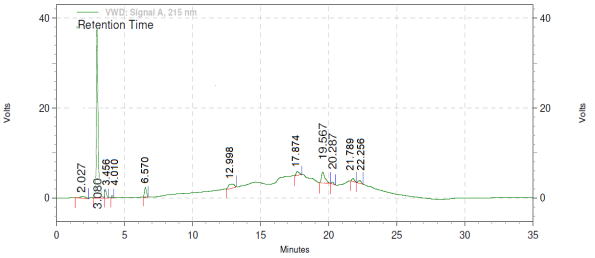

Figure 6: Base degradation of sample (0.1 M NaOH, 1 hour)

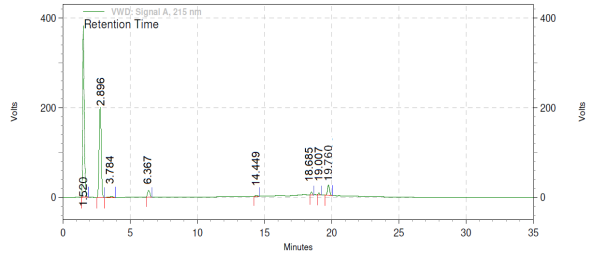

Figure 7: Oxidative degradation (3% H_2O_2, 30 minutes)

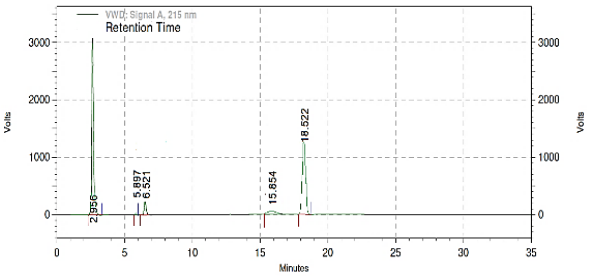

Figure 8: Photo degradation of sample solution at 24 hours

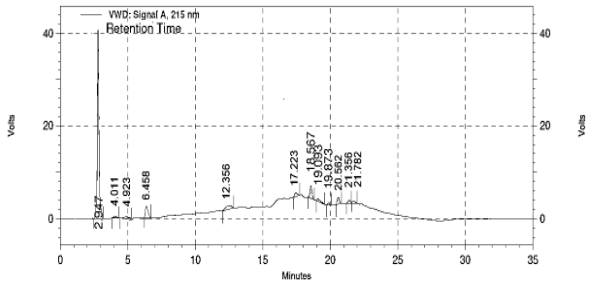

Figure 9: Thermal degradation at 70°C, 3 hours

Assay of marketed sample

Tablet formulation (Remozen MV 500 manufactured by Glenmark Pharmaceuticals) containing 500 mg of MET, 50 mg of VIL and 100 mg REM was analyzed using the proposed method.

RESULTS

Outcomes of Forced Degradation Studies

MET, VIL and REM were undergoing degradation under different stress conditions. The outcomes of these studied are shown in Table 1.

Results of Method Validation

Specificity

The different between time of retention of reference and sample was found to be ± 0.001, ± 0.027 and ± 0.008 minutes for MET, VIL and REM, respectively.

Precision

The results found are given in Table 2.

Table 1: Results of stability study

Types	*Conditions of degradation*	*% of degradation*		
		MET	*VIL*	*REM*
Acid	1 N HCl for 2 hours at 60°C	8.30	7.69	16.43
Alkali	0.1 N NaOH for 1-hour at 70°C	18.22	8.82	15.41
Oxidation	3% H_2O_2 for 30 minutes at 60°C	15.54	13.82	16.55
Thermal	70°C for 3 hours.	8.41	11.35	21.32
Photolysis	UV chamber for 24 hours	9.93	17.38	11.92

Table 2: Data of intermediate precision (n = 3)

Drug	*Conc. (µg/mL)*	*Interday*		*Intraday*	
		Peak area ± SD.	*%R.S.D.*	*Peak area ± S. D.*	*%R.S.D.*
MET	25	633129545 ± 4934344.62	0.78	635328662 ± 1083945.48	0.17
	50	71295797036 ± 39488.26	0.51	712624340 ± 3817976.75	0.54
	75	785892076 ± 2616825.80	0.33	785701333 ± 2339077.12	0.30
VIL	2.5	17564438 ± 218879.88	1.25	17697772 ± 320319.22	1.81
	5.0	35511822 ± 160458.71	0.45	35678489 ± 222128.71	0.62
	7.5	52554974 ± 223036.18	0.42	52621641 ± 298047.77	0.57
REM	05	10323954 ± 1363132.24	1.32	104106301 ± 1968767.25	1.89
	10	203657098 ± 1170399.47	0.57	203323764 ± 1584945.40	0.78
	15	302918378 ± 1452779.36	0.48	303251711 ± 1211684.03	0.40

Table 3: Data of linearity of MET, VIL and REM (n = 3)

	MET		*VIL*		*REM*	
No.	*Conc. (µg/mL)*	*Area*	*Conc. (µg/mL)*	*Area*	*Conc.(µg/mL)*	*Area*
1	25.0	630323773.23	2.50	17996960.67	5.0	102260643.35
2	37.5	670030962.71	3.75	26763744.67	7.5	151956988.23
3	50.0	717025809.36	5.00	36078489.11	10.0	205032485.32
4	62.5	745702619.74	6.25	44205131.33	12.5	256798749.36
5	75.0	788403071.32	7.50	53192870.67	15.0	302891159.79

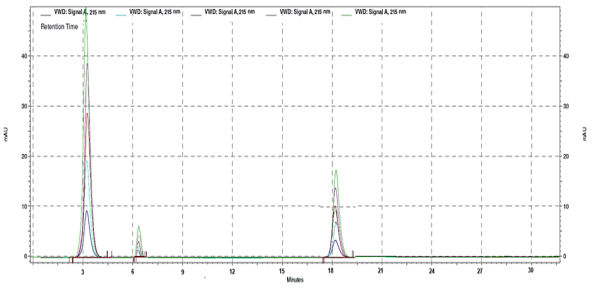

Figure 10: Overlain chromatograms of linearity in standard solutions

Table 4: Recovery data for MET, VIL and REM (n = 3)

	MET		*VIL*		*REM*	
No.	*Amount added (µg /mL)*	*%Mean recovery ± SD.*	*Amount added (µgmL)*	*%Mean recovery ± S.D.*	*Amount added (µg mL)*	*%Mean recovery ± SD.*
1	25	99.13 ± 0.49	2.5	99.73 ± 0.61	5	100.73 ± 1.47
2	50	98.96 ± 0.44	5.0	100.13 ± 1.53	10	100.76 ± 1.69
3	75	99.60 ± 0.50	7.5	100.53 ± 0.83	15	100.66 ± 0.90

Table 5: Results of limit of detection and quantitation in µg/mL

	MET	*VIL*	*REM*
LoD	3.737	0.137	0 .348
LoQ	11.326		1. 550

Table 6: Data of robustness parameters

	%R.S.D.					
	Flow rate (mL/min)		*pH*		*Mobile phase (%v/v)*	
Drug	0.8	1.2	4.8	5.2	68:32	72:28
MET	0.921	1.003	1.140	1.176	1.122	1.436
VIL	1.353	1.366	1.353	1.365	1.431	1.424
REM	1.110	1.434	0.991	1.229	1.431	0.980

Table 7: Assay of marketed sample

Tablet	*Remo-zen MV 500*		
Label claim	*MET (500 mg)*	*VIL (50 mg)*	*REM (100 mg)*
Assay (% Mean ± S. D.)	99.42 ± 0.114	99.09 ± 0.106	99.51 ± 0.162

Table 8: Validation parameters summary

S. No.	*Parameters*	*MET*	*VIL*	*REM*
1	Linearity (µg/mL)	25–75	2.5–7.5	5–15
2	Equation of Regression (y = mx+ c)	y = 3000000 x+ 600000000	y = 7000000 x+ 514157	y = 20000000 x + 1000000
3	Slope (m)	3000000	7000000	20000000
4	Intercept (c)	600000000	514157	1000000
5	R^2 value	0.9959	0.9997	0.9995
6	LoD (µg/mL)	3.737	0.137	0.348
7	LoQ (µg/mL)	11.326	0.415	1.055

Linearity and range

A linear correlation was obtained between peak area and concentration, which is shown in Table 3. The overlain of chromatograms of linearity was shown in Figure 10.

Accuracy study and recovery

%Recoveries were found as shown in Table 4.

LoD and LoQ

Calculated values are shown in Table 5.

Robustness

Flow rate, mobile phase ratio and pH were changed to study robustness. %RSD was calculated, which was found to be less than 2. The RSD values are given in Table 6.

Assay of Marketed Sample

Tablet formulation (Remozen MV 500 manufactured by Glenmark Pharmaceuticals) containing 500 mg of MET, 50 mg of VIL and 100 mg REM was analyzed using the proposed method. Results are shown in Table 7.

Validation Parameters Summary

All the validation parameters data are shown in Table 8.

CONCLUSION OF METHOD

A specific, selective, sensitive and simple forced degradation RP-HPLC method was developed which is suitable for the determination of VIL, MET and REM in the presence of its degradation products in the triple-drug formulation. As per ICH guidelines, this method is robust, sensitive, accurate, selective and precise. The developed method is less time-consuming as well as cost-effective. It can be routinely applied for simultaneous VIL, MET and REM estimation.

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