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High Performance Liquid Chromatographic Method for Estimation of Repaglinide in Tablet Dosage Forms

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ABSTRACT

A simple and sensitive, HPLC method has been developed and validated for the quantitative estimation of repaglinide in its single component tablet formulations (2 mg). Determination was performed using a Thermo BDS C18 column with mobile phase phosphate buffer: acetonitrile (pH 6.0; 45:55, v/v), and ultraviolet (UV) detection at 288 nm. The method was validated in terms of linearity (10–60 µg/ml), precision (intra-day variation below 0.6, inter-day variation below 0.2), accuracy (97.9 to 100.1%), ruggedness (within 0.5%) and specificity. The limit of detection and limit of quantification for repaglinide were found to be 1 µg/ml and 3 µg/ml, respectively. The developed method was successfully used for the assay of repaglinide tablet formulations. The method is simple, sensitive and precise; it can be used for the routine quality control testing of marketed formulations.

KEY WORDS

Repaglinide, acetonitrile, methanol, potassium dihydrogen ortho phosphate buffer, HPLC

INTRODUCTION

Repaglinide is a meglitinide antidiabetic used in the management of type 2 diabetes mellitus, chemically *S*(+)2-ethoxy-4(2((3-methyl-1-(2-(1-piperidinyl) phenyl)-butyl) amino)-2-oxoethyl) benzoic acid[1-2]. It is official in USP[3] which describes liquid chromatographic method for its quantitation. Literature survey reveals that one HPLC method in human plasma[4], two HPLC[5-6], one RPTLC[7] and one spectrophtometric method[8] in pharmaceutical dosage form. The purpose of this work was to develop and validate simple, specific, sensitive, accurate, precise, rapid and cost effective HPLC method for the estimation of rosiglitazone in its formulations.

MATERIALS AND METHODS

Materials and reagents

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Repaglinide standard was procured as a gift sample from Torrent Research center, Bhat, Ahmedabad. HPLC grade acetonitrile and methanol were obtained from Spectrochem Pvt. Ltd., Mumbai. Analytical grade Potassium dihydrogen ortho phosphate and sodium hydroxide were purchased from Finar Chemical Ltd., Ahmedabad. High purity water was prepared by using Sartorius Arium 611 purification system. Tablets of Eurepa manufactured by Torrent Pharma. Ltd., Ahmedabad and Regan, manufactured by Ranbaxy Ltd., Mumbai, labeled claim 2 mg of repaglinide were purchased from the market.

Instruments

HPLC analysis (Shimadzu LC-2010 C_{HT}) was performed with dual reciprocating pump, a multi wavelength UV/VIS detector, rheodyne auto injector was used for the chromatographic separations and Thermo BDS C18 column (250 mm × 4.6 mm i.d., particle size 5 µ) with quadrapole gradient system. The HPLC system was equipped with the software "Class VP" (Shimadzu).

Chromatographic separation condition

The mobile phase consisted of a mixture of aqueous 0.02 M potassium dihydrogen ortho phosphate: acetonitrile (45:55, v/v) adjusted to a pH of 6.0 with 0.1 M sodium hydroxide. The mobile phase was sonicated for 10 min and filtered through a 0.45 µm nylon membrane filter prior to use. Symmetry Thermo BDS C18 column (250×4.6 mm, 5 µm packing) was used for the separation. The flow rate of mobile phase was 1.0 ml/min and the column was operated at ambient temperature (30 °C). The sample injection volume was 10 µl. The UV detector was set at a wavelength of 288 nm.

Diluting solution

Methanol was used as diluting solution for pure sample and table formulation.

Preparation of standard solution

Repaglinide (10 mg) was weighed accurately and transferred in 100 ml volumetric flask. It was dissolved in and diluted up to mark with methanol. The final solution contained 100 µg of repaglinide per ml of the solution (S1).

Preparation of calibration curve of standard repaglinide

Standard repaglinide solution (1-6 ml) were pipetted into a series of 10 ml volumetric flask and mixed with methanol and diluted to the mark with same solvent to get concentrations of 10, 20, 30, 40, 50 and 60 µg/ml. Each solution (10 µl) was injected three times into the column and the peak area and retention times were recorded.

Preparation of sample solution from formulation

Twenty tablets were weighed and finely powdered. The powder equivalent to repaglinide (10 mg) was weighed accurately and dissolved in methanol (30 ml) and sonicated for 10 minutes and make the final 100 ml volume with methanol. The solution was filtered through Whatman NO. 45 filter paper. Discard first few ml of solution and from filtrate (100 µg) solution; (3 ml) was further diluted to 10 ml with methanol to obtain 30 µg/ml of repaglinide.

RESULTS AND DISCUSSION

The system suitability test was carried out by injecting 15 µg/ml of standard repaglinide solution six times into the chromatographic system under the optimized conditions to check various parameters. System suitability results are as follows

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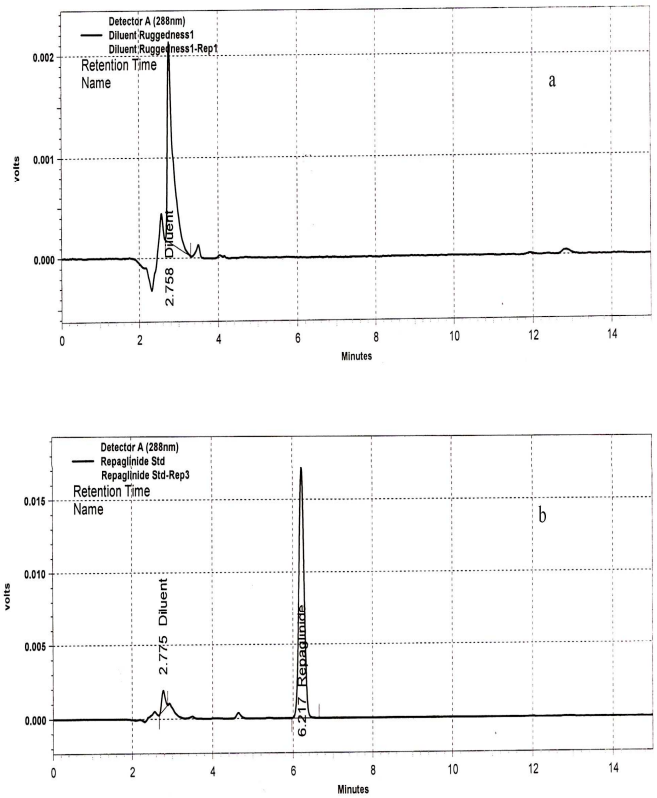
Retention time	Area (n = 6) Mean ± S. D.	Theoretical plate	Asymmetry
6.21	155535.3 ± 761.8	10681.8	1.02

To demonstrate the specificity of the method, peak of repaglinide tablet sample was compared with pure sample results. It was observed that the excipients present in formulation did not interfere with the peak of repaglinide. Reproducibility was observed in both the cases (Fig.1).

Figure 1

Typical Chromatograms of repaglinide (Diluent (a), Standard Repaglinide (b) and its Formulation (c), (Rt = 6.21 min))



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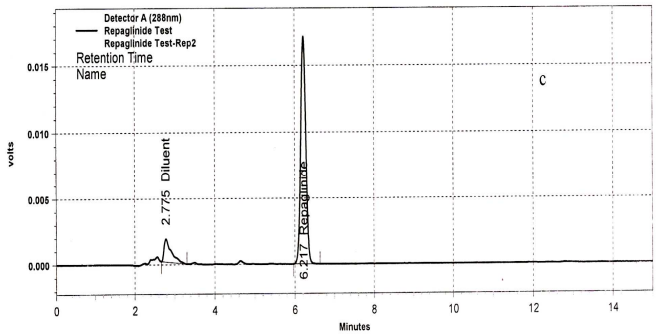

Linearity was checked by preparing standard solutions at six different concentration levels ranging from 10 - 60 µg/ml. The linearity was also checked for 3 consecutive days for the solutions of the same concentrations prepared from the stock solution. The precision for inter-day linearity is below 0.6% RSD. The equation for calibration curve is *y*= 4935x + 4862**.** The correlation coefficient was found to be 0.999, indicating good linearity. The accuracy of the method for assay determination was checked at five concentration levels i.e. at 10, 20, 30, 40 and 50 µg each in triplicate for 3 consecutive days. Solutions for the standard curves were prepared fresh every day. The assay accuracy variations shown in terms of % recovery were found to be 97.9 -100.1 %.

Table 1
Recovery Study

Conc. of repaglinide (µg/ml)		Area of repaglinide found Mean ± S.D. (n = 3)	% Recovery ± C.V.
Taken	Added		
5	5	52314.7 ± 79.2	100.1 ± 0.2
15	5	102344.3 ± 213.9	97.9 ± 0.2
25	5	152507.7 ± 210.7	98.4 ± 0.1
35	5	202759.0 ± 298.9	100.0 ± 0.1
45	5	247898.3 ± 1551.9	98.9 ± 0.6

Repeatability in the intra-day variations in assay is obtained at different concentration levels is expressed in terms of RSD values calculated from the data of each day for 3 days. RSD values of assay were found to be below 0.6. The intermediate precision, which is the inter-day variation at the same concentration level, was determined on successive days. The intermediate precision for assay of repaglinide was found to be below 0.2.

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Table 2
Precision Study

	Intra-day			Inter-day		
Sr. No.	**Conc. (µg/ml)**	**Peak Area Mean ± S.D. (n=3)**	**% C.V.**	**Conc. (µg/ml)**	**Peak Area Mean ± S.D. (n=3)**	**% C.V.**
1	10	52238.0 ± 218.6	0.4	30	153924.3 ± 196.2	0.1
2	20	104557.7 ± 63.9	0.1	30	153602.3 ± 228.3	0.1
3	30	154632.7 ± 596.9	0.4	30	152591.0 ± 198.3	0.1
4	40	202677.0 ± 254.1	0.1	30	152239.0 ± 77.4	0.1
5	50	250575.0 ± 1587.4	0.6	30	153634.0 ± 281.7	0.2

The ruggedness of an assay method is defined as the degree of reproducibility of the results obtained by analysis of the same sample under a variety of normal test conditions such as different labs, analysts, instruments and lots of reagents. The samples of day 2 were analyzed at laboratory B with a different instrument by a different analyst with different lots of reagents and another batch column. The data obtained from laboratory B were within 0.5% RSD when compared with the data of the parent lab. This was done by small deliberate changes in the chromatographic condition at 3 different levels -1, 0, +1 and retention time of repaglinide was noted. The factors selected were flow rate, temp and pH of the mobile phase. Results indicate that the selected factors remained unaffected by small variations of these parameters.

A signal-to-noise ratio of approximately 2–3 is generally considered to be acceptable for estimating the detection limit, which is the lowest concentration that can be detected. The LOQ is the lowest concentration of a substance that can be quantified with acceptable precision and accuracy. Atypical signal-to-noise ratio is 10:1. LOD and LOQ were found to be 1 and 3 µg/ml, respectively.

Different validation parameters for the proposed HPLC method for determining repaglinide content are summarized in Table 3.

Table 3
Summary of validation parameters for repaglinide by HPLC method

N0.	**Parameters**	**Result**
1	Linearity range (µg/ml)	10-60 µg/ml
2	Correlation co-efficient	0. 999
3	Precision	
	Intra-day % CV (n = 3)	Below 0.6
	Inter-day % CV (n =3)	Below 0.2
4	% Recovery	97.9 – 100.1
5	Ruggedness (% CV)	Within 0.5
6	Limit of detection	1 µg/ml

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7	Limit of quantification	3 µg/ml
8	Specificity	Specific
9	% Assay	98.6-100.1

This method was applied to determine the content of repaglinide in two different market samples of repaglinide tablet. The content and percentage of repaglinide in two different market samples were found to be 1.97 mg with an accuracy of 98.6±0.1% and 2.10 mg with an accuracy of 100.1±0.5%, respectively (n=3).

CONCLUSION

The results indicate that the proposed HPLC method was found to be simple, specific, rapid, precise and accurate for assay of repaglinide in its formulations.

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