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Simple and Fast Stability Indicating UPLC Method for the Simultaneous Quantification of Vildagliptin and Remogliflozin Etabonate in Bulk Drug and Formulations

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Abstract

This study reports for the first time about a stability indicating RP-UPLC method for separation and simultaneous of vildagliptin and remogliflozin etabonate. The separation was achieved on Acquity® UPLC BEH C18 (2.1 × 50 mm, 1.7 µm) column as stationary phase, 0.1 M acetate buffer at pH 5.7 and methanol in the ratio of 25:75 (v/v) at 0.3 mL/min flow rate and PDA detector at 215 nm. In these conditions, the resolution of the compounds was obtained as 12.57 with retention time of 2.67 min for remogliflozin and 3.84 min for vildagliptin. The method was validated for system suitability, range of analysis, precision, specificity, stability and robustness. Forced degradation study was done through exposure of the analytes to five different stress conditions and in all the degradation condition, the % degradation was very less, and the method can separate and estimate the vildagliptin and remogliflozin in pharmaceutical formulations. Hence the developed method was found to be suitable for the separation and simultaneous quantification of vildagliptin and remogliflozin in bulk drug and pharmaceutical formulations.

Keywords: Vildagliptin, Remogliflozin, UPLC method development, MethodValidation, Forced degradation study, Formulation assay

Introduction

Vildagliptin belongs to gliptin class cyanopyrrolidine drug approved for the treatment of type II diabetes mellitus(1). In type II diabetes mellitus patients Vildagliptin not only improve insulin secretion but also suppress the inappropriate glucagon secretion (2). Hypoglycaemia, dizziness, headache, nausea and tremor are the vildagliptin side effects. Hepatoxicity was also overserved in rare cases (3). Remogliflozin etabonate belongs to gliflozin class drug prescribed to treat type II diabetes and non-alcoholic steatohepatitis (4). It is a selective sodium-glucose cotransporter-2 (SGLT2) inhibitor having advance selectivity and pharmacokinetic (PK) profile among other SGLT2 inhibitors (5). Remogliflozin etabonate reduced plasma glucose concentrations in subjects with type II diabetes mellitus (6). The possible side effects of Remogliflozin etabonate are urinary tract infections, dizziness and genital mycotic infections (7). Vildagliptin and remogliflozin etabonate were available as fixed combined dosage forms that improves the glycemic control when metformin and one of the mono-components of fixed-dose combination do not provide adequate glycemic control, or when already being treated with separate doses of vildagliptin and remogliflozin. The molecular structure of vildagliptin and remogliflozin were given in figure 1.

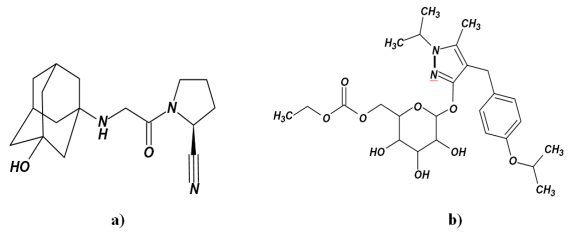


Fig. 1: Molecular structure of vildagliptin (a) and remogliflozin etabonate (b)

The literature survey confirms that only one analytical HPLC method (8) reported for the simultaneous estimation of vildagliptin and remogliflozin. In Literature, few analytical methods available for essay of remogliflozin using HPLC (9,10), UV (11), HPTLC (12)techniques and vildagliptin using HPLC (13-16), UV-visible (17), LCMS (18)and GCMS (19) techniques individually. Few analytical methods reported for assay of remogliflozin in combination with metformin (20-22), vildagliptin in combination with metformin (23-26)and telmisartan (27). Based on the available literature it can be confirm that no UPLC method was available for the separation and simultaneous quantification of vildagliptin and remogliflozin etabonate. Hence the present work aimed to develop simple and precise analytical UPLC method for the separation and simultaneous estimation of vildagliptin and remogliflozin etabonate in bulk drug as well as in pharmaceutical formulations.

Materials and Methods

Instrumentation

The study employed a highly sensitive UPLC system that consisted of a Dionex® UPLC binary solvent manager equipped with a Dionex® automatic sample

manager and a Photodiode Array (PDA) eλ detector procured from Thermo scientific, Bedford, MA, USA. Separation of analytes was performed on Acquity® UPLC BEH C_{18} (2.1 × 50 mm,
1.7 µm) column kept at 25 °C. The UPLC system is equipped with 0.20 µm online filter for
filtering the mobile phase and degassed by an online degasses equipped.

Chemicals and Reagents

The vildagliptin and remogliflozin pure standard drugs were obtained from Glenmark pharmaceuticals LTD, Mumbai. The HPLC grade methanol, acetonitrile and ultra-pure (Milli-Q®) water were obtained from Merck chemicals, Mumbai.

Preparation of standard solutions

25 mg of active pharmaceutical ingredient of vildagliptin and remogliflozin etabonate were accurately weighed and was dissolved in 25 mL methanol solvent separately. The content was sonicated for 2 min to dissolve the drug completely in the solvent separately and the standard solution of vildagliptin and remogliflozin at a concentration of 1000 µg/mL was obtained separately. For preparing calibration curve dilutions, equal volume of known and fixed selected concentrations of vildagliptin and remogliflozin were mixed separately. The combined solution of vildagliptin and remogliflozin having known concentrations were used for method development and validation study.

Preparation of formulation solution

The formulation tablets of vildagliptin and remogliflozin etabonate with brand Remo- V (vildagliptin - 50mg & remogliflozin etabonate-100mg) was powdered using sterile mortar and pestle. An amount of the tablet powder equivalent to 25 mg of vildagliptin was weighed accurately and was dissolved in 25 mL methanol. Then it was filtered and was further diluted to get a concentration of 10 µg/mL of vildagliptin. As per the label claim of the drugs in the formulation, sample solution having 20 µg/mL of remogliflozin etabonate. This solution was used for the determination of the applicability of the developed method for the analysis of vildagliptin and remogliflozin in pharmaceutical formulations.

Method development

In the development a simple and previse analytical UPLC method for the identification and simultaneous quantification of vildagliptin and remogliflozin in pharmaceutical formulations, different method development trails were performed. While performing the method development trails, various method conditions such as composition, pH and flow rate of mobile phase, wavelength of detector, configuration of stationary phase were optimised. In each optimized conditions studied, the system suitability parameters like peak shape, peak response, number of theoretical plates, tail factor and resolution were verified and the conditions that produce best results were considered as optimized and further validated.

Method validation

The developed method for the simultaneous quantification of vildagliptin and remogliflozin was validated for the determination of range of analysis, sensitively, accuracy, precise, ruggedness and robustness. The detection and quantification limits for both vildagliptin and remogliflozin was identified in the method for evaluation of the sensitivity ofthe developed method.

Force degradation studies

Forced degradation study was carried for the standard drugs vildagliptin and remogliflozin in the develop method to evaluate the effectiveness of the developed method for the separation and identification of known and unknown impurities in the drug. 50 mg of standard drug was mixed with 50 mL of 0.1N HCl for acid hydrolysis study, 50 mL of 0.1 N NaOH in base hydrolysis study and 50 mL of 3% hydrogen peroxide solution for oxidative degradation study. These conditions were carried separately for both the drugs and the solutions were incubated 24 H and then neutralized separately. The equal volume of selected concentration of both drugs were mixed and then neutralized. The neutralized solutions were analysed in the developed method condition. In photolytic and thermal degradation conditions, standard drug was kept under UV light at 254 nm and oven at 60 ^{0}C for 24 hours respectively. Then the standard drug was diluted to standard concentration and were analysed in the developed method condition. The % degradation, number of degradation products formed in the degradation study and the % effectiveness of the method for the separation of degradation products was evaluated.

Formulation analysis

The solution prepared from formulation tablet Remo-V of vildagliptin and remogliflozin was analysed in the developed method. The results observed in the formulation analysis were verified for the confirmation of the applicability of the developed method for theanalysis of vildagliptin and remogliflozin in pharmaceutical formulations.

Results and Discussion

The present study is intended to develop a simple and accurate stability indicating UPLC method for the separation, identification, and simultaneous quantification of vildagliptin and remogliflozin in bulk drug as well as in pharmaceutical tablet formulations.
The suitable wavelength for the simultaneous detection of vildagliptin and remogliflozin was confirmed based on the iso-absorption wavelength and it was confirmed that at a wavelength of 215 nm was selected as suit-

able wavelength for PDA detector in UPLC study. The stationary phase was selected as Acquity® UPLC BEH C18 (2.1 × 50 mm, 1.7 µm) column and mobile phase flow rate was initially fixed at 0.3 mL/min. The composition of mobile phase using various pH modifiers at different pH ranges.

The initial method development was performed using 0.1 M acetate buffer at pH 5.7as pH modifier and methanol as organic modifier in the ratio of 25:75 (v/v). In this condition no separation of analytes was observed (figure 1A). While changing the pH modifier as 0.1 M phosphate buffer also doesn't separate the analytes in the study (figure 1B). Then 0.05 M ammonium acetate buffer was utilised as organic modifier and this produce clear separation of analytes (figure 1C and 1D). Various ratios 0.05 M ammonium acetate buffer and methanol were studied for the optimized separation of vildagliptin and remogliflozin. The optimization trail chromatograms were given in figure 2.

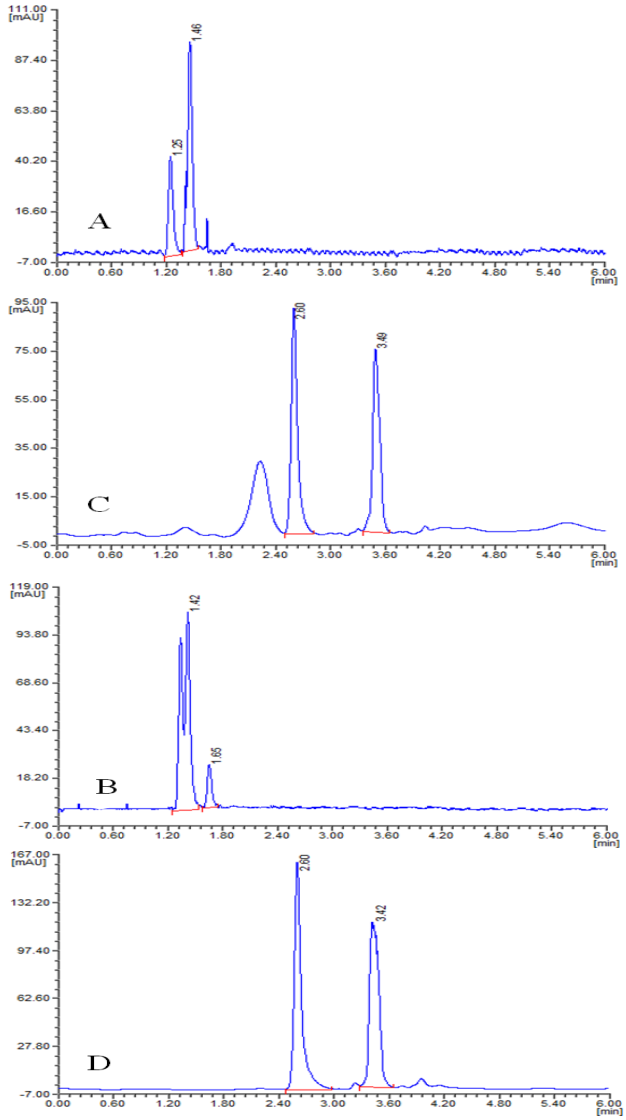


Figure 2: Chromatograms observed in the development of method for the analysis of vildagliptin and remogliflozin using UPLC

Finally, the optimization of the UPLC method for the simultaneous analysis of vildagliptin and remogliflozin was concluded by achieving the optimized conditions. The separation of vildagliptin and remogliflozin was achieved using Acquity® UPLC BEH C18 (2.1 × 50 mm, 1.7 µm) column maintained at room temperature as stationary phase, 0.05 M ammonium acetate buffer at pH 5.1 and methanol in the ratio of 45:65 (v/v) as mobile phase at 0.3 mL/min flow rate in isocratic elution. The column eluents were recorded using PDA detector at 215 nm.

In the optimised condition, symmetric peaks were identified at a retention time of 2.67 min for remogliflozin and 3.84 min for vildagliptin with a resolution factor of 12.57 (figure 3B). The peak area response was observed to be very high for both the analytes andthe resolution between the compounds was observed to be acceptable. The number of theoretical plates was found to be 7629 and 9417 whereas the tail factor was observed to be 1.08 and 0.93 respectively for remogliflozin and vildagliptin. In these conditions, the blank analysis chromatogram (figure 3A) doesn't show any detection at the retention time of vildagliptin and remogliflozin confirms that the method was specific for the analysis of vildagliptin and remogliflozin.

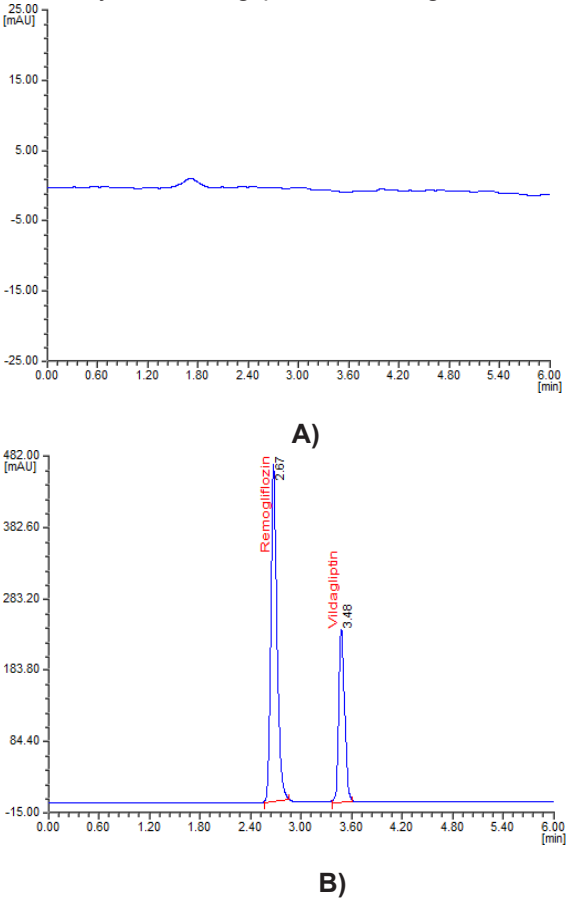
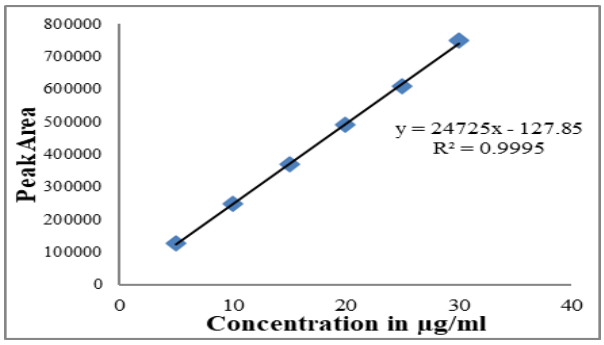


Figure 3: UPLC chromatograms observed in the optimized conditions. A): Blank solution; B): Standard solution containing vildagliptin and remogliflozin

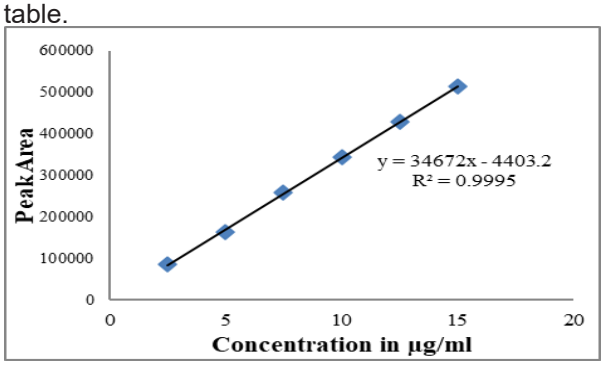
Accurately correlated calibration curve was observed in the concentration range of 2.5-15 µg/mL for vildagliptin and 5-30 µg/mL for remogliflozin. The regression equitation was found to be y = 34672x - 4403.2 (R^2 = 0.9995) and y = 24725x - 127.85 (R^2 = 0.9995) for vildagliptin and remogliflozin respectively. The calibration curve was found to linear in the concentration studied for both vildagliptin and remogliflozin with a very high correlation coefficient of more than 0.999 for both the drugs. The results of linearity study were given in table 1 and calibration curve was shown in figure 4 for remogliflozin and vildagliptin in the developed method.

Table 1: Linearity results observed in the developed method

S No	Vildagliptin		Remogliflozin	
	Concentration in µg/ml	PeakArea	Concentration in µg/ml	Peak Area
1	2.5	85867.1	5	125968.0
2	5.0	161959.6	10	247482.3
3	7.5	258576.9	15	370991.8
4	10.0	342621.0	20	491007.2
5	12.5	429575.3	25	610227.4
6	15.0	515242.9	30	749698.7



A



B

Figure 4: Linear calibration curve for vildagliptin (A) and remogliflozin (B) in thedeveloped method

The spiked recovery at 50%, 100% and 150% spiked levels at a target concentration of 10 µg/mL of vildagliptin and 20 µg/mL of remogliflozin were studied. The % Recovery and the % RSD of recovery in each spike level was calculated (table 2) and was found to be within the acceptable limits for both vildagliptin and remogliflozin confirms that the method was found to be accurate.

The repeatability and reproducibility were studied by intraday, interday precision and ruggedness study. The standard solution at a concentration of 20 µg/mL of remogliflozin and 10 µg/mL of vildagliptin was analysis six times in the same day for intraday precision, six times in two successive days for interday precision and six times for change in two analyst for ruggedness study. The % RSD in each study was calculated for both the drugs and was found to be with in the acceptance limit for both vildagliptin and remogliflozin. This confirms that the method developed was found to be precise and rugged for the simultaneous analysis of vildagliptin and remogliflozin. The standard concentration of remogliflozin and vildagliptin were analysed by change in analytical conditions i.e mobile phase composition (±5 %), mobile phase pH (±0.1) and detector wavelength (±3 nm). The % change was calculated in each changed condition for both the drugs and was found to be within the acceptable limit of less than 2 confirms that the method was found to be robust. The summaryresults of precision, ruggedness and robustness study were given in table.

Table 2: Recovery results for remogliflozin and vildagliptin

S No	Recovery level	Concentration in µg/mL			Concentration Obtained (µg/mL)	% Recovery	% RSD of recovery
		Target	Spiked	Total			
Vildagliptin							
1	50%	5	2.5	7.5	7.435±0.020	99.13±0.263	0.27
2	100%	5	5	10	9.951±0.028	99.51±0.284	0.28
3	150%	5	7.5	12.5	12.325±0.040	98.60±0.323	0.33
Remogliflozin							
4	50%	10	5	15	14.904±0.046	99.36±0.308	0.31
5	100%	10	10	20	19.722±0.031	98.61±0.156	0.16
6	150%	10	15	25	24.838±0.098	99.35±0.390	0.39

Table 3: Results observed in precision, ruggedness and robustness study

S. No	Parameter	Results obtained	
		Vildagliptin	Remogli-flozin
1	% RSD in intraday precision	0.19	0.12
2	% RSD in interday precision (day 1)	0.65	0.68
3	% RSD in interday precision (day 2)	0.69	0.45
4	% RSD in analyst 1 change	0.07	0.11
5	% RSD in analyst 2 change	0.43	0.11
6	% change in mobile phase +ve change	-0.20	-0.37
7	% change in mobile phase -ve change	-0.52	-0.41
8	% change in pH +ve change	0.20	-0.55
9	% change in pH -ve change	-0.22	-0.45
10	% change in wavelength +ve change	-0.56	-0.47
11	% change in wavelength -ve chang	0.65	-0.43

The limit of detection was identified as 0.015 and 0.03 µg/mL whereas the quantification limit was calculated as 0.05 and 0.01 µg/mL respectively for remogliflozin andvildagliptin. The results proved that the method was very sensitive and can detect and quantify vildagliptin and remogliflozin at very low concentrations.

In the stress degradation study, the % degradation of vildagliptin was found to be 8.96(acidic), 5.73 (basic), 6.03 (peroxide), 8.42 (thermal) and 9.69 (UV light) whereas the % degradation of remogliflozin was found to be 9.52 (acidic), 7.24 (basic), 5.63 (peroxide), 7.01(thermal) and 8.53 (UV light). Less % degradation was observed for both the drugs in peroxide conditions whereas the % degradation was found to be high in VU light and acidic conditions. In the stress degradation studies, both the standard drugs were retained in the same retention time compared with un-stressed conditions and the additional degradation products formed were effectively separated and retained in the developed method. Hence the method can be used for the identification of known or unknown impurities formed during the stress study. Hence the method was considered as stability indicating method. The stress degradation chromatograms were given in figure 5.

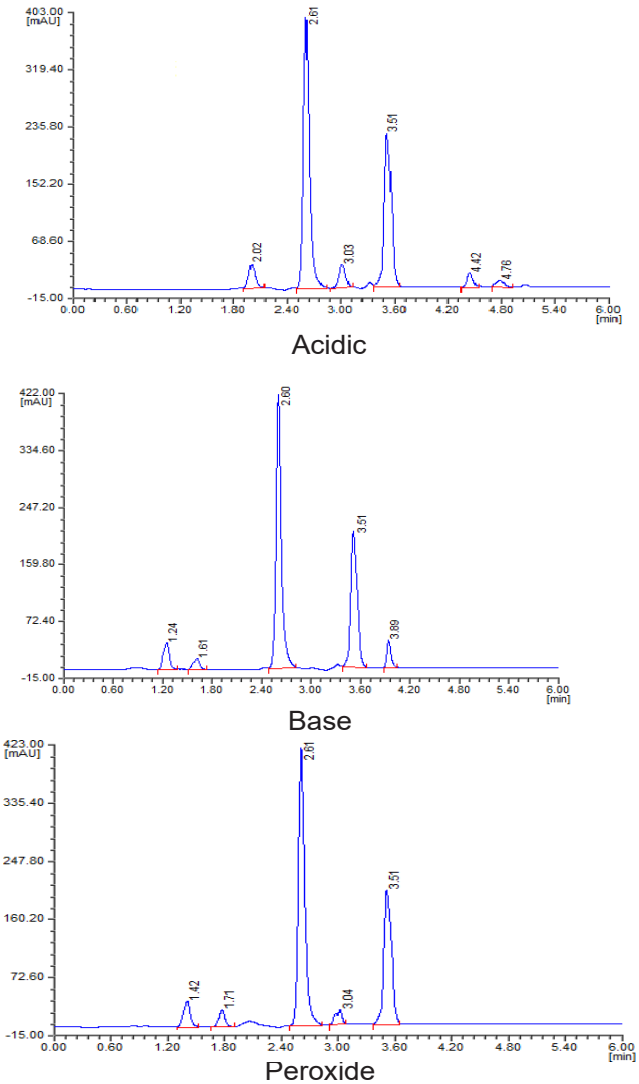


Figure 5: Forced degrdation chromatograms in the developed method

The formulation assay was found to be 98.84 % for vildagliptin and 99.37 % for remogliflozin in the developed method. In the formulation chromatogram, both the drugs vildagliptin and remogliflozin were well retained and the retention time was found to be similar to the standard (figure 6). There is no detection of formulation excipients and clear base line was observed confirms that the method was suitable for the separation andsimultaneous quantifica-

tion of remogliflozin and vildagliptin in pharmaceutical formulations.

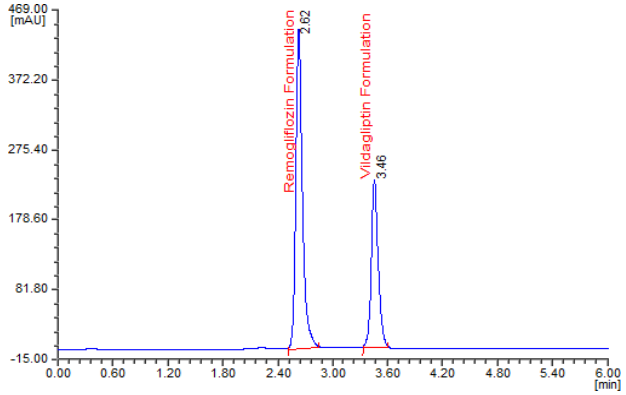


Figure 6: Formulation chromatogram of vildagliptin and remogliflozin

The developed method conditions were compared with the methods available in literature. The method developed by Mandale et al., 2021[8] was based on the separation of vildagliptin and remogliflozin using HPLC. The sensitivity of the present method was found to be more than the reported HPLC method. The total run time of the present study was less compared with the reported HPLC method that facilitates fast analysis of the samples. The separation of analyes was also very improved than the reported method. Hence, it can be confirmed that the method developed was found to be the most suitable and reliable method for the simultaneous analysis and stability study of vildagliptin and remogliflozin in pharmaceutical formulations.

Conclusion

In the present study, a simple, fast, accurate, and reliable UPLC method was developed and validated for the simultaneous analysis of vildagliptin and remogliflozin in pharmaceutical formulations as per ICH guidelines. The method obeys all the system suitability and other validation parameters. The method can effectively separate the degradation products formed during the stress degradation study. As there is no stability indicating UPLC method reported for the simultaneous analysis of vildagliptin and remogliflozin, the method developed was found to be the reliable and convenient for the routine analysis and stability study of vildagliptin and remogliflozin in bulk drug and pharmaceutical formulations.

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