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Development and Validation of stability indicating method for the determination of Repaglinide in Pharmaceutical dosage form using High Performance Liquid Chromatography

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Abstract: A stability-indicating HPLC method has been developed and validated for Repaglinide in bulk drug and pharmaceutical dosage forms. An isocratic RP-HPLC was achieved on younglin HPLC system using Varian C18 (250 X 4.6 mm i.d, 5 µm particle size) column with the mobile phase containing mixture of acetonitrile:10mM ammonium acetate(pH 3.0, adjusted with phosphoric acid) (70 : 30, v/v). The flow rate was 1.0ml/min and the eluent was monitored at 230nm. Linearity was found in the range of 0.5-3µg/ml. The values obtained of LODs and LOQs were 0.056µg/ml and 0.172µg/ml respectively. The stress testing of Repaglinide was carried out under acidic, alkaline, oxidative and thermal conditions. Repaglinide was well resolved from its degradation products. The proposed method was validated as per ICH guidelines. The method was found to be fast, accurate, precise, reproducible and suitable for analysis of Repaglinide in bulk and pharmaceutical dosage forms as well as the stability-indicating studies.
Keywords: Repaglinide, Stress testing, Stability indicating method, HPLC.

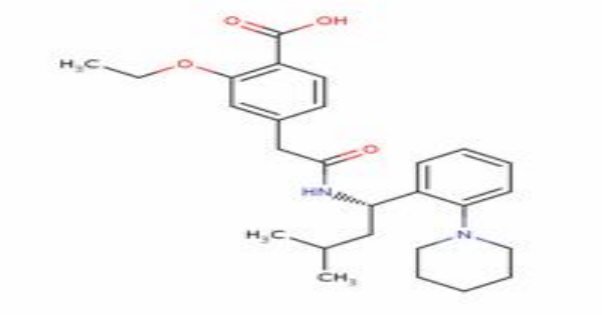
Introduction

Repaglinide(RGE),(*S*)-(+)-2-ethoxy-4-[2-(3-methyl-1-[2-(piperidin-1-yl)phenyl]butylamino)-2-oxoethyl]benzoic acid, is an oral antidiabetic drug(Figure 1). It is a novel prandial glucose regulator for the treatment of type 2 diabetes mellitus[1,2]. It reduces the fasting glucose concentrations in patients with type 2 diabetes mellitus. It helps to control blood sugar by increasing the amount of insulin released by the pancreas. Repaglinide is rapidly absorbed from the gastrointestinal tract after oral administration. It differs from other antidiabetic agents in its structure, binding profile, duration of action and mode of excretion[3].Tablets containing 0.5, 1 and 2 mg of RGE are available for oral administration. The methods investigated for analysis of RGE include HPLC method with UV detector, spectrofluorimetric method [4,5,6] or electrochemical method by using carbon paste electrode and glassy carbon electrode [7]. Liquid chromatography-tandem mass spectrometry (LC/MS/MS) and normal phase chiral HPLC methods[8] for determination of RGE are also reported. Several analytical methods are available for the determination of repaglinide in biological fluids,

including liquid chromatography-tandem mass spectrometry (LC/MS/MS)[9,10]. These methods are costly and require the availability of expensive equipment. To date, no stability indicating method for determination of repaglinide has been available.

The present study involves the development of stability indicating reverse phase-high performance liquid chromatographic (RP-HPLC) method for quantitative determination of repaglinide which is sensitive and requires shorter time of 5min. The developed method was validated as per International conference on harmonization (ICH) guidelines [11] and was applied to commercial formulation of REG.

Figure 1. Structure of Repaglinide



Experimental

Apparatus

A gradient high performance liquid chromatograph from younglin HPLC system, equipped with a UV detector and Autochro-3000 software was used. A reversed phase Varian C-18 (250 X 4.6 mm i.d, 5 µm particle size) analytical column was used for the present analysis. Shimadzu electronic balance, ultrasonic cleaner (225x125x60 mm s.s-304, 1.5 Liter capacity of tank) and pH meter LI127 (Elico Limited) were used during the study.

Reagents and materials

Repaglinide was obtained from Torrent Pharmaceutical Ltd (India). Market formulations Europa-0.5 and Europa-1 were obtained commercially. All solvents were of HPLC grade and all reagents were of analytical grade. Methanol, Acetonitrile and ammonium acetate were obtained from Merk (India). Sodium hydroxide, hydrochloric acid, hydrogen peroxide and phosphoric acid were obtained from s.d.fine-chem ltd (Mumbai). Triple distilled water was used throughout the experiment. All solvents and solutions were filtered through a membrane filter (ultipor N66 Nylon 6,6, 0.2µm pore size) and degassed using ultrasonic cleaner before use.

Chromatographic conditions

The samples were chromatographed on a reversed phase C-18 (250 X 4.6 mm i.d, 5 µm particle size) column with a flow rate of 1.0 ml/min. All analyses were carried out at isocratic conditions. The mobile phase consisted of a mixture of acetonitrile : 10mM ammonium acetate(pH 3.0, adjusted with phosphoric acid) (70 : 30, v/v). The mobile phase was filtered through a Nylon 0.2µm membrane filter and degassed before use. The volume of injection was 20µl and the detection was made at 230nm.

Preparation of solutions

Ammonium Acetate Buffer

Accurately weighed Ammonium Acetate(0.77gm) was dissolved in sufficient triple distilled water and then dilute upto 1000ml with the same. The pH was adjusted 3.0 using phosphoric acid.

REG standard stock solution

Accurately weighed REG (50mg) was transferred in 100 ml volumetric flask. The drug was dissolve in methanol with sonication and final volume was adjusted with methanol upto mark to prepare a 500µg/ml stock solution.

REG working standard solution

From the above stock solution (500µg/ml), an accurately measured 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ml was transfer into separate 100 ml volumetric flask and final volume was adjusted with methanol upto mark to prepare 0.5-3µg/ml solutions.

Sample solution

Weigh and finely powder 20 tablets. Transfer exactly equivalent to 0.5 mg of Repaglinide to a 100 ml volumetric flask. Add about 60 ml of methanol and sonicate for 15 minutes and make up volume with methanol. From this solution take 1ml and dilute upto 10ml with methanol. This solution was injected for HPLC determination.

Optimization of the solvent system

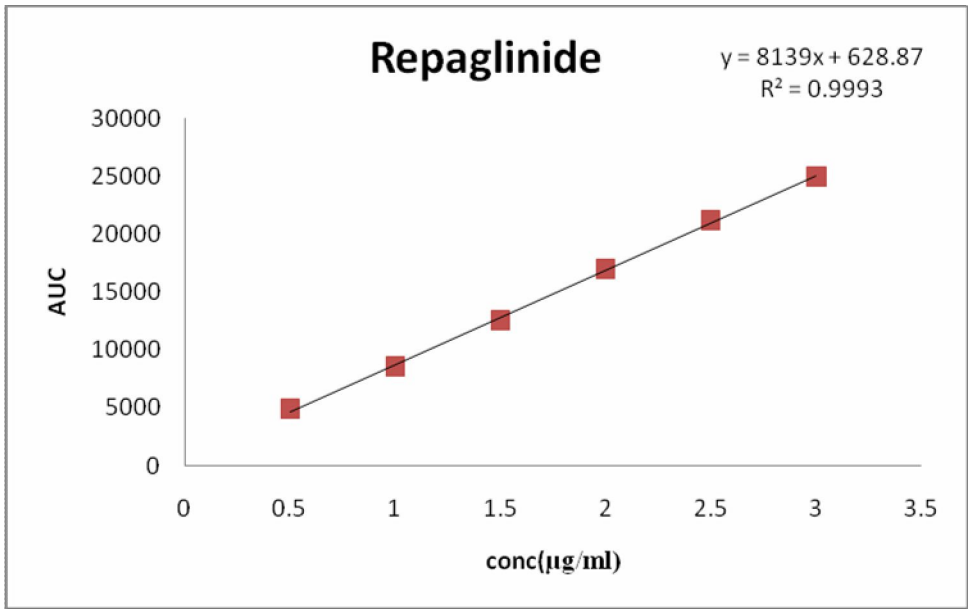
Varying compositions of acetonitrile : 10mM ammonium acetate(pH 3.0, adjusted with phosphoric acid) 65 : 35, 50 : 50, 40 : 60, and 30 : 70 v/v were evaluated as mobile phase in order to achieve good peak shape and short run time. Finally, acetonitrile : 10mM ammonium acetate(pH 3.0, adjusted with phosphoric acid) (30 : 70, v/v) isocratic method was used.

Method Validation

Linearity

The linearity was evaluated by linear regression analysis. The calibration curve was obtained with concentrations of pure repaglinide solution ranging from 0.5 to 3µg/ml for the chromatographic method(Figure2).

Figure 2. Calibration curve for Repaglinide



Precision

The precision of the procedure was determined by repeatability (intraday). Intraday precision was evaluated by assaying same concentration and during the same day. Repeatability of sample measurement was carried out in six different sample preparations from same homogenous blend of sample. Another replicate determination on three different days to estimate interday precision.

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To a preanalysed sample solution, a definite concentration of standard drug was added and recovery was studied. A 80%,100% and 120% of pure drug solutions were added to the preanalyzed samples (Table 3).

Limit of detection and limit of quantification

For HPLC method, the limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the response and the slope by using calibration curves.

Robustness

For the HPLC method, the robustness was determined by the analysis of the samples under a variety of conditions making small changes in the buffer pH (4.3 and 4.7), in the percentage of mobile phase compounds (phosphate buffer : methanol in the ratios 38 : 62 and 42 : 58), in the flow rate (0.9 and 1.1 ml/min), in the temperature conditions (35 and 45°C), and changing the wavelength (223 and 225 nm).

System suitability

System suitability parameter is established to ensure that the validity of the analytical method is maintained whenever used. Typical variations are the stability of analytical solution, different equipment, and different analyzer. In case of liquid chromatography typical variations are the pH of the mobile phase, the mobile phase composition, different lots or supplier of columns, the temperature and flow rate.

Analysis of marketed formulation

The response of sample solution was measured at 230nm using HPLC. The amount of REG was determined by regression equation and results are shown in Table 4.

Forced degradation under acidic condition

Weigh accurately 10mg of repaglinide, transfer in 100ml volumetric flask. Dissolve in 50ml of methanol then add 50ml of 0.1 N HCl and reflux at 80°c for 8 hours. From this solution take 0.2ml and dilute upto 10ml with methanol (2µg/ml). This solution was injected for HPLC determination.

Forced degradation under alkaline condition

Weigh accurately 10mg of repaglinide, transfer in 100ml volumetric flask. Dissolve in 50ml of methanol then add 50ml of 0.1 N NaOH and reflux at 80°c for 8 hours. From this solution take 0.2ml and dilute upto 10ml with methanol (2µg/ml). This solution was injected for HPLC determination.

Forced degradation under oxidative condition
Weigh accurately 10mg of repaglinide, transfer in 100ml volumetric flask. Dissolve in 50ml of methanol then add 50ml of 6% H_2O_2 and kept at room temperature for 4 hours. From this solution take 0.2ml and dilute upto 10ml with methanol (2µg/ml). This solution was injected for HPLC determination.

Forced degradation under thermal (dry heat) condition
Weigh accurately 10mg of repaglinide, kept at 80°c for 4 hours transfer in 100ml volumetric flask. Dissolve in 50ml of methanol then dilute upto mark with methanol. From this solution take 0.2ml and dilute upto 10ml with methanol (2µg/ml). This solution was injected for HPLC determination.

Table 1. Data of regression analysis and calibration curve of REG

Conc. (µg/ml)	Area under Curve (AUC) at RT 3.31± 0.5 min					Mean
	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	
0.5	4912	5125	5142	4816	4962	4991.4
1	8609	9432	8932	9215	9314	8961.5
1.5	12567	13120	12142	12819	12925	12746
2	16978	16632	15620	16618	16854	16916
2.5	21213	20918	20122	21315	20225	20719
3	24954	24812	23465	24512	24124	24539
r2	0.9993	0.9992	0.998	0.9982	0.999	0.99915
slope	8139	7794.6	7352.2	7918.8	7569.8	7854.4
intercept	628.87	1366	1370.9	1024.6	1486.8	1057.835

Table 2. Result of system suitability parameters for REG

System suitability Parameter	RT	AUC	No. of theoretical plates	Tailing factor
REG(2µg/ml)	3.31	16978	726.2	0.97
REG(2µg/ml)	3.28	16632	730.8	0.97
REG(2µg/ml)	3.29	15620	724	0.97
REG(2µg/ml)	3.31	16618	745.6	0.96
REG(2µg/ml)	3.30	16854	721.8	0.98
REG(2µg/ml)	3.30	16916	727	0.98
Mean	3.298333	16736.67	729.2333	0.97
S.D.	0.01169	131.494	8.56917	0.01
% R.S.D.	0.354435	0.785664	1.175093	1.03

Table 3. Results of recovery studies of REG

Brand Name	Batch No.	Conc. Of Form. (µg/ml)	Conc. Of Std. added (µg/ml)	Conc. Recover (µg/ml)	% recovery
Europa-0.5	C8629003	0.5	0.4	0.897	99.71%
		0.5	0.5	0.995	99.59%
		0.5	0.6	1.099	99.98%
Europa-1	C8789002	1	0.8	1.798	99.89%
		1	1	1.999	99.98%
		1	1.2	2.199	99.95%

*Mean recovery average of three determination

Table 4. Result of Market formulation of REG

Brand Name	Amount taken (µg/ml)	Amount found (µg/ml)	%Amount found
Europa-0.5	0.5	0.498	99.6±1.52
Europa-1	1	0.997	99.7±0.56

Table 5. Summary of Validation Parameters

Parameters	REG
Wavelength	230 nm
Range	0.5 -3 µg/ml
Linearity	0.9993
Intercept	628.87
Slope	8139
Intra day precision	%RSD < 2
Inter day precision	%RSD < 2
LOD	0.056µg/ml
LOQ	0.172µg/ml

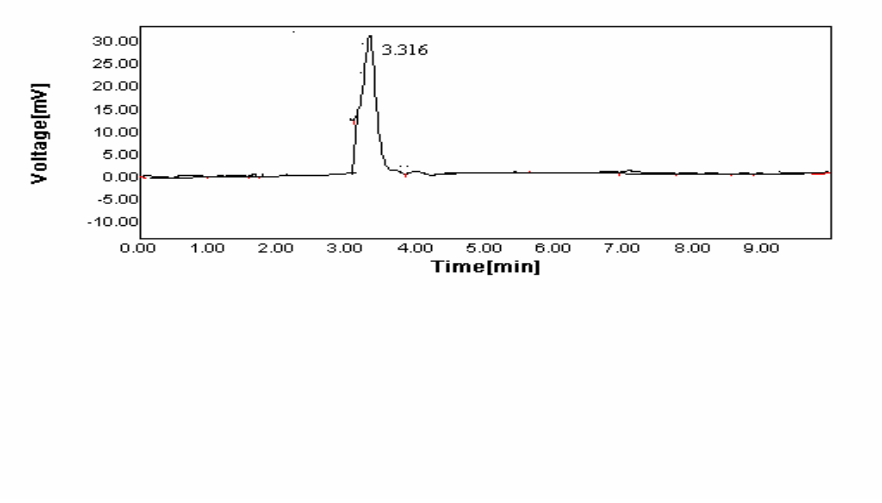
Table 6. Result of Forced degradation study

Degradation condition	Conc. Of drug(µg/ml)	RT of observed peak	AUC	%of drug	%of degrdation
acidic	2	3.316	16978	100.00%	0.00%
alkaline	2	3.283	16129	95.17%	
		2.383	849		4.83%
oxidative	2	3.283	15602.7	91.90%	
		2.924	1375.3		8.09%
dry heat	2	3.283	16587.5	97.70%	
		2.238	390.5		2.30%

Result and discussion

The mobile phase consisting of acetonitrile: 10mM ammonium acetate (70:30, v/v) having pH 3.0 adjusted with phosphoric acid, at 1ml/min flow rate which gave sharp, well-resolved peak with minimum tailing factor for repaglinide. The retention time for repaglinide was 3.3 min and detection wavelength was 230 nm(Fig.3).

Figure 3. A typical chromatogram of REG(2µg/ml)



The calibration curve for repaglinide was found to be linear over the range of 0.5-3 µg/ml. The data of regression analysis of the calibration curves is shown in Table 1. The proposed method was successfully applied to the determination of repaglinide in market formulation.

The LOD was found to be 0.056 µg/ml, while LOQ was 0.172 µg/ml. The results for system suitability test parameters and recovery study are summarized in Table 2 & 3. The summary of validation parameter for analysis of REG was shown in Table 5.

The degradation study indicated that repaglinide was susceptible to base, H_2O_2 and dry heat under experimental conditions. The Repaglinide showed no degradation in 0.1 N HCl when reflux at 80°c for 8hr condition(Fig. 4) and chromatogram showed no additional peak.

In alkaline hydrolysis the drug degrades as observed by the decreased area in the peak of the drug when compared with peak area of the same concentration of the nondegraded drug, with giving additional degradation peak (Fig. 5).

Figure 4. Forced degradation under acidic condition

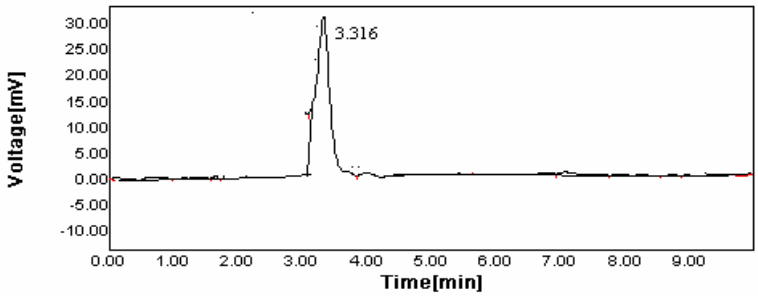
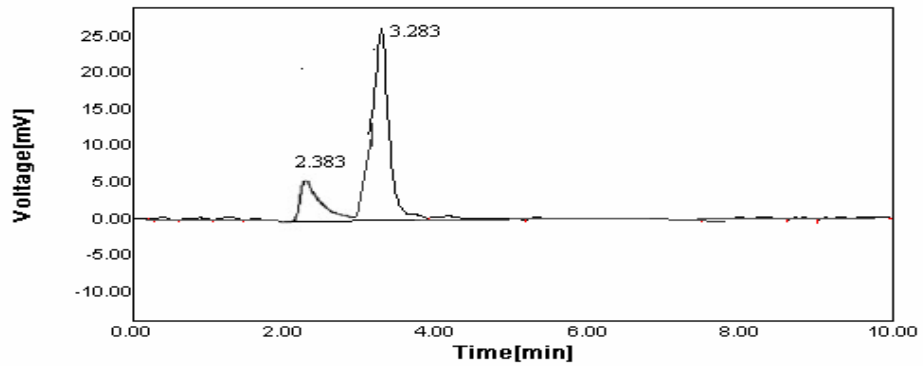


Figure 5. Forced degradation under alkaline condition



In oxidative and dry heat degradation, the drug degrades as shown by the decreased areas in the peaks when compared with peak areas of the same concentration of the nondegraded drug, with giving additional degradation peaks at different retention time(Fig.6 & 7).

Percent degradation was calculated by comparing the areas of the degraded peaks in each degradation condition with the corresponding areas of the peaks of the drug under non degradation condition. Summary of degradation studies was given in Table 6.

Figure 6. Forced degradation under oxidative condition

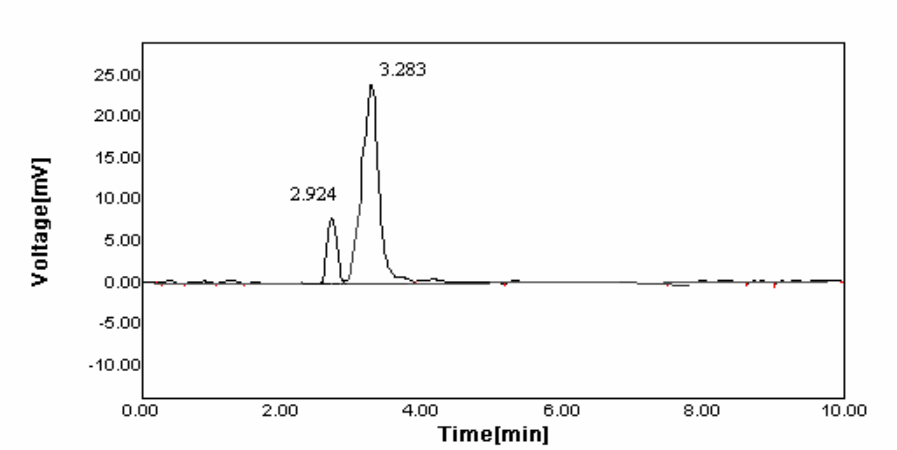
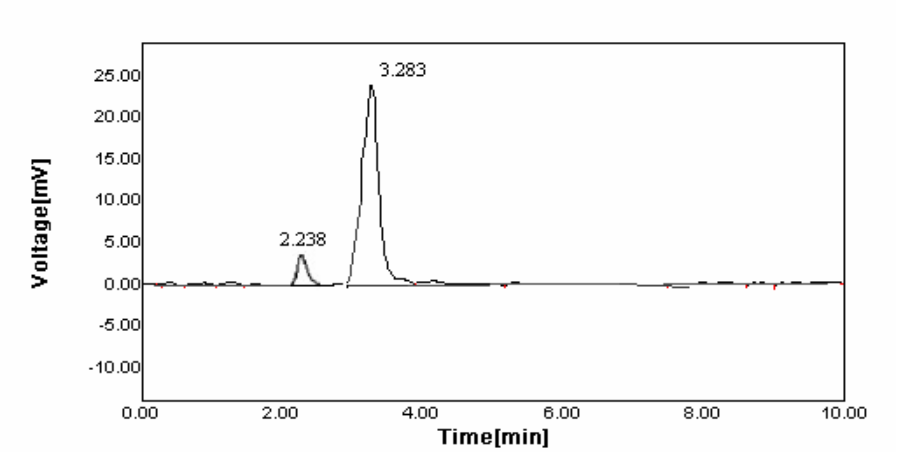


Figure 7. Forced degradation under thermal condition



Conclusion

The developed method was found to be simple, sensitive and selective, accurate, precise, and repeatable for analysis of repaglinide in market formulation without any interference from the excipients. The method was successfully used for determination of drugs in a pharmaceutical formulation. The results indicated the suitability of the method to study stability of repaglinide under various forced degradation conditions like acid, base, dry heat and oxidative degradation. It can be concluded that as the method could separate the drugs from their degradation products.

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