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Bio-Analytical Method Development of Repaglinide Drug Delivery Systems

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ABSTRACT

A sensitive, specific and rapid high-performance liquid chromatography-ultraviolet spectroscopy method was developed and successfully validated to estimate the repaglinide in rabbit plasma. The solvent extraction method was used for repaglinide from serum by using ethyl acetate and 0.1N HCl. The mobile phase consists of acetonitrile: phosphate buffer pH 4.0 at 60:40 %v/v with 1% triethylamine at flow rate of 0.8ml/min and at fixed wavelength of 254nm. On ten minutes of run time, repaglinide was retention at 7.4min. The extraction efficiency 95% for repaglinide. The intra-day and inter-day precision was in the terms of %RSD less than 1.76%. The developed method was validated and proposed method is useful for pharmacokinetics studies.

Keywords: Anti-diabetics, HPLC,Methanol,Phosphate buffer, Repaglinide

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INTRODUCTION

Repaglinide is a meglitinide antidiabetic used in the management of type 2 diabetes mellitus, chemically 2-ethoxy-4-({[(1S)-3-methyl-1-[2-(piperidin-1-yl) phenyl]butyl]carbamoyl} methyl)benzoic acid (Fig 1). 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. Tablets containing 0.5, 1 and 2 mg of Repaglinide are available for oral administration [1,2]. It is official in USP [11] which describes liquid chromatographic method for its quantitation. A few analytical methods have been reported for its quantitative estimation in pharmaceutical formulations and biological samples, which include visible spectrophotometric [3,4,5], HPLC [6,7,8] and electrochemical methods [9]. The purpose of the present study was to develop a simple, sensitive, accurate and precise RP-HPLC method for the determination of Repaglinide in rabbit plasma.

MATERIALS AND METHODS

Materials

Repaglinide (98.3% purity), chemically, (s)-2-ethoxy-(1-[2-[[3-methyl-1-[2- (1-piperidinyl) phenyl] butyl] amino]-2-oxoethyl] benzoic acid, were kindly supplied by aurobindo Pharma Ltd (hyd, India). De-ionized water obtained from a Millipore-Q water purification system (Millipore®, Mumbai). Methanol (RFCL limited, New Delhi, India), Lichrosolv® water, Lichrosolv® acetonitrile for chromatographic separations, were obtained from Merck (Merck, Mumbai, India). Solvent and sample filtrations were done by using Ultipor® N®66 0.2 µm for and 0.45 µm membranes, respectively.

Instrumentation

The shimadzu UFLC system consists of following components: Prominence® CBM-20A controller, gradient system with dual pumps LC-10 AT VP, SPD-10 A VP detector with Class-VP: V6.13 software with BDS Hypersil® column, C-18, 150mm×4.6mm i.d., particle size 5µm (Thermo® scientific, India) was used at fixed wave length 254nm. Sonicator was used for solubility and de-aeration (PCI analytics, Mumbai, India). Centrifuge was from REMI®,

Mumbai, India. Waters symmetric® C18, i.d. 4.6×250 mm, 5 µm was used for check the accuracy of proposed method.

Chromatographic conditions

The column was equilibrated for at least 45min with mobile phase consists of acetonitrile: phosphate buffer pH 4.0 (60:40) with 1% triethylamine. It was sonicated before the equilibration of column for 20min and followed by filtered through 0.2µm whatman membrane. Gradient elution technique was applied, and flow rate was 0.8ml/min at fixed wavelength at 254nm.

Preparation of standard

Stock solutions were prepared by dissolving appropriate concentration repaglinide, in methanol to yield a final drug concentration of 550 µg/ml of repaglinide. Working 550, 440, 330, 220,110 µg/ml repaglinide was prepared by dilution of 550 µg/ml standard solution.

Extraction procedure

The extraction of plasma samples using the following procedure: First, 200 µl of plasma sample was pipetted out into a 1.5 ml Eppendorf tube; thereafter, mixture of working standard solutions and 50.0 µl of ethyl acetate and 10.0 µl of 0. 1N Hcl was added. repaglinide is insoluble in water, it has two pKa values due to zwitterionic behavior, and addition of hydrochloric acid gives ionization, results in improvement of water solubility. Due to hydrophobicity of the repaglinide, and it was impossible to dissolve directly into plasma. To the standard stock solutions, the subsequent plasma addition can cause protein precipitation, protein precipitation results in poor precision of analytical method. The mixture was vortex and 1.00 ml of methanol: phosphate buffer pH 4.0 was added. The mixed solution vortexes again subsequently centrifuged for 15 minutes at 10,000 g.The supernatant present after centrifugation was transferred to a 1.5 ml Eppendorf tube, and evaporated to dryness at 65 ºC for 90 min. The dried sample was reconstituted in 200 µl of the mobile phase prior to analysis.

Method development

BDS Hypersil® column was equilibrated and tested by using methanol/water mixture at various compositions and flow rate at 1ml/min. Results in broad peaks with poorer resolution, then switched to methanol/potassium dihydrogen phosphate buffer, gives less broader peaks than first one at different ratios. To optimize the separation, different fractions of acetonitrile and water tested, and optimum separation was obtained using 60% acetonitrile and 40% phosphate buffer pH 4 at flow rate10 of 0.8 ml/min at fixed wavelength of 254 nm.

Method validation

Once the chromatographic method was developed, it must be validated to check the efficiency of proposed method with USP guidelines to determine the assay, linearity, accuracy, precision, sensitivity, specificity and recovery.

Calibration curve

Standard combinations of solutions were prepared by serial diluting with phosphate buffer pH 4. The linearity was determined between the 55-550 ng/ml for repaglinide daily constructed by repeated analysis for five times (n=5) and continued for three days (n=15).

Recovery, Precision, Accuracy

Recovery studies were conducted for extracted samples by applying the least square regression analysis for peak areas vs. concentrations. The standard solutions were covering the linearity between 55-550 ng/ml for repaglinide Each sample injected for five times. Accuracy was determined by injecting three samples of standard solutions were in the range of 50%, 100% and 150% of repaglinide for five times, by the same operator, same day and same equipment and by the different column to check specificity of analytical method.

Specificity and selectivity

The specificity of repaglinide retention times were investigated by repeated analysis. The interferences of endogenous compounds were identified and resolved in combination with sulphonylureas like gliclazide, glipizide, thiazolidinediones like pioglitazone. Non-sulfonylureas like nateglinide and mitiglinide were used in combination with metformin in the treatment of type II diabetes mellitus in five different blank plasma samples.

RESULTS AND DISCUSSION

Chromatogram

The retention times and capacity factors was at 7.4 ±0.15, 4.35±0.04 for repaglinide.

Linearity shown in fig 1.

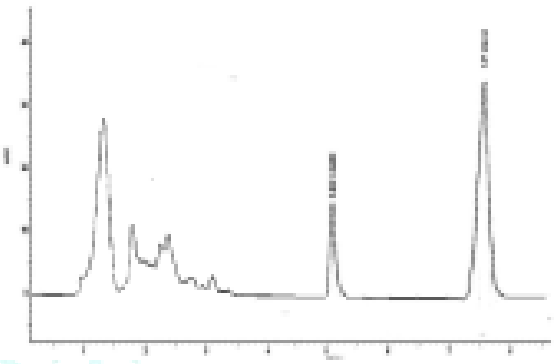

Fig.1 chromatogram of repaglinide in rabbit plasma

The chromatographic analysis of repaglinide exhibited excellent regression values R2=0.9995 over the concentration range of 55-550 ng/ml for repaglinide . The three day daily analysis of five samples, resulting in the calibration curves were don't have a statistical significant in values of slope, regression and intercepts.

The assays showed the acceptable precision in the terms of %RSD, <5 for repaglinide.

Recovery, precision and accuracy

Recoveries of repaglinide from extraction samples are 95% (n=5). The precisions of intraday analysis of five samples were in the terms of %RSD on the range over 0.26-0.78% for repaglinide in table 1. The inter-day analysis on three consequent days resulted in the range of 1.30-1.56 % for repaglinide. The accuracy of proposed method was checked by using different column on intra-day and inter-day assays were shown in table 1for repaglinide.

Table.1: Accuracy and precision of repaglinide in rabbit plasma samples

BDS Hypersil® column C18				Waters symmetric® column C18			
Intra-day (µg/mL)	Experiment (µg/mL) ± SD*	Accuracy (%)	Precision (%RSD)*	Experiment (µg/mL) ± SD*	Accuracy (%)	Precision (% RSD)*	n
2.75	2.72±0.02	98.91	0.78	2.73±0.014	99.27	0.51	05
5.50	5.48±0.01	99.64	0.26	5.45±0.035	99.09	0.64	05
8.25	8.30±0.04	100.61	0.43	8.23±0.014	99.75	0.17	05
Inter-day (µg/mL)							
2.75	2.69±0.04	97.82	1.56	2.69±0.042	97.81	1.57	15
5.50	5.39±0.08	98.00	1.43	5.16±0.240	93.81	4.55	15
8.25	8.10±0.11	98.18	1.30	8.01±0.169	97.09	2.10	15

*SD=Standard Deviation, %RSD=Percentage Relative Standard Deviation

Specificity and selectivity

The blank serum showed that no interference of endogenous and administered substance for elution on run time. The retention times were different for them not detected in the present chromatogram.

Limit of detection (LOD) and limit of quantitation (LOQ)

The LODs of repaglinide was 18.15ng/ml respectively. LOQ of repaglinide was 55ng/ml respectively11.

CONCLUSION

Highly sensitive and specific analytical method was developed and validated for quantification of repaglinide in rabbit plasma samples. This analytical method was applicable to study of pharmacokinetic parameters in the research study of novel drug delivery systems in rabbit as an animal model. The specificity of this method was tested in five different sources, were analyzed. The chromatogram of fig.1 shows about retention times of 7.4 for repaglinide . The current described HPLC method in rabbit plasma for repaglinide can be readily used for determination of pharmacokinetic parameters of novel drug delivery systems.

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