



SIMULTANEOUS DETERMINATION OF METFORMIN AND REPAGLINIDE IN BULK AND TABLETS BY RP-HPLC METHOD

Desabattina Sai Jahnvi

Analytical Research and Development, SRC laboratories, Raichur Growth Centre, Raichur, Karnataka, India.

Desabattina Venkata Narayana Rao

Analytical Research and Development, SRC laboratories, Raichur Growth Centre, Raichur, Karnataka, India.

Yeluri Ramachandra Reddy

Analytical Research and Development, SRC laboratories, Raichur Growth Centre, Raichur, Karnataka, India.

ABSTRACT

The present communication describes a rapid, precise and accurate RP-HPLC method for the simultaneous quantification of metformin and repaglinide in bulk and tablet dosage forms. Chromatographic separation of the selected drugs was carried out on Phenomenex C18 analytical column (250 mm x 4.6 mm, 5 μ particle size). An isocratic elution system was developed using 0.1M ammonium acetate: acetonitrile [50:50 v/v]. The pH of the mobile was adjusted to 4.2 with orthophosphoric acid. The elution of the drugs was achieved with a flow rate of 1.0 ml/min. Detection was by UV absorbance at a wavelength of 254 nm. The detector response was linear in the concentration of 500-1500 μ g/ml and 2-6 μ g/ml for metformin and repaglinide, respectively. The method was validated following ICH guidelines. All the parameters of validation were found in the acceptance range.

KEYWORDS : Metformin, Repaglinide, Antidiabetic, RP-HPLC, Method development, Validation

INTRODUCTION

Metformin is an oral hypoglycemic agent belonging to the biguanides class of compounds. Chemically, metformin is described as 3-(diaminomethylidene)-1,1-dimethylguanidine (Figure 1). Metformin is prescribed for the treatment of non insulin dependent diabetes mellitus [1,2]. The hypoglycemic effect of metformin is mediated by the activation of AMP-activated protein kinase [3-5].

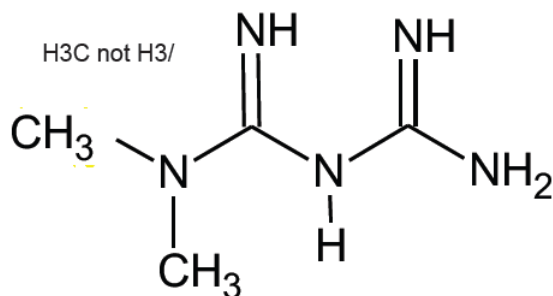


Figure 1: Structure of metformin

The combination of metformin and repaglinide is approved by US Food and Drug Administration in 2008 for the treatment of type 2 diabetes mellitus [10,11, 12]. The review of the literature revealed that few methods have been reported for the simultaneous estimation of the metformin and repaglinide.

LC-MS/MS-ESI methods have been developed for simultaneous quantification of metformin and repaglinide in human plasma [13] and rat plasma [14]. The LC-MS/MS-ESI methods were applied to clinical bioequivalence study [13] and pharmacokinetic study [14]. UV spectrophotometric methods such as absorption correction spectroscopy [15], difference spectroscopy [16] and second derivative spectroscopy [17] were reported for the simultaneous

estimation of metformin and repaglinide in pharmaceutical dosage forms. HPTLC-densitometry method is also presented for the quantification of metformin and repaglinide in combined pharmaceutical formulation [18]. The reported LC-MS/MS-ESI [13, 14] and HPTLC [18] methods described above are time consuming, required sophisticated equipment, involve cumbersome procedures and are not applied to pharmaceutical dosage forms. The UV spectrophotometric methods [15-17] are simple but are less selective.

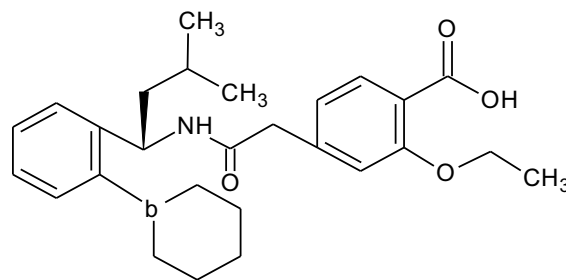


Figure 2: Structure of repaglinide

HPLC with UV detection is considered as the most convenient analytical method in the pharmaceutical analysis owing to its simplicity, sensitivity, accuracy, precision and its availability in most

of the quality control laboratories. Joshi et al., [19] Ramanji Reddy et al., [20] Navya [21], Prbhakar et al., [22] Alagar raja et al., [13] and Soni et al.,[24] developed and validated RP-HPLC method with UV detection for the quantification of metformin and repaglinide simultaneously. Except the Ramanji Reddy et al., [20] method, remaining all RP-HPLC methods [19, 21-24] are applied for the quantification of metformin and repaglinide in combined pharmaceutical dosage forms. Ramanji Reddy et al., [20] method is applied for rabbit plasma. Most of the RP-HPLC methods suffer from one or the other disadvantage such as more runtime (> 6 minutes), narrow range of linearity, use of triplet solvent system as mobile phase, less accurate and less precise. The more runtime and use of triplet solvent system increase the utilization of solvents and cost of the analysis. In some of the methods validation parameters like system suitability studies, selectivity and robustness are not reported.

The present paper describes a rapid, precise, accurate and validated RP-HPLC with UV detection method for the determination of metformin and repaglinide simultaneously in bulk and tablet dosage form.

MATERIALS AND METHODS

Instrumentation

A Waters 2695 alliance with binary HPLC pump equipped with Waters 2998 PDA detector and Waters Empower2 software is used in this study. Phenomenex C18 (250 x 4.6 mm; 5 µm particle size) analytical column was used for separation and simultaneous analysis of the metformin and repaglinide. Electronic balance ELB 300 was used for weighing the materials. Digisun pH meter was used for all pH measurements.

Mobile phase

The solvents and chemicals used in the preparation of mobile phase were of HPLC grade and analytical grade, respectively. The mobile phase used is 0.1M ammonium acetate (Merck India Ltd., Mumbai) and acetonitrile (Merck India Ltd., Mumbai) in the ratio of 50:50 v/v. The pH of the mobile phase was adjusted to 4.2 with orthophosphoric acid (Sd Fine Chemicals Ltd., Mumbai) The mobile phase is filtered before using, through millipore membrane filter and degassed for 15 min by sonication. The same mobile phase was also used as diluent for the preparation of stock and working standard solutions of the drugs.

Chromatographic conditions

The column temperature was maintained at 30±1°C. The separation was carried out under isocratic elution. The flow rate was maintained 1.0 ml/min. The injection volume of sample was 10 µl. The metformin and repaglinide were analyzed using a PDA detector set 254 nm.

Standard solutions

Metformin and repaglinide reference drugs were obtained as a gift sample from Lara drugs pvt Ltd., Hyderabad, India. The standard stock solution was prepared by dissolving 500 mg of metformin and 2 mg of repaglinide in mobile phase in a 100 ml volumetric flask. Working standard solutions equivalent to 500-1500 µg/ml metformin and 2-6 µg/ml repaglinide was prepared from stock solution by appropriately diluting the stock standard solution with the mobile phase.

Sample Solution

PrandiMet tablets (Novo Nordisk Inc. Princeton, New Jersey) labeled to contain 2 mg repaglinide and 500 mg metformin per tablet was purchased from the local pharmacy market. Ten tablets were weighed and crushed to a fine powder. The powder equivalent to 500 mg of metformin and 2 mg of repaglinide was transferred into a 100 ml volumetric flask containing 20 ml of mobile phase. The contents of the flask was sonicated for 20 min and made up to mark with the same solvent. The resultant mixture was filtered through 0.45 µm filter paper. The resultant solution was diluted

appropriately with the mobile phase to get a final concentration of 1000 µg/ml metformin and 4 µg/ml repaglinide.

RESULTS AND DISCUSSION

Optimization of RP-HPLC conditions

The main aim of this study is to simultaneously analyze metformin and repaglinide with sufficient resolution in reasonable analysis time. The peak shape and system suitability parameters of metformin and repaglinide were good with phenomenex C 18 (250 mm x 4.6 mm, 5 µm) column. Hence this analytical column was selected.

Different mobile phases with different flow rate and pH were also tested to obtain good chromatographic condition:

1. Water: Acetonitrile (60:40, v/v)
2. 0.1% Orthophosphoric acid: Acetonitrile (60:40, v/v)
3. 0.1% Orthophosphoric acid: Acetonitrile (70:30, v/v)
4. 0.1M Ammonium acetate: Acetonitrile (60:40, v/v)
5. 0.1M Ammonium acetate: Acetonitrile (50:50, v/v)

The good performance and better separation was achieved with the mobile phase combination 0.1M ammonium acetate and acetonitrile in the ratio of 60:40 (v/v) with pH 4.2. The isocratic elution with a flow rate of 1 ml/min was optimized. The two drugs showed good absorbance at 254 nm. Hence the same wavelength was selected for detection. Under the optimized chromatographic conditions, the chromatogram (Figure 3) obtained, demonstrated a good separation of the metformin (RT= 1.797 min) and repaglinide (RT= 3.974 min) from each other.

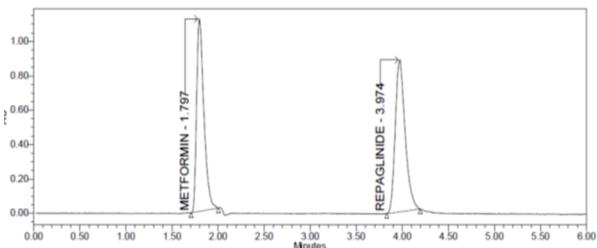


Figure 3: Chromatogram of metformin and repaglinide under optimized HPLC conditions

Method validation

The optimized RP-HPLC method for simultaneous assay of metformin and repaglinide was validated according to ICH guidelines [25] with respect to system suitability, linearity, sensitivity, selectivity, accuracy, precision and robustness.

System suitability

Prior to analysis, the chromatographic system must satisfy system suitability test requirements. System suitability test was assessed from five replicate injections of the standard solution containing 1000 and 4 µg/ml metformin and repaglinide, respectively. Both the peaks were well resolved and the precision of injections for all the peaks were acceptable. The percent relative standard deviation of the metformin and repaglinide peaks area responses were found to be <1.5. The USP tailing factor and USP plate count were also calculated. The results of system suitability in comparison with the required limits are shown in Table 1 and are found to be within the accepted limits.

Table 1: System suitability parameters

Parameters	Metformin	Repaglinide	Recommended limits
Peak area	6816708 (%RSD-0.376)	6271166 (%RSD-1.081)	RSD ≤2
USP resolution	-	12.12	> 1.5
USP plate count	2289	7182	> 2000
USP tailing factor	1.52	1.29	≤ 2

Linearity and range

The linearity of the RP-HPLC method was determined, for the simultaneous assay of metformin and repaglinide, by analyzing five different concentrations of each drug. The calibration curve was plotted by area under the peak responses of the selected drugs against their corresponding concentrations. Calibration curves were linear over the concentration range of 500-1500 µg/ml for metformin and 2-6 µg/ml for repaglinide. The linearity parameters are given in Table 2. The results show a good correlation between the peak areas of the three drugs and their corresponding concentrations.

Table 2: Linearity studies for metformin and repaglinide

Metformin		Repaglinide	
Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area
500	3403716	2	3135149
750	5110231	3	4700145
1000	6816223	4	6276642
1250	8525237	5	7838410
1500	10218914	6	9405286
Regression equation: y = 6816 x - 1177 (R ² = 0.9999)		Regression equation: y = 1567.59 x - 103.29 (R ² = 0.9998)	

Where y = peak area and x = concentration of the drug in µg/ml

Sensitivity

The sensitivity of the method was assessed by calculating limit of detection (LOD) and limit of quantification (LOQ). LOD was found to be 0.918 µg/ml and 0.0401 µg/ml for metformin and repaglinide, respectively (signal to noise ratio of 3:1). LOQ was found to be 26.392 µg/ml and 0.133 µg/ml and 9.320 µg/ml for metformin and repaglinide, respectively (signal to noise ratio of 10:1). The values of LOD and LOQ demonstrate sufficient sensitivity of the HPLC method.

Precision and accuracy

Precision and accuracy of the developed method was determined by injecting six standard solutions of metformin and repaglinide with 1000 and 4 µg/ml concentrations, respectively. The peak areas were determined. Relative standard deviation of metformin and repaglinide peaks and percent recovery of metformin and repaglinide were then calculated to represent precision and accuracy, respectively. The results are summarized in Table 3. The low relative standard deviation values and good percent recoveries indicated that the method is precise and accurate.

Table 3: Precision and accuracy of the proposed method

Metformin		Repaglinide					
Peak Area	Assay (%)	Mean assay (%)	RSD (%)	Peak Area	Assay (%)	Mean assay (%)	RSD (%)
6811286	99.521	99.583	0.056	6274566	99.854	99.871	0.047
6811378	99.522			6275039	99.862		
6819423	99.640			6279040	99.925		
6818132	99.621			6271213	99.801		
6813819	99.558			6275233	99.865		
6819120	99.635			6278767	99.921		

Recovery study

The accuracy of the proposed method was further established by means recovery study. The recovery study is performed through the standard addition technique, by adding a known amount of standard drug at three different levels (50%, 100% and 150%) to the

preanalyzed sample. Accuracy was expressed as percentage recovery in Table 4. The results indicating acceptable accuracy.

Table 4: Recovery of metformin and repaglinide

Spiked Level (%)	Concentration of drug (µg/ml)	Recovery (%)	Mean (%)	
	Added	Found		
Metformin				
50	495.000	497.59	100.52	100.45
	495.000	496.79	100.36	
	495.000	497.36	100.48	
100	990.000	995.07	100.51	100.56
	990.000	995.31	100.54	
	990.000	996.16	100.62	
150	1485.000	1501.88	101.14	101.03
	1485.000	1502.17	101.16	
	1485.000	1496.90	100.80	
Repaglinide				
50	1.980	2.00	100.81	100.82
	1.980	1.99	100.74	
	1.980	2.00	100.91	
100	3.960	3.99	100.80	100.80
	3.960	3.99	100.84	
	3.960	3.99	100.81	
150	5.940	5.99	100.77	100.81
	5.940	5.99	100.84	
	5.940	5.99	100.83	

Robustness

The robustness of the proposed method was checked by studying the effect of deliberate changes in the flow rate of mobile phase (±0.1 ml/min) and column temperature (±2°C) on the chromatographic system suitability parameters. The robustness of the method was studied by using the standard solution with concentration 1000 µg/ml and 4 µg/ml of metformin and repaglinide, respectively. According to the results shown in Table 5, these small and deliberate variations did not have any significant effect on the measured system suitability parameters.

Table 5: Robustness data for metformin and repaglinide

Parameter varied	Peak area	USP plate count	USP Tailing	USP resolution
Metformin				
Column temperature (30-1°C)	7069826	1892	1.58	-
Column temperature (30+1°C)	7057889	1805	1.64	-
Flow rate (1-0.1 ml/min)	9218390	1660	1.73	-
Flow rate (1+0.1 ml/min)	8998618	1683	1.86	-
Repaglinide				
Column temperature (30-1°C)	5525371	6881	1.31	11.65
Column temperature (30+1°C)	5583594	6981	1.27	11.95
Flow rate (1-0.1 ml/min)	7093657	6830	1.30	11.55
Flow rate (1+0.1 ml/min)	6436175	6344	1.33	11.51

Selectivity

In order to evaluate the selectivity of the proposed method, the chromatograms of mobile phase blank, placebo blank, tablet sample and standard sample were compared. Placebo blank solution was prepared in the same way of the tablet sample solution by common excipients of the tablet dosage form but without the selected drugs. The test sample and standard solution are prepared at the concentration of 1000 µg/ml and 4 µg/ml metformin and repaglinide, respectively. The chromatograms are presented in Figure 4a-4d. The common excipients of the tablet dosage form and components of the mobile phase did not interfere with the peaks of the selected drugs. Therefore, the proposed method was selective for the simultaneous assay of metformin and repaglinide.

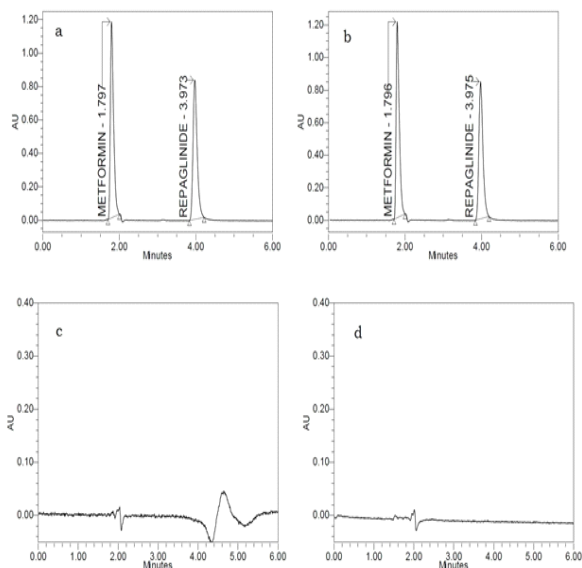


Figure 4: Chromatogram of [a] Standard solution [b] Tablet sample solution [c] Blank mobile phase [d] Placebo blank solution

CONCLUSION

A RP-HPLC method was developed for the simultaneous quantification of metformin and repaglinide in bulk and tablet dosage forms. The method validation was performed according to the guidelines of ICH in terms of linearity, accuracy, precision, selectivity, robustness, detection limit and quantitation limit. All the validation parameters were found to be within the acceptance limits. The method can be used in the quality control laboratories for the routine simultaneous analysis of metformin and repaglinide.

References:

1. Hundal R.S., Inzucchi S.E., *Drugs*, 2003; 63(18): 1879–94.
2. Metformin Hydrochloride. The American Society of Health-System Pharmacists. Retrieved Jan 2016.
3. Rena G., Pearson E.R., Sakamoto K., *Diabetologia*, 2013; 56(9): 1898–906.
4. Burcelin R., *Gut*, 2013; 63(5): 706–707.
5. Kirpichnikov D., McFarlane S.I., Sowers J.R., *Ann. Intern. Med.*, 2002; 137(1): 25–33.
6. "DailyMed - REPAGLINIDE - repaglinide tablet". dailymed.nlm.nih.gov. Retrieved 2015-11-04.
7. Scott L.J., *Drugs*, 2012; 72(2): 249-72.
8. Plosker G.L., Figgitt D.P., *Pharmacoeconomics*, 2004; 22(6): 389-411.
9. Hollingdal M., Sturis J., Gall M.A., Damsbo P., Pincus S., Veldhuis J.D., Pørksen N., Schmitz O., Juhl C.B., *Diabet. Med.*, 2005; 22(10): 1408-13.
10. <http://www.medscape.org/viewarticle/577024>
11. http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/022386s007lbl.pdf

12. Ryuzo K., Kohei K., Toshiaki H., Tatsuya O., Shigeru K., Nigishi H., *J. Diabetes Investig.*, 2014; 5(1): 72–79.
13. Liang X.R., Dai X.J., Zhang Y.F., Ding J.F., Chen X.Y., Zhong D.F., *Yao Xue Xue Bao*, 2013; 48(4): 547-53.
14. Sharma K., Pawar G., Yadav S., Giri S., Rajagopal S., Mullangi R., *Biomed. Chromatogr.*, 2013; 27(3): 356-64.
15. Nishith P., Kanu R.P., *J. Pharma. Sci. Biosci. Res.*, 2015; 5(1): 104-109.
16. Makwana M.M., Rajesh R.P., Vaishali N.S., Dushyant A.S., *Int. J. Int. Pharm. Life Sci.*, 2012; 2(3): 38-47.
17. Fouad M.N., Rashed N.S., *JGTPS*, 2014; 5(3): 1844–48.
18. Keyur B.A., Emanuel M.P., Arpit S., *J. Chromatogr. Sep. Techn.*, 2013; 4(1): 1-5.
19. Joshi S.S., Nahire R.R., Shastri N.R., Surendranath K.V., Satish J., *Acta Chromatogr.*, 2012; 24(3): 419–32.
20. Reddy T.R., Dhachinamoorthi D., Sekhar K.B.C., *Int. J. Pharm. Pharma. Sci.*, 2012; 4(2): 311-13.
21. Navya S., *The Experiment*, 2014; 2(3): 109-15.
22. Prbhakar, Harshini S., Sireesha D., Haque M.A., Vasudha B., *Int. J. Med. Nanotech.*, 2014; 1(3): 163-68.
23. Raja M.A., Santhoshi R., David B., Rao K.N.V., Kumar D.S., *Asian J. Res. Pharma. Sci. Biotechn.*, 2015; 3(2): 33-40.
24. Soni L.K., Tamanna N., Madhavi J., *J. Liq. Chromatogr. Rel. Techno.*, 2012; 35(3): 385-392.
25. ICH Validation of analytical procedures; Text and methodology; Q2(R1), International Conference on Harmonization, 2005.