RESEARCH ARTICLE

Concurrent Estimation of a Three Combination Type-2 Anti-diabetic Oral Dosage Form by Ultra-performance Liquid Chromatography

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ABSTRACT

Estimation of a three-combination dosage form containing metformin (MET), vildagliptin (VLDG), and remogliflozin etabonate (REMET) was performed by developing an economically simple and isocratic simultaneous method using ultra-performance liquid chromatograph (Alliance 1100 series) comprising of X-Bridge (50 × 4.6 mm, 2.5 µ) C18 column and photo diode array detector. 0.1 % v/v trifluoro acetic acid: acetonitrile (60:40% v/v) movable phase at 0.5 mL/min was used. The analytes were detected at a wavelength of 240.0 nm at 8.0 min. run time. The respective retention times of 1.004, 2.005, 5.118 min. was achieved concurrently for metformin, vildagliptin, and remogliflozin etabonate. The method was validated for various parameters according to international council for harmonization. Linearity was established at 187.50 to 1125.00 µg/mL (R^2 = 0.9997), 18.75 to 112.50 µg/mL (R^2 = 0.9990), 37.50 - 225.00 µg/ mL (R^2 = 0.9997). The respective limit of detection and quantification were 22.50; 75.0 (MET) 2.25; 7.5 (VLDG) and 4.50; 15.0 µg/mL (REM ET). A recovery (% w/w) of 99.0 ± 1 % was obtained for metformin, vildagliptin, and remogliflozin etabonate, respectively. Various degradation studies were conducted and degradants were identified at different retention times.

Keywords: Ultra-performance liquid chromatograph, Metformin, Vildagliptin, Remogliflozin etabonate, Degradation studies.

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INTRODUCTION

Diabetes is a long-lasting chronic condition that affects people of all ages, including older adults and children having obese character traits. Among the three, type-2 diabetes is the most serious of diabetes, where our body does not utilize insulin well and blood sugar levels may not be within normal ranges. Even though some lifestyle adjustments like diet control, and exercise may help bring blood glucose levels to normal, the condition is also treatable with pharmaceuticals. Therefore, several drugs were developed and became available for type-2 diabetes. REMO-ZEN MV 500 is a new three-combination developed by Glenmark in September 2021 with metformin (MET), vildagliptin (VLDG) and remogliflozin etabonate (REM ET) (Figure 1), to effectively treat type-2 diabetes to combat the drawbacks occurring with various two combination type-2 diabetes oral dosage forms.

Simultaneous determination of drugs in the formulation is performed as it is time-saving, feasible, and specific and assures that all the drug compounds in the formulation are as per the label claim mentioned individually. The stability of drugs in the presence of the degradation products is determined by forced degradation. A thorough literature study was done and a few liquid chromatographic methods were identified for the respective drugs in triple combination.[1-6] As few works were reported on the simultaneous quantification of three combinations, an attempt was made to develop and validate an economically rapid, isocratic simultaneous ultra-performance liquid chromatographic (UPLC) method for metformin, vildagliptin and remogliflozin etabonate.

MATERIALS AND METHODS

Chemicals and Reagents

The work was done using trifluoro acetic acid (Merck), acetonitrile (HPLC grade), and HPLC water (Milli-Q) of 99.9, 99.99 and 99.99% w/w purity respectively. Both sodium hydroxide (NaOH), and hydrochloric acid (HCl) were from Merck Millipore, and 30% w/v hydrogen peroxide (H_2O_2) from Thermo Fischer Scientific were also used for the study.

The standards of MET (100.90% w/w pure), VLDG (99.00% w/w pure), and REM ET (100.3% w/w pure) were provided by Glenmark Pharmaceuticals Private Limited, Hyderabad; Zydus Cadila Healthcare Limited, Gujarat; Supriya Life Sciences Limited, Mumbai, respectively and Remo - Zen MV 500 tablets were procured from Glenmark pharmaceuticals

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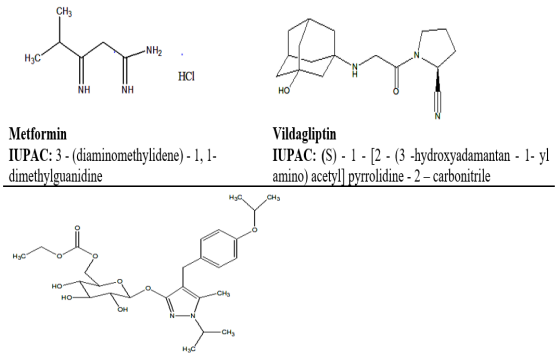


Metformin
IUPAC: 3 - (diaminomethylidene) - 1, 1-dimethylguanidine

Vildagliptin
IUPAC: (S) - 1 - [2 - (3 -hydroxyadamantan - 1- yl amino) acetyl] pyrrolidine - 2 – carbonitrile

Remogliflozin
IUPAC: Ethyl [(2R, 3S, 4S, 5R, 6S) - 3,4,5 - tri - hydroxy - 6 - [5 - methyl - 1 - propan - 2 - yl - 4 - [(4 - propan - 2 - yloxyphenyl) methyl] pyrazol - 3 - yl] oxyoxan - 2 - yl] methyl carbonate
(MET), Vildagliptin (VLDG) and Remogliflozin etabonate (REM ET) (Figure 1), to effectively

Figure 1: Chemical structures

private limited, Hyderabad. The tablets contain metformin hydrochloride, vildagliptin, and remogliflozin etabonate in a ratio of 10:1:2.

Instruments

The research study was executed on Alliance 1100 series ultra-performance liquid chromatography auto-sampler having X-Bridge C18 column of Waters (50 × 4.6 mm, 2.5 µ), a photodiode array detector, and a quarternary pump, operated with empower software (version 2.0). Shimadzu balance (AP 225 WD), and smart ultrasonic 3 bath were used for weighing and sonication, respectively.

Trifluoro Acetic Acid (0.1% v/v) Preparation

A 1.0 mL trifluoro acetic acid was pipetted, dissolved in 600 mL HPLC water and filtered under vacuum. This was made upto 1000 mL with HPLC water.

Standard Stock Preparation

In 188.0 mg MET, 19.0 mg VLDG and 38.0 mg REM ET was transferred into a 25.0 mL volumetric flask, dissolved and made up with diluent.

Sample Stock Preparation

A totla of 20 tablets were ground to a fine powder. A 154.0 mg powder was taken into a 10.0 mL volumetric flask, dissolved in little amount of diluent, sonicated (30.0 minutes), filtered using 0.45 µ filter and made up with diluent.

Method Optimization

A few trials were conducted using Agilent eclipse XDB (150 x 4.6 mm, 3.5 µ) and ammonium acetate pH – 4.6: ACN (30:70 v/v), (40:60 v/v); Phenomenex C18 (150 × 4.6 mm, 5 µ) and ammonium acetate pH – 4.6: ACN (20:80 v/v); Phenomenex C18 (150 × 4.6 mm, 5 µ) and 0.1% v/v trifluoro acetic acid (TFA): ACN (70:30 v/v); Waters X-Bridge C-18 (50 x 4.6 mm, 2.5 µ) and 0.1 % v/v TFA: ACN (60:40 v/v), (50:50 v/v), (40:60 v/v) at 0.5 mL/min. with 10.0 µL volume of injection to develop an isocratic UPLC method but resulted in peak splitting, broadening and higher retention times, lower resolution and theoretical plates etc., and then finally symmetrical peaks with good retention time, resolution were attained with Waters X-Bridge column (50 × 4.6 mm, 2.5 µ) using trifluoro acetic acid (0.1 % v/v): ACN (60:40 % v/v) at 0.5 mL/min. and 5 µL injection volume.

Validation of the Method

The method obtained was assessed for validation parameters as per ICH Q2 R1[7] guidelines.

System suitability

In 1.0 mL each of 750.0 (MET), 75.0 (VLDG), and 150.0 (REM ET) µg/mL were pipetted, made to 10 mL with diluent, and injected in six replicates. The resolution, theoretical plates, and tailing factor were recorded. %RSD was calculated using the area counts obtained from the respective chromatograms of MET, VLDG, REM ET.

Linearity

Six linear concentrations of each standard were prepared in the labeled 10.0 mL volumetric flasks for linearity study 187.50, 375.00, 562.50, 750.00, 937.50, 1125.00 µg/mL (MET); 18.75, 37.50, 56.25, 75.00, 93.75, 112.50 µg/mL (VLDG); and 37.50, 75.00, 112.50, 150.00, 187.50, 225.00 µg/mL (REM ET), respectively. Linearity data were noted from the concerned individual calibration curves of MET, VLDG and REM ET.

Detection and quantification limit

Using signal to noise (S/N) ratio, the lowest amount detected, and the lowest amount quantified were calculated and verified for the sensitivity of the developed method.

Precision

In 1.0 mL each of 750.0 µg/mL MET, 75.0 µg/mL VLDG, 150.0 µg/mL REM ET, were pipetted, dissolved, made up with diluent to 10 mL and analyzed in six replicates under similar operating conditions on a single day and three consecutive days. The %RSD were, respectively calculated for MET, VLDG and REM ET.

Specificity

A blank and placebo were prepared, transferred to labeled vials and analyzed to confirm that excipients, solvents, and degradants do not interfere at the retention time of the chromatograms of MET, VLDG, REM ET.

Robustness

Parameters like flow rate (± 10%), organic phase composition (± 10%) and wavelength (± 5 nm) was altered intentionally and the %RSD was calculated individually for MET, VLDG, REM ET.

Accuracy

Using the standard addition method, the accuracy was verified at three levels (50, 100, 150%). The %recovery was calculated from the area counts obtained for MET, VLDG, and REM ET.

Assay

The amount of MET, VLDG, REM ET and the percentage purity of respective drugs were calculated and compared with the respective amounts as specified on the label of the tablet dosage form.

Solution stability

The stability study of the sample solutions of MET, VLDG and REM ET was conducted by analyzing an aliquot of sample solution at room temperature and 2 to 8°C at specific intervals of 0, 6, 12, 18, 24 hours and the percentage deviation was calculated for the three drugs.

Forced degradation studies

These studies were done as per ICH Q 1A,[8] and Q 1B[9] guidelines. The stability of the sample solution under stressed conditions was simultaneously studied from the chromatograms of MET, VLDG, REM ET, respectively to ensure that the sample formulation was stable although subjected to stress conditions.

- *Acidic degradation*

A 1.0 mL each of sample stock and 0.1N HCl were transferred into a volumetric flask (10.0 mL), heated at 60°C for 30.0 minutes cooled, and neutralized with 1.0 mL 0.1 N NaOH. This was made up with diluent.

- *Basic degradation*

From the sample stock, 1.0 mL was pipetted to a clean volumetric flask with 1.0 mL of 0.1 N NaOH, and heated for 30.0 minutes at 60°C, cooled, neutralized with 1.0 mL 0.1 N HCl and made upto volume with diluent.

- *Oxidative degradation*

A 1.0 mL each of sample stock and 3% v/v hydrogen peroxide were added to a 10.0 mL volumetric flask, cooled after heating at 60°C for 30.0 minutes and made up with diluent.

- *Thermal degradation*

A 1.0 mL sample stock was taken into a volumetric flask (10.0 mL), placed in hot air oven at 105°C for 30.0 minutes, cooled and made up with the diluent.

- *Photolytic degradation*

A 1.0 mL sample stock was pipetted to a volumetric flask (10 mL), kept in a UV chamber at 254.0 nm for 30.0 minutes, made to 10 mL with diluent.

- *Hydrolytic degradation*

A 1.0 mL each of sample stock, and HPLC water were added to a 10.0 mL volumetric flask, made up with diluent after heating for 30.0 minutes at 60°C and cooling.

RESULTS

Method Optimization

After a few trials on various columns at different flow rates, and mobile phases/compositions, an optimized method (Figure 2) satisfying all the required parameters (theoretical plates, tailing factor, resolution) a method was developed (Table 1).

Method Validation

System suitability

Six aliquots of standard prepared were analyzed in six replicates. The system suitability data and %RSD was determined and tabulated (Table 2.). From the data, it was evident that the %RSD, USP plate count, USP tailing and USP resolution for MET, VLDG, REM ET, respectively complied with the ICH acceptance criteria. Hence, the results were reproducible.

Linearity

Calibration curves were plotted (Figure 3) using regression analysis (Tables 3 and 4). The linearity was achieved at 187.50 to 1125.00 (MET), 18.75 to 112.50 (VLDG), and 37.50 to 225.00 µg/mL (REM ET). By linearity data, it is evident that a linear relationship between the concentrations and area counts of MET, VLDG, REM, respectively was met.

Detection and quantification limit

The least amount of analytes detected and quantified were calculated using the S/N ratio. The obtained values of LoD and LoQ were 22.5, 75.0 µg/mL (MET); 2.25, 7.5 µg/mL (VLDG) and 4.5, 15.0 µg/mL (REM ET).

Precision

Six standard dilutions were analyzed (n = 6) and using the area counts obtained from chromatograms, %RSD was calculated (Table 5). The %RSD achieved was < 1.3 in both Intra - day and Inter - day for MET, VLDG, REM ET.

Specificity

The blank and placebo were determined and the chromatograms were observed (Figure 4) for any interferences. There were no interferences due to the excipients, solvents and degradants at the retention time of MET, VLDG, REM ET and hence the method was specific.

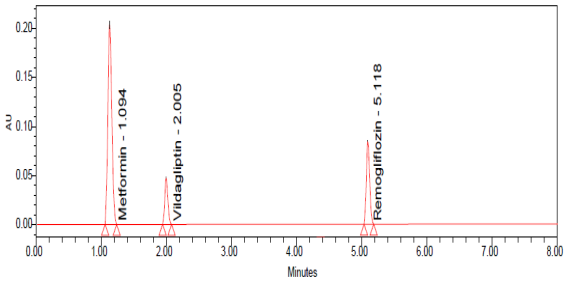


Figure 2: Optimized chromatogram for the standards

Table 1: Optimized chromatographic conditions

Parameter	*Value*
Column	Waters X-Bridge C18(50 x 4. mm, 2.5 µ)
Mobile phase	Triflouoro acetic acid (0.1% v/v): acetonitrile (60.40% v/v)
Elution mode	Isocratic
Flow rate (mL/min)	0.5
Detection wavelength (nm)	240.0
Injection volume (µL)	5.0
Run time (min)	8.0
Retention time (min)	1.004 (MET), 2.005 (VLDG) and 5.118 (REM ET)

mm – millimeter; µ - micron; % v/v - percentage volume/volume; mL/min. - milliliter per minute; nm nanometer; µL - microliter; min. - minutes

Table 2: Results of system suitability

Drug	*Retention time*	*Area counts*	**Area counts ± SD,%RSD*	*USP plate count*	*USP tailing*	*USP resolution*
MET	1.094	1159635	1148379 ± 16681.4, 1.45	5664	1.00	-
	1.097	1147251		5674	1.05	-
	1.092	1126859		5685	1.07	-
	1.095	1142385		5632	1.08	-
	1.097	1174582		5672	1.04	-
	1.097	1139562		5662	1.02	-
VLDG	2.005	163689	165819 ± 2453.0, 1.48	8029	1.21	5.26
	2.002	165427		8032	1.22	5.49
	2.007	162534		8013	1.46	5.74
	2.001	168457		8084	1.24	5.64
	2.005	168549		8033	1.12	5.54
	2.003	166258		8034	1.36	5.34
REM	5.118	721163	723429 ± 1447.2, 0.20	10293	1.10	15.35
	5.113	725142		10165	1.15	15.27
	5.116	723251		10295	1.22	15.13
	5.117	723185		10321	1.16	15.28
	5.119	724874		10318	1.36	15.39
	5.114	722956		10274	1.18	15.51

*- mean of six observations; ± - plus or minus; SD - standard deviation; % RSD - percentage relative standard deviation; USP - United States Pharmacopoeia

Table 3: Results of linearity

MET		*VLDG*		*REM ET*	
Conc. (µg/mL)	*Area counts*	*Conc. (µg/mL)*	*Area counts*	*Conc. (µg/mL)*	*Area counts*
187.50	272695	18.75	44985	37.50	182487
375.00	589248	37.50	86684	75.00	369157
562.50	845964	56.25	132685	112.50	544581
750.00	1106648	75.00	163201	150.00	728204
937.50	1391548	93.75	203594	187.50	900214
1125.00	1662149	112. 50	246986	225.00	1054541

Conc. - concentration,; µg/mL - microgram per milliliter

Table 4: Linearity summary

Parameters	*MET*	*VLDG*	*REM ET*
Linearity range (µg/mL)	187.50-1125.00	18.75-112.50	37.50-225.00
R^2	0.9998	0.9990	0.9997
Slope	1474.58	2161.32	4722.02
Intercept	8869.61	3873.61	8655.86

R^2 - correlation coefficient

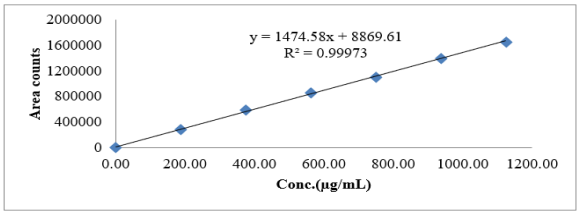
Robustness

The standard aliquot was checked for robustness (n = 3) at modified flow rate (± 10%), organic phase (± 10%), and wavelength (± 5 nm). The %RSD was calculated (Table 6). The %RSD was < 2.0. The intentional alterations of flow rate, organic phase composition and wavelength had little effect on the retention times. The system suitability parameters of MET, VLDG, REM ET were not much affected.

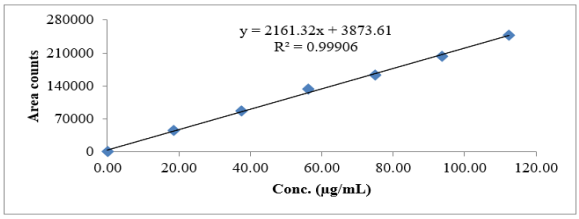
Table 5: Results of precision

Precision	*MET*	*VLDG*	*REM ET*
	**Area counts ± SD, % RSD*		
Intra – day			
	1144554 ± 12102.3, 1.05	164354 ± 2054.2, 1.25	725002 ± 3077.9, 0.42
Inter – day			
Day - 1	1144303 ± 15841.1, 1.38	164450 ± 2143.2, 1.30	725009 ± 2828.0, 0.39
Day - 2	1144770 ± 15730.1, 1.37	164295 ± 2155.9, 1.31	724879 ± 2848.2, 0.39
Day - 3	1147803 ± 16718, 1.46	166047 ± 2258.9, 1.36	723180 ± 3126.1, 0.43

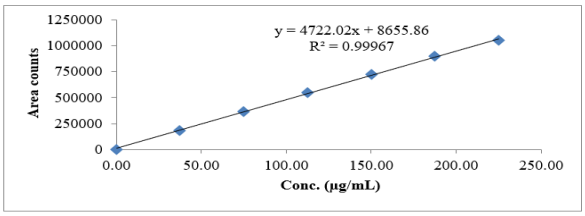
* - mean of six observations



MET



VLDG



REM

Figure 3: Linearity curves

Accuracy

The recovery studies were done using the sample solution and the respective percentage recovery (% w/w) and %RSD calculated at each level for MET, VLDG, REM ET were 99.0 ± 1% w/w and < 2.0 (Table 7).

Assay

A sample aliquot was analyzed and the calculated %assay was 100.9% w/w (MET), 99.9% w/w (VLDG), 100.3% w/w (REM) (Table 8) in marketed tablets. The %assay obtained ensured the purity of each drug in the tablet dosage forms.

Table 6: Results of robustness

Drug	*Parameter variation*		**Area counts ± SD, %RSD*	**USP tailing*	**USP plate count*	**USP resolution*
MET	Flow rate (± 10%)	Low medium	1763614 ± 14124.3, 0.80	1.27	5536	-
		High	1140957 ± 6597.7, 0.58	1.17	5658	
			957900 ± 4308.3, 0.45	1.09	5655	
	Mobile phase (± 10%)	Low medium	1434902.7 ± 18235.2, 1.27	1.32	5239	-
		high	1140957 ± 6597.7, 0.58	1.17	5658	
			1014398.7 ± 1232.2, 0.12	1.17	5758	
	Wavelength (± 5 nm)	Low medium	1221319 ± 16942.2, 1.39	1.38	5638	-
		high	1140957 ± 6597.7, 0.58	1.17	5658	
			1089612 ± 1113.3, 0.10	1.07	5630	
VLDG	Flow rate (± 10%)	Low medium	186017 ± 936.6, 0.50	1.29	8153	4.45
		high	165161 ± 1466.9, 0.89	1.22	8095	5.28
			122008 ± 1360.2, 1.11	1.39	8053	4.68
	Mobile phase (± 10%)	Lowmedium	201314 ± 1131.3, 0.56	1.19	8367	5.29
		high	165161 ± 1466.9, 0.89	1.22	8095	5.28
			158091 ± 279.2,018	1.17	8245	3.43
	Wavelength (± 5 nm)	Low medium	171554 ± 914.4,0.53	1.14	8065	5.60
		high	165161 ± 1466.9, 0.89	1.22	8095	5.28
			158517 ± 1024.1, 0.65	1.16	8056	5.48
REM ET	Flow rate (± 10%)	Low medium	767315.7 ± 2382.7, 0.31	1.31	10232	14.56
		high	724984 ± 3502.2, 0.48	1.19	10283	15.59
			696281.3 ± 2939.9, 0.42	1.09	10136	13.73
	Mobile phase (± 10%)	Low medium	753975 ± 1475.7, 0.20	1.09	10189	16.53
		high	724984 ± 3502.2, 0.48	1.19	10283	15.59
			705379 ±1403.1, 0.20	1.33	10353	10.48
	Wavelength (± 5 nm)	Low medium	729889 ± 1349.3, 0.18	1.08	10267	15.50
		high	724984 ± 3502.2, 0.48	1.19	10283	15.59
			715481 ± 1561.7,0.22	1.17	10241	15.65

* - mean of three observations

Table 7: Results of accuracy

Drug	*Level (%)*	*Amount added (mg)*	*Amount recovered (mg)*	*%Recovery (w/w) ± SD, % RSD*
MET	50	112.5	111.9	99.4 ± 0.70, 0.70
	100	150.0	149.8	99.8 ± 0.69, 0.69
	150	187.5	186.2	99.3 ± 0.79, 0.80
VLDG	50	11.3	11.1	98.9 ± 0.59, 0.60
	100	15.0	14.8	98.8 ± 0.33, 0.34
	150	18.8	18.7	99.5 ± 0.64, 0.64
REM ET	50	22.5	22.5	100.1 ± 0.90, 0.90
	100	30.0	29.9	99.6 ± 1.55, 1.56
	150	37.5	37.3	99.4 ± 0.76, 0.77

% - percentage; mg- milligram; * - mean of three observations; % - w/w percentage weight by weight

Table 8: Results of assay

Sample	*Drug*	*Area counts*	*Label claim (mg)*	*Amount obtained (mg)*	*%Assay (% w/w)*
Remo-Zen MV 500	MET	1159033	500.0	504.0	100.9
	VLDG	163889	50.0	49.0	99.0
	REM ET	724960	100.0	100.0	100.3

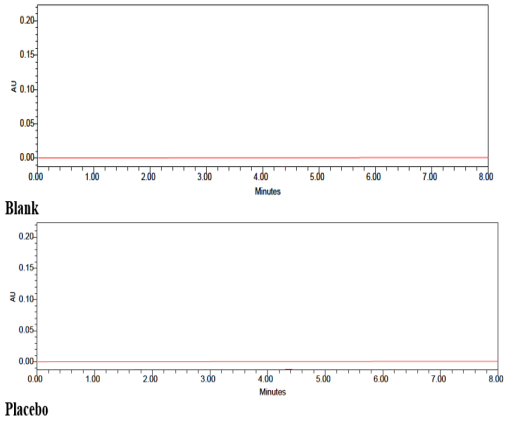


Figure 4: Specificity chromatograms

Solution stability

Stability of the sample solution was conducted and the %deviation in stability for MET, VLDG, REM ET at room temperature and 2 to 8°C at 24 hours was calculated to be 1.7, 1.5, 3.0, 3.0, 1.9, 1.4%, respectively. The stability of the sample was determined and found that there was a little change in percentage deviation for the three drugs in formulation both at room temperature and 2 to 8°C.

Forced degradation studies

The sample aliquots were subjected to various stress environments, later analyzed for any degradation. From the

Table 9: Results of forced degradation studies

Condition	*MET*			*VLDG*			*REM ET*		
	*%Degraded (% w/w)	*Tailing factor*	*Peak resolution*	*% Degraded (% w/w)*	*Tailing factor*	*Peak resolution*	*% Degraded (% w/w)*	*Tailing factor*	*Peak resolution*
Control	0.1	1.06	-	0.1	1.04	5.54	0.1	1.03	15.69
Acid	18.5	1.12	-	16.2	1.05	5.24	19.9	1.13	5.45
Alkali	18.7	1.19	2.86	17.5	1.12	5.24	16.9	1.05	5.45
Oxidative	19.1	1.22	-	18.3	1.15	3.54	20.4	1.13	8.38
Thermal	5.8	1.31	-	11.1	1.07	4.41	7.3	1.11	15.48
Photolysis	12.2	1.23	-	5.3	1.02	5.41	4.6	1.01	15.74
Hydrolysis	3.7	1.12	-	3.5	1.04	5.25	4.4	1.13	15.78

* - Mean of three observations

Table 10: Comparison of the proposed study with the literature methods

Method	*Column*	*Mobile phase*	*Flow rate (mL/min)*	*RT (min)*			*Run time (min)*	*Remarks*
				MET	*VLDG*	*REM ET*		
RP-HPLC	Zorbax Sb-Aq (250 × 4.6 mm, 5 µ)	PO4 buffer pH 3.3: ACN (50:50 v/v)	1.0	2.21	3.68	8.14	15.0	Kedar AJ., *et al.* (2023)
HPLC	Phenomenex luna C18 (250 × 4.6 mm, 5 µ)	5.2 pH acetate buffer: ACN (55:45 v/v)	1.0	7.38	8.41	3.40	12.0	Ramanjaneyulu KV., *et al.*(2022)
RP-HPLC	Ascentis C18 (150 × 4.6 mm, 2.7 mm)	ACN: PO4 buffer (35:65 v/v)	1.5	-	-	-	-	Kamini S., *et al.* (2023)
HILIC	Acclaimed mixed mode HILIC-1 (150 4.6 mm, 5 µ)	ACN: 20 mM PO4 buffer(65:35 v/v)	1.4	3.5	2.3	1.5	5.0	Attimarad M., *et al.* (2022)
RP-HPLC	Acclaimed mixed mode HILIC-1 (150 4.6 mm, 5 µ)	ACN: 20 mM PO4 buffer (75:25 v/v)	1.0 (Gradient)	5.81	4.86	3.81	6.0	Bano T., *et al.* (2023)
RP-HPLC	Agilent C18 (150 × 4.6 mm, 5 µ)	0.1 N KH2PO4: ACN (50:50 v/v)	0.9	2.57	2.13	2.96	7.0	Rakesh Y., *et al.* (2022)
UPLC	Waters X-Bridge C18 (50 × 4.6 mm, 2.5 µ)	TFA (0.1 % v/v): ACN (60:40 v/v)	0.5	1.00	2.00	5.11	8 .0	Proposed method

HILIC - hydrophilic interaction liquid chromatography; RP - reverse phase; HPLC - high performance liquid chromatography; UPLC - ultra-performance liquid chromatography

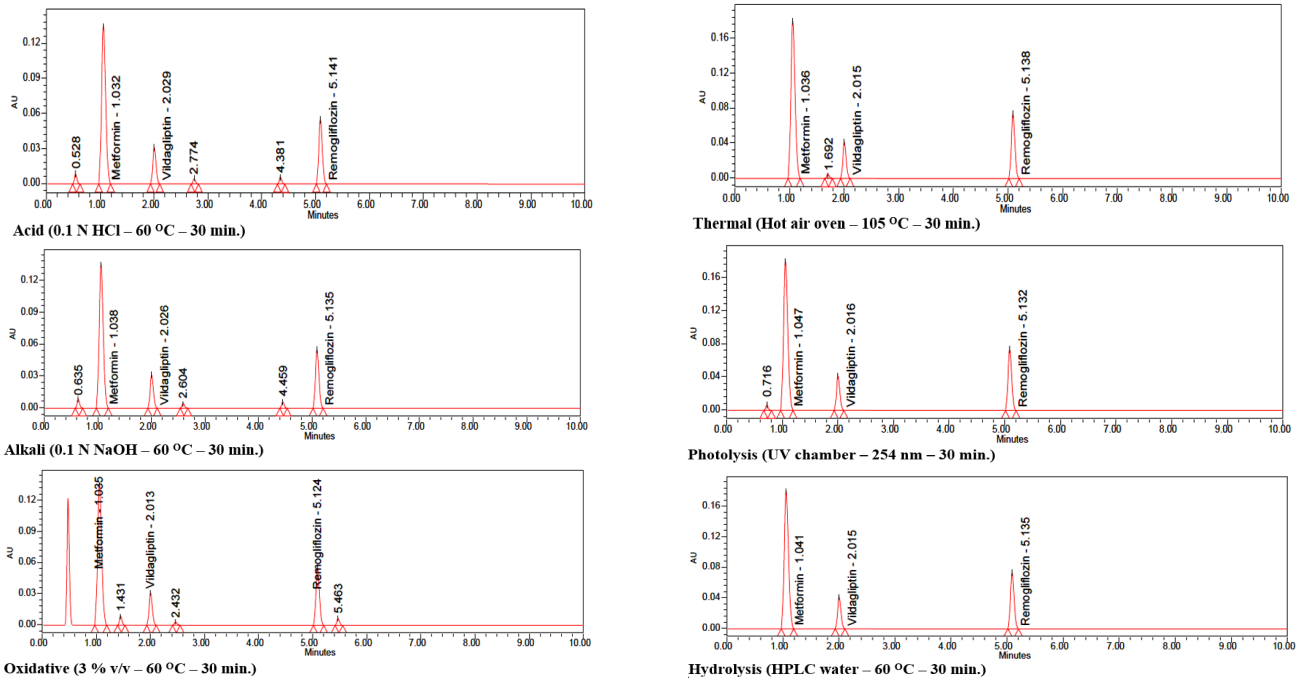


Figure 5: Forced degradation studies chromatograms

chromatograms obtained (Figure 5.), %degradation (% w/w) was calculated. From the results, it was inferred that the peak resolution, peak tailing (Table 9.) were more than 2.0 and less than 2.0, respectively. A remarkable degradation of the sample occurred in acid, alkali, and oxidative, photolysis and negligible degradation in thermal, and hydrolysis at 18 hours. Degradants were not identified during hydrolysis. The degradants were well eluted at different retention times and hence no interference was observed at the retention times of the main peaks. This concluded the specificity of the method even when subjected to stress

CONCLUSION

A simple, rapid, isocratic, economical, specific, time-saving ultra-performance liquid chromatographic - photo diode array detection method was developed to simultaneously quantify a three combination oral dosage form composing metformin, vildagliptin and remogliflozin etabonate. The validation results were within the ICH specifications. The forced degradation studies conducted showed identifiable degradants. The current method has lower flow rate (Table 10.) and can be exploited for the estimation of MET, VLDG and REM ET in bulk and tablets without any interference as a routine quality control procedure.

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AUTHORS CONTRIBUTION

Rajya Lakshmi N majorly contributed in the concept selection, and method development. Sowjanya G and Rajya Lakshmi N equally contributed in the data analysis, data interpretation and preparation of the manuscript. The authors read the manuscript and approved the manuscript for publication.

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