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Development and validation of an HPLC method for the analysis of repaglinide in pharmaceutical dosage forms

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ABSTRACT

A simple reverse phase HPLC method was developed for the determination of repaglinide present in pharmaceutical dosage forms. A Hypersil ODS C18, 4.6mm×250 mm, 5m column from Supelco (India), with mobile phase methanol: ammonium acetate buffer (pH-4) (80:20) was used. The flow rate was 1.0 ml/min and effluent was monitored at 240 nm. Ritonavir is used as internal standard. The retention times were 6.19 min and 5.23 min for repaglinide and ritonavir respectively. The linearity range was found to be 0.5-200µg/ml for repaglinide. The proposed method was also validated.

KEYWORDS

Repaglinide;
Anti-diabetic;
Recovery studies.

INTRODUCTION

Repaglinide, 2-ethoxy-4-[[3-methyl-1-[2-(1-piperidyl)phenyl]-butyl] carbamoylmethyl] benzoic acid[1], is a meglitinide class oral hypoglycemic used as an adjunct to diet and exercise in the treatment of type 2 diabetes mellitus[2]. Repaglinide binds a characterizable site on the beta-cells in the pancreas and closes ATP-dependent potassium channels. Intracellular uptake of repaglinide is very limited, however, intracellular uptake of some drugs, notably, tolbutamide is not required to stimulate insulin secretion[3]. The drug is available in the market as tablet form. A search on literature reveals that one HPLC method is reported so far for estimation in pharmaceutical dosage forms[4], and a few HPLC methods have been reported for repaglinide in biological fluids[6-8]. The aim of present study is to develop a simple, sensitive, precise and accurate RP-HPLC method for the determination of repaglinide in bulk drug and in pharmaceutical dosage forms.

EXPERIMENTAL

Instrumentation

Quantitative HPLC was performed on a binary gradient HPLC with Shimadzu LC10AT and LC10AT VP series HPLC pumps, with a 20µl Injection of sample loop (manual), and SPD 10A VP UV-Visible detector. The output signal was monitored and integrated using Shimadzu CLASS-VP Version 6.12 SP1 software. Hypersil ODS C_{18} (46 mm X 25 cm, 5mcm) column was used for the separation. The pH of the solution was adjusted by using digital pH Meter, Model DI 707 (Digisun electronics, Hyderabad, India).

Standards and chemicals

Repaglinide and ritonavir used as an internal standard (IS) were gifts obtained from Aurobindo Pharma

(Hyderabad, India) and Ranbaxy Laboratories Limited (Mohali, India). Rapilin tablets (Sun pharma.) and Eurepa tablets (Torrent pharma) containing 2 mg and 2 mg of repaglinide were purchased from local market. Purified water was prepared using a Millipore Milli-Q (Bedford, M.A., USA) water purification system. Methanol of HPLC grade was purchased from Burdick and Jackson (Muskagon, MI, USA), ammonium acetate of A.R. grade and formic acid of A.R. grade were purchased from local suppliers.

A 0.01 M solution of ammonium acetate (pH: 4.0) was prepared by dissolving 0.77g of ammonium acetate in 800 ml water and diluting to 1000 ml with water. The pH was adjusted to 4.0 with formic acid. The contents are filtered through 0.45μ membrane filter.

Preparation of standard drug solutions

Stock solution of repaglinide was prepared by dissolving 25 mg of repaglinide in 25 mL of volumetric flask containing 20 mL methanol. The solution was sonicated for about 20 min and then made up to volume with methanol. Daily working standard solutions of repaglinide was prepared by suitable dilution of the stock solution with appropriate mobile phase. Similarly stock solution of internal standard was prepared by dissolving 25mg of ritonavir in 25 mL of methanol.

Chromatographic conditions

The mobile phase used in this study was a mixture of ammonium acetate buffer (0.01M P^H 4.0 adjusted with formic acid) and methanol 20:80%v/v. The stationary phase is a Hypersil ODS C18, 4.6mm×250 mm, 5μm column. The contents of the mobile phase were filtered before use through a 0.45μ membrane and degassed for 15 min. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1ml/min. The column temperature was maintained at 23±1^{0}C. The eluents were monitored at 240nm using UV-detector. The identification of the separated repaglinide and ritonavir were confirmed by running the chromatograms of the individual compounds under identical conditions.

Calibration of standards

Calibration standards were prepared by spiking working standard solutions into methanol containing 5 mL volumetric flasks to yield concentrations of 0.5, 1, 2, 5, 7.5, 10, 15, 20, 50, 100, 150, and 200μg/mL. To the above solutions 20μg/mL of ritonavir (internal standard) was added and the final volume was made up to the mark. The linearity data were shown in TABLE 1. Calibration curve was plotted between peak area ratios of drug vs. internal standard against concentration of the drug.

TABLE 1: Linearity table for repaglinide

Concentration (μg/mL)	Peak area ratio's	Statistical analysis
0.5	0.064	
1.0	0.13	
2	0.25	
5	0.64	
7.5	0.94	Slope: 0.1209
10	1.23	Intercept: 0.0614
15	1.91	Correlation
20	2.41	Coefficient:0.9998
50	6.3	
100	12.43	
150	18.18	
200	24.07	

Regression equation from 0.5-200 (μg/mL): Y=0.1209X+0.0614, (r^2=0.9998)

TABLE 2: Amount of repaglinide in tablet dosage forms by proposed HPLC method

Formulation	Labeled amount (mg)	Mean ±s.d (amount mg recovered)n = 3	Mean ±s.d (% of recovery)
Ripilin	2	1.96 ± 0.15	98.11 ± 0.42
Eurepa	2	1.95 ± 0.29	97.39± 0.75

Recovery of repaglinide in tablets

Twenty tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 25 mg of repaglinide was extracted with methanol in a 25mL volumetric flask using ultra sonicator. This solution was filtered through Whatmann No 1 filter paper. The solution obtained was diluted with the mobile phase so as to obtain a concentration in the range of linearity previously determined. An aliquot of the internal standard was added to the sample solution prior to the dilution. All determinations were carried out in six replicates. The represented data were shown in TABLE 2.

RESULTS

The developed method was used based upon its ability to detect and quantify repaglinide with the use of

standard high-performance liquid chromatographic system equipped with UV-Visible detector.

Method validation[9,10]

1. Specificity and selectivity

The HPLC chromatograms recorded for the drug-matrix (mixture of the drug-excipients) showed almost no peaks within a retention time range of 10 min. as shown in figure 1. The figure shows that repaglinide is clearly separated from its internal standard. The retention time, asymmetric factor and peak area ratio of the marketed formulations were not affected with excipients present in formulation. Thus, the HPLC method presented in this study is selective and specific for repaglinide.

2. Linearity

The standard curve was obtained in the concentration range of 0.5-200g/mL. The linearity of this method was evaluated by linear regression analysis, which was calculated by least square method. The mean ± standard deviation (SD) for the slope, intercept and correlation coefficient of standard curves (n=6) were calculated.

3. Limit of detection (L.O.D) and limit of quantification (L.O.Q)

Limit of detection was found to be 0.128µg/mL (signal to noise ratio is 3) and limit of quantification was found to be 0.427µg/mL (signal to noise ratio is 10).

4. Precision and accuracy

The precision of the method was demonstrated by inter day and intra day variation studies. In the intra day studies, six repeated injections of standard solutions were made and the response factor of drug peaks and percentage Coefficient of variance (C.V) were calculated and presented in TABLE 3. In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage C.V were calculated and presented in TABLE 3. From the data obtained, the developed RP-HPLC method was found to be precise. The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in TABLE 4. From the data obtained, added recoveries of standard drugs were found to be accurate.

TABLE 3: Precision studies

S.no	Concentration taken (µg/ml)	Intra-day Measured concentration (µg/ml) Mean ± S.D	Intra-day % C.V	Inter-day Measured concentration (µg/ml) Mean ± S.D	Inter-day % C.V
1	20	20.97 ± 0.1	0.47	20.02 ± 0.16	0.79
2	50	50.16 ± 0.12	0.23	49.62 ± 0.21	0.42
3	100	99.89 ± 0.14	0.14	98.99 ± 0.06	0.06

TABLE 4 : Accuracy studies

Sl. no.	Amount of standard drug added to pre-analysed formulation	Amount of pure drug recovered Mean ±s.d (µg) (n=6)	% of recovery Mean ±s.d
1	40	39.98±0.11	99.96±0.29
2	50	49.97±0.15	99.94±0.30
3	60	59.63±0.61	99.38±1.03

TABLE 5 : System suitability parameters of repaglinide

Sl.no.	Parameter	Value
1	Resolution	2.27
2	Capacity factor	2.13
3	Theoritical plates	2759
4	Tailing factor	0.914
5	HETP	0.091×10^{-5}
6	Asymmetry	1.02

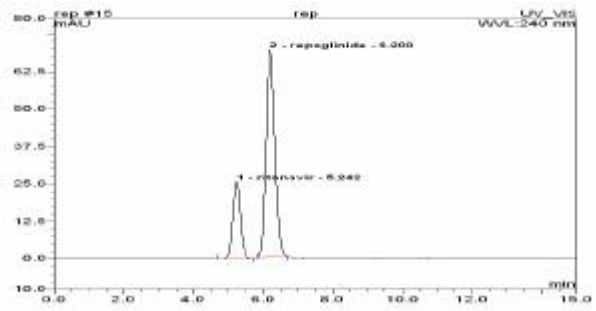

Figure1: A typical chromatogram of repaglinide in formulations

5. System suitability

For system suitability, six replicates of standard sample were injected and studied the parameters like plate number (N), tailing factor (k), resolution (R) and relative retention time (α), height equivalent theoretical plate (HETP), capacity factor (k^1), plates per meter and peak symmetry of samples. The represented data was shown in TABLE 5.

6. Robustness

The percent recovery of repaglinide was good under most conditions and didn't show any significant change when the critical parameters were modified. The tailing factor for repaglinide was always less than 2.0 and the components were well separated under all the changes carried out. Considering the modifications in the system suitability parameters and the specificity of the method, as well as carrying the experiment at room temperature may conclude that the method conditions were robust.

DISCUSSION

The chromatographic method was optimized by changing various parameters, such as pH of the mobile phase, organic modifier and buffer used in the mobile phase. Retention of repaglinide has more dependence on pH of the mobile phase when compared to ritonavir. The separation of peaks was also dependent on pH of the buffer and the percentage of methanol.

Under the presently prescribed conditions, the recovery studies of repaglinide were found to be from 99.38 to 99.96 %respectively. This method is very useful for determination of repaglinide in pharmaceutical dosage forms, clinical studies and pharmacokinetic studies. The observation of C.V less than 2.0 for both intra- and inter-day measurements indicates high degree of precision. In the present method, a Hypersil ODS C18 column has been used and the buffer pH in the mobile is 4.0, which is within the limits (pH 2-8) specified by the manufacturers. In the present method, we have established a linearity range of 0.5-200µg/mL; this linearity range covers all the strengths of repaglinide. Hence this method can be applied for quantifying the low levels of repaglinide in pharmaceutical dosage forms and other pharmacokinetic studies.

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