Original article

Development and validation of a simple stability indicating UPLC method for the determination of repaglinide in pharmaceuticals

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Abstract:

A simple, precise and accurate stability-indicating isocratic reverse phase ultra-performance liquid chromatographic (RP-UPLC) method was developed for the determination of repaglinide (RPG) in bulk drug and in its tablets. The method was developed using Waters Aquity BEH C18 (100 × 2.1) mm, 1.7 μm column with mobile phase consisting of a mixture of potassium dihydrogen phosphate buffer of pH 3.2 and acetonitrile (40:60 v/v). The total run time for the assay was only 4 min. The eluted compound was detected at 245 nm with an UV detector. The standard curve of mean peak area versus concentration showed an excellent linearity over a concentration range 0.1-750 μg ml^{-1} RPG with regression coefficient (r) value of 0.9997. The limit of detection (S/N = 3) was 0.03 μg ml^{-1} and the limit of quantification (S/N = 10) was 0.1 μg ml^{-1}. With three quality control concentrations of 100, 200 and 300 μg ml^{-1}, accuracy and precision of the assay were satisfactory. Both within-day and between-day RSD were less than 1.0%. The method was validated by the determination of RPG in tablets and the percent of the label claim was 100 ± 2%. The accuracy of the method was further ascertained by recovery studies *via* standard addition procedure and the recoveries obtained were 96.6-100.9%. Forced degradation of the bulk sample was conducted in accordance with the ICH guidelines. Acid and base hydrolysis, oxidative, thermal stress and photolytic degradation were used to assess the stability indicating power of the method. RPG was found to degrade significantly in acidic and basic stress conditions and stable in oxidative, thermal and photolytic conditions. The degradation products were well resolved from main peak proving the stability-indicating power of the method.

Keywords: Repaglinide; UPLC; Validation; Stability indicating; Pharmaceutical

Introduction

Repaglinide (RPG), chemically known as (*S*)-(+)-2-ethoxy-4-[2-(3-methyl-1-[2-(piperidin-1-yl)phenyl] butylamino)-2-oxoethyl]benzoic acid (Fig. 1) is a non-sulfonyl urea oral hypoglycemic agent of the meglitinide class Type 2 anti-diabetic drug [1]. It reduces the fasting glucose concentrations in patients. It helps to control blood sugar by increasing the amount of insulin released by the pancreas. RPG is official in United States Pharmacopoeia [2] and European Pharmacopoeia [3] which recommend non-aqueous titrimetry for its assay. In the literature, two high performance liquid chromatography (HPLC) methods are available for the assay of RPG in human plasma [4, 5]. Several methods have been reported for the determination of RPG in pharmaceuticals and include spectrophotometry [6-10] differential pulse polarography [11] reversed-phase thin layer chromatography [12] and HPLC [13-15].

Though HPLC is a well-established reliable technique used in controlling the quality and consistency of active pharmaceutical ingredients (APIs) and formulations, it is often a slow technique because of the complexity of the samples; it could still be improved. Ultra performance liquid chromatography (UPLC) is a new category of separation technique based upon well-established principles of liquid chromatography, which utilizes sub-2 μm particles of stationary phase. The upper limit of pressure used for the mobile phase flow is 15,000 psi which is two times greater than the pressure limit of ordinary HPLC. The elevated mobile phase linear velocity results high resolution, sensitivity and low analysis time [16]. Owing to its speed and sensitivity, this technique is gaining considerable attention in recent years in different fields of pharmaceutical and biomedical analysis [17-21].

Despite these advantages, the UPLC has not been applied for the assay of RPG. The stability of drug substance or drug product is defined as its capacity to remain within established specifications, *i.e., to* maintain its identity, strength, quality and purity until the retest or expiry date [22]. Stability testing of an active substance or finished product provides how the quality of a drug substance or drug product varies with time under a variety of experimental conditions such as temperature, humidity and light. Knowledge from stability studies is used in the development of manufacturing process, selection of proper packaging and storage conditions, and determination of product shelf-life [23, 24]. There is no reported stability-indicating HPLC/UPLC method that can adequately quantify RPG in pure drug and in its tablets. It is, therefore, necessary to develop a stability indicating method for the quantitative estimation of RPG.

In this piece of work, the authors have made an attempt to develop a faster chromatographic technique, UPLC to reduce analysis time but without compromising the accuracy, precision and sensitivity. The developed method was validated as per the regulations of current ICH guidelines [23, 25]. The method was successfully applied to determine RPG in its tablets without getting any additional peaks from the inactive ingredients in the chromatogram and almost zero interference was observed.

Materials and Methods

Materials and reagents

Pure active ingredient sample of RPG was kindly supplied by Torrent Pharmaceuticals Ltd, Ahmadabad, India, as gift. Eurepa-1® (1 mg RPG) and Eurepa-2®

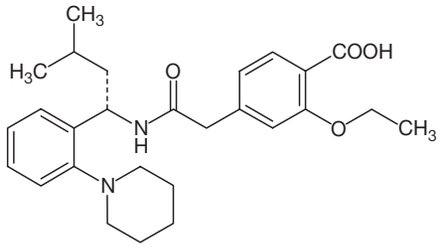


Figure 1 Structure of repaglinide

(2 mg RPG)-both tablets, marketed by Torrent Pharmaceuticals, Ahmadabad, India, were purchased from local commercial sources. HPLC grade acetonitrile was purchased from Merck, potassium dihydrogen ortho phosphate and orthophosphoric acid were from Qualigens-India. Doubly distilled water was used throughout the investigation.

Chromatographic conditions and equipment

Analyses were carried out on a Waters Aquity UPLC with Tunable UV (TUV) detector. The output signal was monitored and processed using Empower software. The Chromatographic column used was Acquity UPLC BEH C-18 (100 × 2.1) mm and 1.7 μm particle size. Isocratic elution process was adopted throughout the analysis.

Mobile phase preparation

Dissolved 2.0 g of potassium dihydrogen orthophosphate in 500 ml of water and adjusted the pH to 3.0 using 10% ortho phosphoric acid. A 400 ml portion of this resulting buffer was mixed with 600 ml of acetonitrile, shaken well and filtered using 0.22 μm nylon membrane filter. This solution was also used as diluent in all subsequent preparations of the sample.

Instrumental parameters

The isocratic flow rate of mobile phase was maintained at 0.50 ml min^{-1}. The column temperature was adjusted to 40°C. The injection volume was 1.2 μl. Eluted sample was monitored at 245 nm and the run time was 4.0 min. The retention time of the sample was about 2.1 min.

Stress study

A 25 mg of pure RPG was transferred into three different 50 ml volumetric flasks and added 10 ml of 5 M HCl, 5 M NaOH or 5% H_2O_2 separately, and the flasks were heated for 2 h on a water bath maintained at 80°C. Then the solutions were cooled and neutralized by adding base or acid, the volume in each flask was brought to the mark with mobile phase, and the appropriate volume (1.2 μl) was injected for analysis. Solid state thermal degradation was carried out by exposing pure drug to dry heat at 105°C for 3 h. For photolytic degradation studies, pure drug in solid state was exposed to 1.2 million lux hours in a photo stability chamber. The sample after exposure to heat and light was used to prepare 500 μg ml^{-1} solutions in mobile phase and the chromatographic procedure was followed.

Preparation of stock solution

A stock standard solution of RPG (5 mg ml^{-1}) was prepared by dissolving an accurately weighed 250 mg of pure drug in 50 ml volumetric flask using the mobile phase.

Procedures

Procedure for preparation of calibration curve

Working solutions containing 0.1-750 μg ml^{-1} of RPG were prepared by serial dilutions of aliquots of the stock solution. Aliquots of 1.2 μl were injected (six injections) and eluted with the mobile phase under the reported chromatographic conditions. The average peak area versus the concentration of RPG in μg ml^{-1} was plotted. Alternatively, the corresponding regression equation was derived using mean peak area-concentration data and the concentration of the unknown was computed from the regression equation.

Preparation of tablet extracts and assay procedure

Twenty five Eurepa-2® tablets (Each tablet contained 2.0 mg RPG) were weighed and transferred in to a clean, dry mortar and powdered. The entire material was transferred in to a 100 ml volumetric flask and 60 ml of the mobile phase was added. The solution was sonicated for 20 min to achieve complete dissolution of RPG, and the solution was then diluted to volume with the mobile phase to yield concentration of 500 μg ml^{-1} and filtered through q 0.22 μm nylon membrane filter. The solution obtained was analyzed by UPLC. The same procedure was repeated with fifty Eurepa-1® (each tablet contained 1.0 mg RPG) tablets.

Procedure for method validation

1. Accuracy and precision

To determine the accuracy and intra-day precision,

pure RPG solutions at three different concentrations were analyzed in six replicates during the same day. Mobile phase was injected as blank solution before sample injection and the RSD (%) values of peak area and retention time were calculated.

2. Limits of detection (LOD) and quantification (LOQ)

The LOD and LOQ were obtained by signal to noise (S/N) ratio method. LOQ and LOD were obtained by a series of dilutions of the RPG stock solution. Precision study was performed at LOQ level also. LOQ solution was injected six times (n=6) and calculated the %RSD values for the obtained peak area and retention time.

3. Linearity

Linearity solutions were prepared from LOQ level to 150% of the actual sample concentration (500 μg ml^{-1} RPG). A total of eight concentrations of the solutions were made separately and injected (LOQ, 125, 250, 375, 500, 625 and 750 μg ml^{-1} levels).

4. Robustness and ruggedness

To determine the robustness of the method the experimental conditions were deliberately changed. The flow rate of the mobile phase (0.5 ± 0.05 ml min^{-1}), column oven temperature ($40 \pm 5^{\circ}C$), mobile phase composition (66:34 and 54:36; acetonitrile: buffer v/v) and detection wavelength (245 ± 1 nm) were the varied parameters. In each case the %RSD values were calculated for the obtained peak area and retention time. The number of theoretical plates and tailing factors were compared with those obtained under the optimized conditions. Three different columns of same dimensions were used for the analyses. The study was performed in a same day and three different days by three different analysts for three different concentrations of RPG (triplicate injections). The area obtained from each concentration was compared with that of the optimized one. The relative standard deviation values were evaluated for each concentration.

5. Solution stability and mobile phase stability

Stability of RPG solution was performed by injecting the sample into the chromatographic system. The peak area was recorded in the time intervals of 0, 12 and 24 h and the RSD values were calculated. The mobile phase stability was studied by injecting a freshly prepared sample solution at the same time intervals (0, 12 and 24 h) and RSD values of the peak areas were calculated.

Results and Discussion

Method development

In order to achieve the better efficiency of the chromatographic system, the experimental conditions such as mobile phase composition, detection wavelength, column, column temperature, pH of mobile phase and diluent were optimized by varying one parameter and keeping other constant at a time. Several proportions of buffer, water, acetonitrile and methanol were evaluated in order to obtain suitable composition of the mobile phase. Choice of retention time, peak shape, theoretical plates and run time were the major tasks while developing the method. Several combinations of gradient methods were also performed. Isocratic method was found better to use for the assay.

RPG solutions injected with methanol mobile phase and the resultant peak showed tailing factor more than 2.0 which is not acceptable for US Pharmacopeia in normal conditions. Acquity BEH C18, (50 × 2.1) mm, 1.7 μm column used initially, but the peak eluted before 1.5 min with a tailing factor of 2. Phenyl (100 × 2.1) mm, 2 μm column was also found to give inconsistent result with fronting of the peak. The column temperature was varied from 20 to $45^{\circ}C$ in a $5^{\circ}C$ increment with the same column, the peak shape was found unaltered. Buffer and acetonitrile solvents ratio were changed and ended up with less number of theoretical plates. Different buffers like sodium dihydrogen othophospahe, dipotassium hydrogen orthophosphate and disodium hydrogen orthophosphate were tried and the results revealed that the use of potassium dihydrogen orthophosphate was most suitable. The pH of the mobile phase was varied from 2 to 6. At pH greater than 3.2, the peak eluted very early and resulted in less number of theoretical plates. At lower and higher flow rates, non-elution of peak and inefficiency of the system (pressure > 15000 psi), respectively, were found. Under these optimized conditions (70:30 acetonitrile:

buffer of pH 3.2 v/v, Acquity BEH C18, (100 × 2.1) mm, 1.7 µm column, 40°C, detection at 245 nm), the system found more suitable for the validation study with the tailing less than 1.1, number of theoretical plates > 5000 and %RSD less than 0.5. The typical chromatograms obtained for blank and pure RPG from final UPLC conditions are depicted in Fig. 2.

Forced degradation

All forced degradation samples were analyzed at 500 µg ml^{-1} concentration level. The observation was made based on the peak area of the respective sample. Degradation was not observed when RPG was subjected to light (1.2 million lux hours), thermal (105°C for 3 h) and oxidative (10% H_2O_2 for 2 h) conditions (observed degradation was less than 0.08%). Significant degradation was observed when the drug was subjected to acid and basic hydrolysis (1 N HCl and 1 N NaOH for 2 h), 35.8% and 41.9%, respectively. The chromatograms obtained for RPG after subjecting to degradation are presented in Fig. 3a-3e. Assay study was carried out by the comparison with the peak area of RPG sample without degradation.

Validation of the method

The described method for the assay of RPG has been validated as per the current ICH Q2 (R1) guidelines [25].

Analytical parameters

A calibration curve was obtained for RPG from LOQ to 150% of its stock solution. A linear correlation was obtained between the peak area and the concentration in the range of 0.1-750 µg ml^{-1} RPG from which the linear regression equation was computed and found to be:

$$Y = mC + a, \ (r = 0.9999) \qquad (1)$$

where Y is the peak area, C is the concentration of RPG in µg ml^{-1}, a is the intercept and r is the correlation coefficient. The LOD and LOQ values, slope (m), y-intercept (a) and their standard deviations are evaluated and presented in Table 1. These results confirm the linear relation between concentration of RPG and the peak areas as well as the sensitivity of the method.

Accuracy and precision

The percent relative error which is an index of accuracy is ≤ 1.5 and is indicative of high accuracy. The calculated percent relative standard deviation (%RSD) can be considered to be satisfactory. The peak area based and retention time based RSD values were < 1. The results obtained for the evaluation of precision and accuracy of the method are compiled in Table 2.

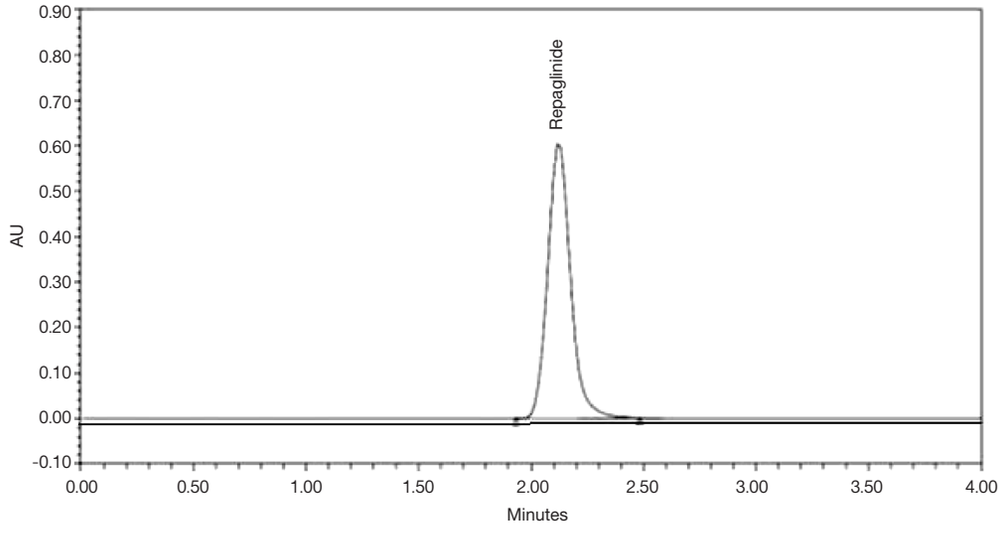
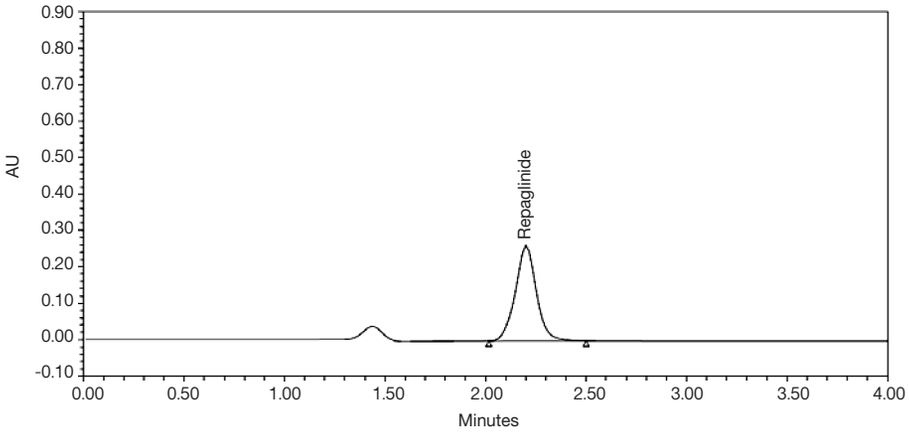
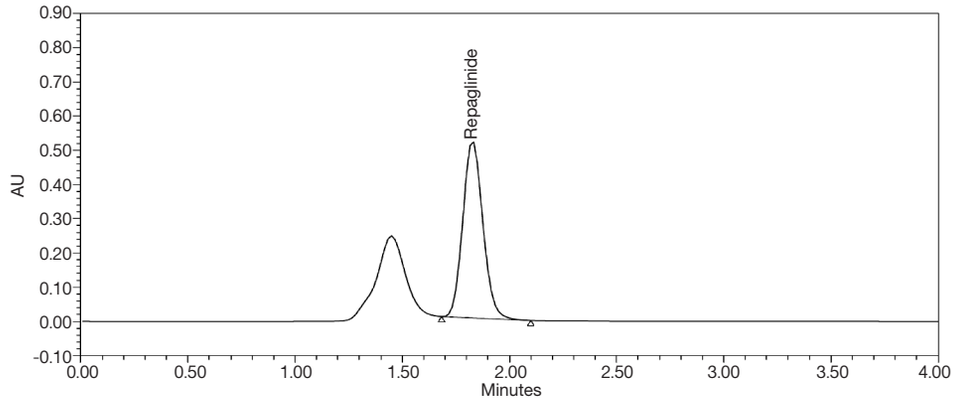


Figure 2 Pure repaglinide overlay with blank

a)


AU
Repaglinide
Minutes

b)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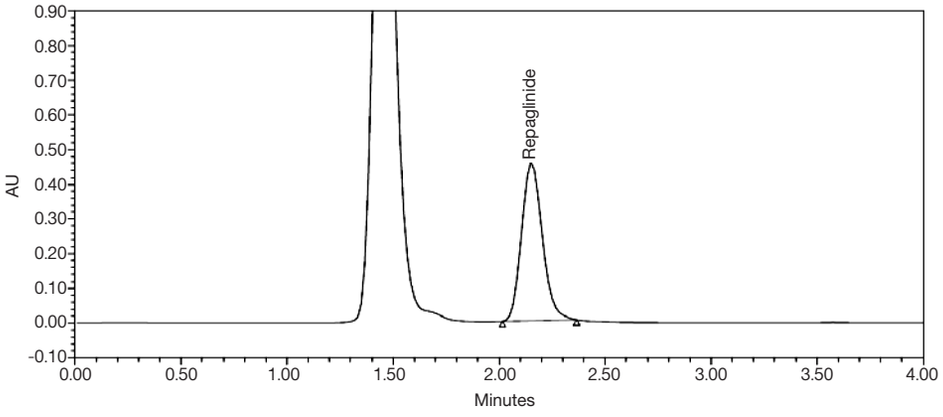
c)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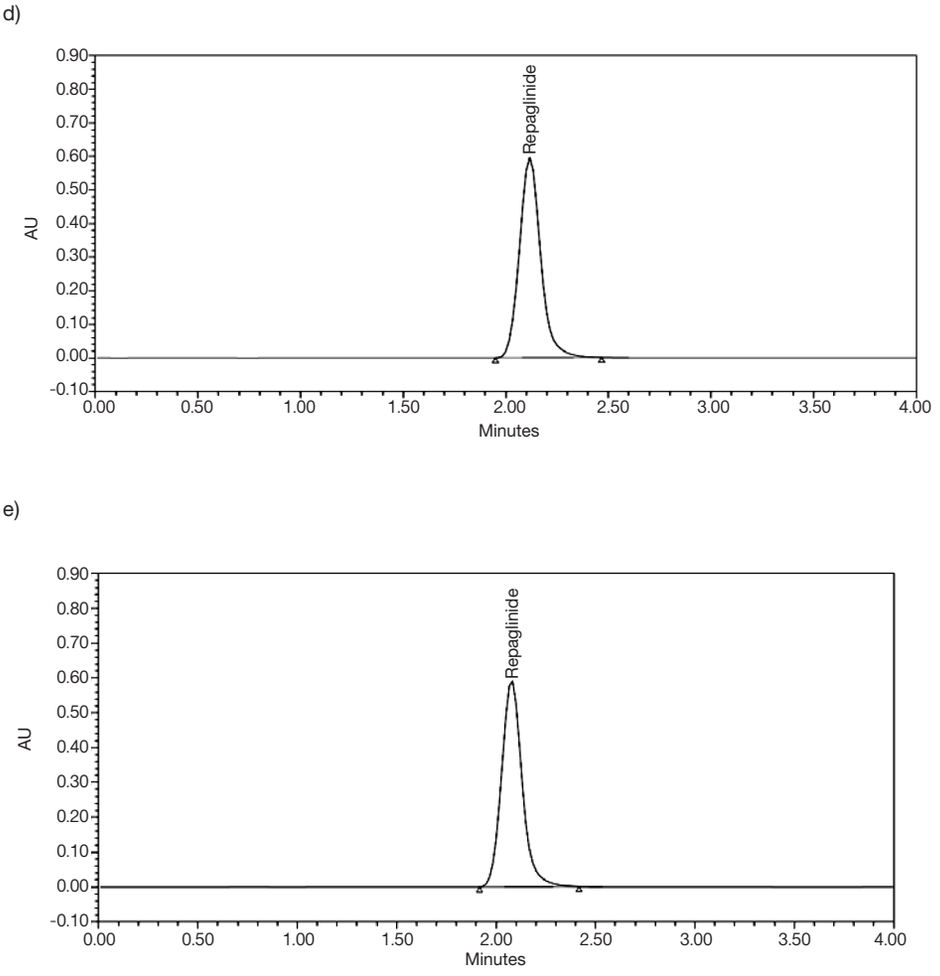

Figure 3 Chromatograms of repaglinide (RPG) after (a) forced degradation with 1 N HCl at 80°C for 2 h (pure RPG, 500 µg ml^{-1}); (b) forced degradation with 1 N NaOH at 80°C for 2 h (pure RPG, 500 µg ml^{-1}); (c) forced degradation with 10% H_2O_2 at 80°C for 2 h (pure RPG, 500 µg ml^{-1}); (d) thermal treatment at 105°C for 3 h (pure RPG, 500 µg ml^{-1}); (e) exposure to UV at 1.2 million lux hrs (pure RPG, 500 µg ml^{-1})

Table 1 Linearity and regression parameters with precision data

Parameter	Value
Linear range, (µg ml^{-1})	0.1-750
Limits of quantification, (LOQ), (µg ml^{-1})	0.10
Limits of detection, (LOD), (µg ml^{-1})	0.03
Regression equation	
Slope (b)	3195.5786
Intercept (a)	-8145.7707
Correlation coefficient	0.9999
Regression	0.9997
Residual sum of squares	1154120143.61539
Standard deviation of b, (S_b)	21.5031
Standard deviation of a, (S_a)	9691.3067

Table 2 Results of accuracy and precision study (n=6)

Results of accuracy study

Concentration of RPG injected (μg ml^{-1})	Intra-day		Inter-day	
	Concentration of RPG found (μg ml^{-1})	RE[a] (%)	Concentration of RPG found (μg ml^{-1})	RE (%)
100	98.74	1.26	100.68	0.68
200	201.88	0.92	101.70	0.85
300	297.75	0.75	303.10	1.04

Results of precision study

Concentration of RPG injected (μg ml^{-1})	Intra-day precision			Inter-day precision		
	Mean area ± SD	RSD[b]	RSD[c]	Mean area ± SD	RSD[b]	RSD[c]
100	292761 ± 1336	0.46	0.18	294549 ± 1922	0.65	0.64
200	587034 ± 969	0.17	0.15	584782 ± 4418	0.76	0.30
300	883424 ± 2093	0.24	0.11	886580 ± 4465	0.50	0.29

[a]Relative error

[b]Relative standard deviation based on peak area

[c]Relative standard deviation based on retention time

Table 3 Results of robustness and ruggedness (n=3)

Condition	Modification	Mean peak area	RSD (%)	Mean Rt	RSD (%)	Theoretical plates	RSD (%)	Tailing factor	RSD (%)
Actual	---	1464362	0.01	2.122	0.31	4951	1.61	1.01	0.10
Temperature	35°C	1498764	0.03	2.221	0.40	4971	1.99	1.04	0.19
	45°C	1457861	0.12	1.998	0.25	4824	1.17	1.00	0.20
Mobile phase composition (acetonitrile: buffer)	66:34	1468798	0.01	1.998	0.30	5122	2.05	1.02	0.49
	54:36	1498799	0.07	2.122	0.23	4598	2.13	0.99	0.50
Flow rate (ml min^{-1})	0.45	1506745	0.13	2.216	0.23	5125	1.96	1.02	0.68
	0.55	1467854	0.08	1.998	0.15	5137	1.97	1.03	0.87
Wavelength (nm)	244	1479753	0.37	2.106	0.29	4809	2.15	1.01	0.30
	246	1468793	0.67	2.112	0.33	4734	1.89	1.05	0.38
Analyst, column, day	Analyst-1, column-1, day-1	1469899	0.27	2.121	0.09	4813	2.27	1.01	0.10
	Analyst-2, column-2, day-2	1461896	0.08	2.023	0.35	4919	2.85	1.05	0.38
	Analyst-3, column-3, day-3	1471934	0.22	2.128	0.42	4892	1.61	1.05	0.29

Robustness and ruggedness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. At the deliberate varied chromatographic conditions (flow rate, temperature, and mobile phase composition), the analyte peak %RSD, tailing factor and theoretical plates were remained near to the actual values. The RSD values ranged from 0.2 to 0.7% resumes the robustness of the proposed method. In method ruggedness, different columns (same lot), at different day by different analyst were performed. The results are summarized in Table 3.

Stability of the solution

At the specified time interval, %RSD for the peak area obtained from drug solution stability and mobile phase stability were within 1%. This shows no significant change in the elution of the peak and its system suitability criteria (%RSD, tailing factor, theoretical plates). The results also confirmed that the standard solution of drug and mobile phase were stable at least for 24 h during the assay performance.

Selectivity

Selectivity of the method was evaluated by injecting the mobile phase, placebo blank, pure drug solution and tablet extract. No peaks were observed for mobile phase and placebo blank and no extra peaks were observed for tablet extracts (Fig. 4).

Application to tablet

A 500 μg ml^{1} solution of tablets was prepared as per *'preparation of tablet extracts and assay procedure'* and injected in triplicate to the UPLC system. The mean peak area of the tablets was found to be equivalent to the pure drug and the results were compared with those of a reference method [3]. The reference method involves the titration of the tablet extract in 1:6 (methanol: anhydrous acetic acid) with perchloric acid. The accuracy and precision of the proposed method was further evaluated by applying Student's t-test and variance ratio F-test, respectively. The t-and F-values at 95% confidence level did not exceed the tabulated values and this further confirms that there is no significant difference between the reference and proposed methods. Table 4 illustrates the results obtained from this study.

Recovery study

A standard addition procedure was followed to evaluate the accuracy of the method. The solutions were prepared by spiking pure drug solution into a pre-analyzed tablet extract at three different levels and injected to chromatographic column. The recovery of the known amount of added analyte was computed. The percentage recovery of RPG from pharmaceutical dosage forms ranged from 96.1% to 100.9%. Detailed results shown in Table 5 revealed good accuracy of the proposed method.

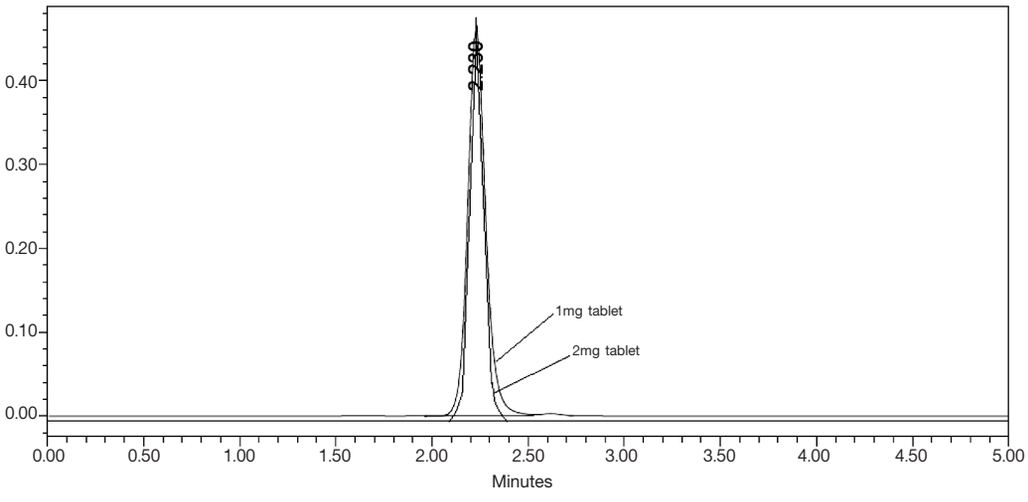

Figure 4 Chromatogram of tablet extract (1-mg and 2-mg tablets)

Table 4 Results of determination of repaglinide (RPG) in formulations and statistical comparison with the reference method

Formulation brand name[a]	Nominal amount, mg	RPG found[b] (%) ± SD		t-value	F-value
		Reference method	Proposed method		
Eurepa-1®	1.0	99.51 ± 0.62	100.10 ± 0.21	2.35	6.13
Eurepa-2®	2.0	101.2 ± 0.85	99.94 ± 0.78	2.44	1.19

[a]Marketed by Torrent pharmaceuticals, India;

[b]Mean value of five determinations. Tabulated t-value at 95% confidence level is 2.78; Tabulated F-value at 95% confidence level is 6.39

Table 5 Results of recovery study by standard addition method

Tablet studied	Tablet (μg ml^{-1})	Pure RPG (μg ml^{-1})	Mean peak area	Total RPG found (μg ml^{-1})	Percent recovery of pure RPG, (%RPG ± SD)
Eurepa 1	200.2	100	943398.85	297.77	97.57 ± 0.84
	200.2	200	1256749.77	395.83	97.82 ± 0.47
	200.2	300	1593299.71	501.14	100.30 ± 0.73
Eurepa 2	199.9	100	948452.85	299.35	99.45 ± 0.80
	199.9	200	1248399.77	393.21	96.66 ± 0.56
	199.9	300	1598765.71	502.85	100.98 ± 0.88

Conclusion

A rapid, isocratic RP-UPLC method developed for quantitative analysis of repaglinide in pharmaceutical dosage forms is precise, accurate, linear, robust and specific. Satisfactory results were obtained from validation of the method. The retention time obtained (2.1 min) enables rapid determination of the drug which is important in routine analysis. The method exhibited an excellent performance in terms of sensitivity and speed. The method is stability indicating and can be used for routine analysis of production samples and can be used for the assay of repaglinide either in pure drug or pharmaceutical formulations. Degradation study of the method reveals that the product is highly unstable in acidic and basic media.

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