DEVELOPMENT, VALIDATION AND COMPARISON OF RP-HPLC AND UV METHODS USING STATISTICAL ANALYSIS ONE WAY ANOVA TEST FOR REPAGLINIDE IN FORMULATION

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ABSTRACT

In the present study, analytical UV and RP-HPLC methods for repaglinide were developed for tablet formulation. UV method shows linearity in the range of 10-30 µg mL^{-1} with a relative coefficient of 0.9999. Linearity is shown in HPLC method in the range of 10- 30 µg mL^{-1} with a relative coefficient of 0.9985. Each method was validated for different validation parameters like specificity, repeatability, accuracy, precision, linearity, robustness, limit of detection and limit of quantification. The results were obtained as per ICH guidelines. The developed UV and HPLC methods were compared with some available methods by statistical analysis one way ANOVA (Analysis of Variance) test, and it was found to be statistically significant.

Keywords: Repaglinide, UV, HPLC, validation

INTRODUCTION

Repaglinide is 2-ethoxy-4-[2-[[3-methyl-1-(2-piperidin-1-ylphenyl) butyl] amino]-2-ethyl] benzoic acid (Fig. 1). It is used as an antihyperglycemic drug. It decreases blood glucose by increasing the release of insulin from the beta islet cells of the pancreas. It comes under the class as meglitinide derivatives, which increase insulin secretion by binding to pancreatic cell. Repaglinide is rapidly absorbed and has rapid onset and shorter duration of action[1].

There are various research papers that have been published for UV method and chromatographic method for the determination of repaglinide in formulations[2-18]. But reported UV methods[6,9,10] for repaglinide are few and thus this would be an alternative for this research work. The developed HPLC and UV methods were validated for accuracy, precision, specificity, robustness and quantitation limit according to ICH guidelines Q2(R1)[19]. Also, we compared our method with some reported methods. Thus the statistical method, One-way Anova test was performed and it was found to be statistically significant.

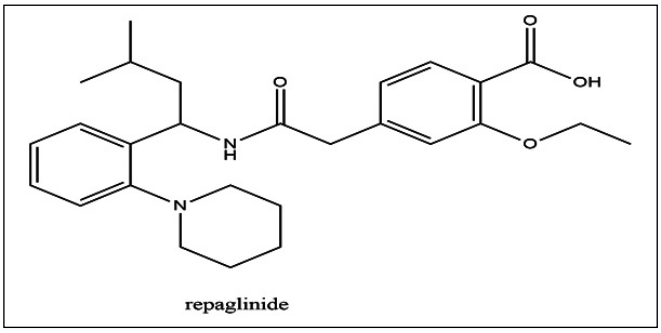

Fig. 1: Structure of repaglinide

MATERIALS AND METHODS

Chemicals and reagents

Repaglinide pure drug was procured from Torrent Pharmaceuticals Ltd., Mehsana, Gujarat as gift sample. Solvents used like methanol, water and acetonitrile were of HPLC grade. Repaglinide tablet marketed formulation was purchased from a local pharmacy.

Apparatus

UV Shimadzu UV-1800 and HPLC Shimadzu HPLC Kyoto, Japan instruments were used. In HPLC, detector used SPD-M20-PDA, column used Phenomenex Gemini C18 (250mm*4.6mmi.d.,5µm particle size) and pump was LC 20AD.

Spectrophotometric conditions

In UV method, initial base line correction was done by methanol. The maximum wavelength obtained from the spectrum of repaglinide was 243nm.

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Chromatographic conditions

By doing various trials like different mobile phases, changing flow rates and change in mobile phase ratio the mobile phase was optimized. Acetonitrile and water at the ratio 90:10 V/V was used. The flow rate was 1mL min^{-1} and determination was performed at 244 nm. The volume for injection was 10 µL in HPLC runs.

Preparation of solution for UV

Preparation of standard stock solution

10mg of repaglinide was weighed and transferred into a volumetric flask, then 50 mL methanol was added, sonicated and finally diluted to 100 mL with methanol to get desired concentration for 1000 µg mL^{-1} of repaglinide.

Preparation of solution for HPLC

Preparation of stock solution

10mg of repaglinide was weighed and transferred to 10 mL volumetric flask and diluted with methanol to get concentration of 100 µg mL^{-1}.

Statistical calculation

Standard deviations and other statistical parameters were performed by use of Microsoft Excel 2007 software.

RESULTS

UV method validation parameters

Calibration curve

Calibration curve of absorbance versus concentration was obtained by analyzing five different concentrations of repaglinide from 10-30 µg mL^{-1}. The calibration curve is shown in Fig. 2.

Interday and Intraday precision

Precision was determined by performing 3 independent measurements of standard solution of drug

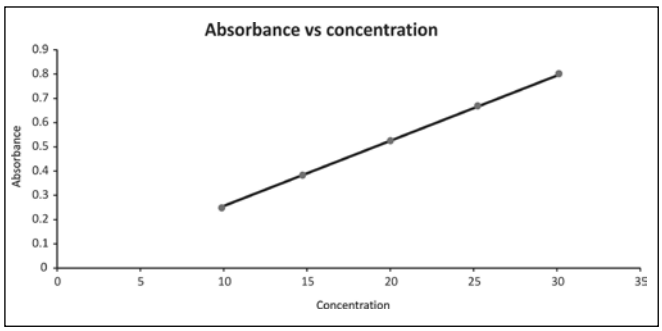

Fig. 2: Calibration curve of repaglinide by UV

(20 µg mL^{-1}) at three times on same day and 3 different days. Results are shown in Table I.

Table I: Precision data of repaglinide by UV

Concentration (µg mL^{-1})	Intraday precision	Intermediate precision
	Absorbance	Absorbance
20	0.349	0.346
20	0.346	0.349
20	0.35	0.351
Average	0.348	0.348
SD	0.0020	0.002
%RSD	0.597	0.721

LOD and LOQ

LOD and LOQ are found as per calibration curve and the results are shown in Table II.

Table II: Data of LOD and LOQ by UV

Drug	LOD (µg mL^{-1})	LOQ (µg mL^{-1})
Repaglinide	0.122	0.371

Table III: Robustness parameter by UV

Drug	Parameter	Changed condition 1	Normal condition	Changed condition 2
Repaglinide	Wavelength	242	243	244
	Absorbance	0.313	0.353	0.381
		0.314	0.355	0.384
		0.315	0.356	0.386
	Average	0.314	0.354	0.383
	SD	0.001	0.001	0.002
	%RSD	0.318	0.430	0.655

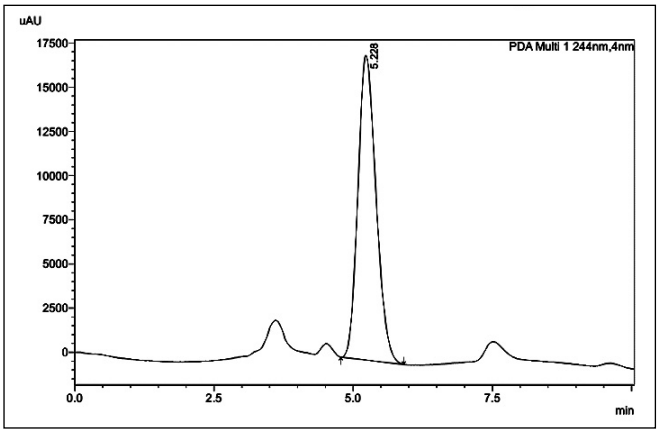

Fig. 3: Optimized chromatogram for HPLC

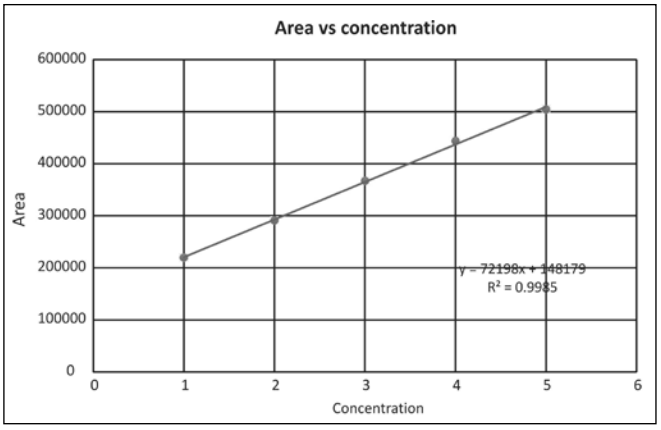

Fig. 4: Calibration graph of repaglinide by HPLC

Robustness

It was determined by change in flow rate and wavelength. Results are shown in Table III.

Accuracy and recovery

It was performed by determining recovery of repaglinide as per standard addition method. Known concentration of repaglinide was added at 80 %,100 % and 120 % level to solution of repaglinide (10 µg mL^{-1}). At each level of the amount, three determinations were performed. %Recovery data obtained by proposed method are as shown in Table IV.

Table IV: Accuracy and recovery of repaglinide by UV

Level	Mean %Recovery	%RSD
80%	99.58	0.5931
100%	100.2	0.599
120%	100.05	0.3936

HPLC method validation parameters

Optimization condition and chromatogram for the method development of HPLC are shown in Table V and Fig. 3, respectively.

Table V: Optimization condition for repaglinide

Column	Phenomenex Gemini C18 Column (25mm*4.6mm, 5µ particle size)
Mobile phase	Acetonitrile: Water (90:10V/V)
Wavelength	244nm
Injection volume	10µL
Flow rate	1 mL min^{-1}
Retention time	5.228

Specificity

There is no interference of mobile phase and solvent observed at the retention time of repaglinide.

Linearity

Linear relation was found between concentration and peak area of repaglinide (10-30 µg mL^{-1}). Calibration curve of absorbance vs concentration was obtained by analyzing five different concentrations of repaglinide. calibration curve is shown in Fig. 4.

Repeatability

It was determined by six-time determination of repaglinide (20 µg mL^{-1}) and result are shown in Table VI.

Table VI: Repeatability of repaglinide by HPLC

Mean Area	428496.33
SD	7740.15
% RSD	1.806

LOD and LOQ

It was calculated as per calibration curve and the data is shown in Table VII.

Table VII: LOD and LOQ of repaglinide by HPLC

Drug	LOD (µg mL^{-1})	LOQ (µg mL^{-1})
REPA	0.000115	0.000348

Robustness

It is determined by change in flow rate and wavelength. Results are shown in Table VIII.

Table VIII: Robustness of repaglinide by HPLC

Parameter	Change condition 1	Change condition 2
Flow rate (1 mL min^{-1})	0.8mL min^{-1}	1.2mL min^{-1}
Area	281071	270518
	286047	275543
	281295	276111
Average	282804.33	274057.333
SD	2810.4643	3078.28139
%RSD	0.9937	1.120
Wavelength (244 nm)	243nm	245nm
Area	278079	268398
	276966	262551
	282344	272349
Average	279129.7	267766
SD	2838.775	4929.48
%RSD	1.0701	1.84

Accuracy (%Recovery)

It was determined in terms of recovery study at three levels (80%, 100% and 120%). Results are shown in Table IX.

Table IX: Accuracy and recovery of repaglinide by HPLC

Level	%Recovery	Mean recovery
80%	99.75	99.79166667
	99.5	
	100.125	
100%	99.9	99.81666667
	99.35	
	100.2	
120%	99.583333	99.91666667
	100.25	
	99.916667	

Table X: Comparison of UV method

Parameter	Developed method	Reported method[6]
Wavelength	243.5nm	254nm
Concentration (µg mL^{-1})	10-30 µg mL^{-1}	10-60 µg mL^{-1}
LOD	0.100165	0.23
LOQ	0.30353	0.72
Linearity coefficient	Y=0.0269x-0.0134	Y=0.026x-0.002
Regression coefficient	0.9999	0.999
Intraday precision	0.59760746	0.4561
Interday precision	0.7218	0.4645
Repeatability	1.52	0.5118
Accuracy	99.58-100.05%	99.62%-100.4%

Table XI: Comparison table of HPLC method

Parameter	Developed method	Reported method[2,3]	
Column	Phenomenex Gemini C18 column	Hypersil®C18	C18 column
Mobile Phase	Acetonitrile: water (90:10 V/V)	Acetonitrile: phosphate buffer (60:40 V/V)	Acetonitrile : water (90:10 V/V)
Flow rate	1 mL min^{-1}	1 mL min^{-1}	1 mL min^{-1}
Retention time	5.228	7.4	6.3
Wavelength	244nm	235nm	223nm
Concentration	10-30 µg mL^{-1}	55-500 µg mL^{-1}	2-35 µg mL^{-1}
LOD	0.000115	10	0.10
LOQ	0.000348	20	0.30
Regression coefficient	0.9985	0.9983	0.995
Accuracy	99.7-99.9%	92.37-104.66%	99.32-100.15%

Table XII: Data summary for UV

Data summary				
Group	**N**	**Mean**	**Std. Dev.**	**Std. error**
Group 1	5	0.6486	0.5448	0.2436
Group 2	5	0.4765	0.1745	0.0781

Where, Group 1= Developed method, Group 2= Reported method and N= LOD, LOQ, Repeatability, Interday precision and Intraday precision

Table XIII: ANOVA summary for UV

Source	Degree of freedom (Df)	Sum of squares (SS)	Mean squares (MS)	F-Stat	P value
Between groups	1	0.074	0.074	0.4525	0.5201
Within groups	8	1.309	0.1636		
Total	9	1.3831			

Table XIV: Data summary for HPLC

Data Summary				
Group	**N**	**Mean**	**Std. Dev**	**Std. error**
Group 1	**7**	64.261	91.8133	34.7022
Group 2	**7**	67.0326	85.4369	32.2921
Group 3	**7**	61.4279	84.9004	32.0893

Where, Group 1= Developed method, Group 2= Reported Method (1), Group 3= Reported Method (2), N= LOD, LOQ, Repeatability, Regression Coefficient, Retention time, Accuracy (1), Accuracy (2)

Table XV: ANOVA summary for HPLC

ANOVA Summary					
Source	**Degree of freedom (Df)**	**Sum of squares (SS)**	**Mean squares (MS)**	**F-Stat**	**P value**
Between groups	2	109.9487	54.9744	0.0072	0.9928
Within groups	18	137623.3432	7645.7413		
Total	20	137733.2919			

Comparison of developed methods with reported method

Comparison of developed UV method and HPLC method with reported method is shown in Table X and Table XI, respectively.

Statistical data analysis

For UV by using one -way ANOVA

Data summary is shown in Table XII and Table XII.

For HPLC by using one -way ANOVA

Data summary is shown in Table XIV and Table XV.

DISCUSSION

UV and HPLC methods were developed, and the validation were performed as per ICH Q2(R1) guidelines. After that, comparison of our method with reported method was done by statistical One-Way Anova test. In UV method, p-value is 0.52 which is more than 0.05. It is statistically significant and null hypothesis is accepted. In HPLC, p-value is 0.9 which is greater than 0.05, it is significant statistically, and so null hypothesis is accepted. There are many reported methods that are developed and validated of UV and HPLC for repaglinide. The present methods aim to compare the developed method with reported method and that are to be statistically proved by One way ANOVA.

REFERENCES

1. Roglic G.: WHO Global report on diabetes: A summary. **Int. J. Non Commun. Dis.,** 2016, 1(1), 3.
2. Tatiparthi R.: Method development and validation of metformin and repaglinide in rabbit plasma by RP-HPLC. **Fabad J. Pharm. Sci.** 2010, 35, 69-75.
3. Kumar L. S., Narsinghani T. and Jain M.: Development and validation of RP-HPLC method for simultaneous estimation of metformin hydrochloride and repaglinide in tablet dosage form. **J. Liq. Chromatogr. Relat. Technol.,** 2012, 35(3), 385-392.
4. Gandhimathi M., Ravi T. K. and Kurian S. R.: Determination of repaglinide in pharmaceutical formulations by HPLC with UV detection. **Anal. Sci.,** 2003, 19(12), 1675-1677.
5. Joshi S. S., Nahire R. R., Shastri R., Surendranath K. V. and Satish J.: Validated stability-indicating RP-HPLC method for simultaneous determination of metformin and repaglinide. **Acta Chromatogr.,** 2012, 24(3), 419-432.
6. Prasanth V. G., Eapen S. C., Kutty S. V. and Rai A.: Development and validation of UV spectroscopic methods for the estimation of repaglinide and metformin hydrochloride in synthetic mixture. **Int. J. Pharm. Sci. Healthcare,** 2012, 2(2), 150-158.
7. Mahrouse M. A., Nesrine T. and Lamie P.: Experimental design methodology for optimization and robustness determination in ion pair RP-HPLC method development: Application for the simultaneous determination of metformin hydrochloride, alogliptin benzoate and repaglinide in tablets. **Microchem. J.,** 2019, 147, 691-706.
8. Bashar A. A., Majed S., Hatim S. A., Mohammad M. and Yasser B.: A comparative study of first-derivative spectrophotometry and column high- performance liquid chromatography applied to the determination of repaglinide in tablets and for dissolution testing**. J. AOAC. Int.,** 2008, 91(3), 530-535.
9. Kharbade S., Asnani A. and Kumar P.: Development and validation of UV spectrophotometric method for simultaneous estimation of metformin HCl and repaglinide in pharmaceutical formulation. **J. Drug Deliv. Ther.,** 2019, 9(3), 344-347.
10. Cijo X., Basavaiah K., Ramesh P.J. and Vinay K.B.: Development and validation of two stability-indicating UV- spectrophotometric methods for the determination of repaglinide in bulk and dosage forms. **Int. J. Chem. Tech. Res.,** 2013, 5, 72-79.
11. Fouad M. M. and Noha S. R.: Development and validation of chromatographic and spectroscopic methods for estimation of repaglinide and metformin HCl in combined dosage form. **J. Glob. Trends Pharm.,** 2014, 5(3), 1844-1848.
12. Berecka A., Gumieniczek A. and Hopkała H.: Development and validation of a new high-performance liquid chromatography method for the determination of gliclazide and repaglinide in pharmaceutical formulations. **J. AOAC. Int.,** 2006, 89(2), 319-325.
13. Shaikh N. K., Jat R. and Bhangale J. O.: Development and validation of stability indicating RP-HPLC and UV method for simultaneous quantitation of repaglinide and sitagliptin phosphate in combination. **Am. J. PharmTech. Res.,** 2021, 10(06).
14. Venkatesh P., Harisudhan T., Choudhury H., Mullangi R. and Nuggehally R. S.: Simultaneous estimation of six anti-diabetic drugs—glibenclamide, gliclazide, glipizide, pioglitazone, repaglinide and rosiglitazone: development of a novel HPLC method for use in the analysis of pharmaceutical formulations and its application to human plasma assay. **Biomed. Chromatogr.** 2006, 20(10), 1043-1048.
15. Wadhwa G., Kowthavarapu V. K., Dubey S. K. and Taliyan R. K.: Development and validation of RP-HPLC method for quantification of repaglinide in mPEG-PCL polymeric nanoparticles: QbD-driven optimization, force degradation study, and assessment of *in vitro* release mathematic modeling. **Microchem. J.,** 2021, 168, 1064-1091.
16. Patel D. R., Patel L. J. and Patel M. M.: Stability indicating HPLC method for simultaneous determination of repaglinide and metformin hydrochloride in pharmaceutical dosage form. **Asian J. Res. Chem.,** 2011, 4(3), 500-505.
17. Basavaiah X. K.: Development and validation of a simple stability indicating UPLC method for the determination of repaglinide in pharmaceuticals. **Thai J. Pharm. Sci.,** 2013, 37(2).
18. Patan A., Basha S. R., Ketha R. K., Cheriyan B. V. and Muthukumar V. A.: Development and validation of new RP-HPLC method for the simultaneous estimation of metformin hydrochloride and repaglinide in pure and pharmaceutical formulations. **Res. J. Pharm. Technol.,** 2021, 14(3), 1323-1328.
19. ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R1) Current Step 4 Version, November, 2005.