Original Article

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Analytical method for simultaneous estimation of ranolazine and metformin hydrochloride by validated RP-HPLC-DAD method

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ABSTRACT

Background: In the present study, a simple, sensitive, rapid, accurate, and precise reverse-phase high-performance liquid chromatography (RP-HPLC) method with diode array detection was developed and validated for simultaneous estimation of Metformin hydrochloride (MET) and Ranolazine (RANO) in a synthetic mixture. **Material and Method:** The method was developed using isocratic elution mode on a reversed-phase. Chromatographic separation was performed on ACE CN C_{18} (250 mm, 4.5 mm, 5 µm) column and a mobile phase consisting of methanol: acetonitrile:ammonium formate (45:45:10% v/v/v) with 10% formic acid to adjust the pH to 6.0, at a flow rate of 1.0 ml/min. Detection and quantification of all the analytes were carried out at 230 nm using a photodiode array detector. **Result:** The method was found to be linear in the range of 1–50 µg/ml for RANO and 2–100 µg/ml for MET. Percentage recovery was found in the range of 99.07–101.53% for RANO and 99.59–100.45% for MET. The RSD of precision and repeatability was found lower than 1% for each drug. Validation of the method was carried out as per International Council on Harmonization guideline Q2 (R1). **Conclusion:** The proposed RP-HPLC method can be highly suitable for the analysis of RANO and MET without interference.

Keywords: International Council on Harmonization guideline Q2 (R1), Metformin hydrochloride, Ranolazine, reverse-phase high-performance liquid chromatography, validation

INTRODUCTION

Ranolazine (RANO) is a blocker of the late sodium current and is approved to treat chronic angina as first-line treatment and Metformin hydrochloride (MET), an oral antidiabetic drug in the biguanide class, is the most widely used as first-line treatment for type 2 diabetes mellitus (T2DM).[1,2] However, nowadays, coronary artery disease (CAD) and diabetes mellitus (DM) commonly coexist. It is estimated that 26% of subjects with CAD also have DM as a comorbid condition. It is, therefore, anticipated that RANO and MET could be coadministered in subjects with T2DM.[3] This fixed-dose combination undergoing a clinical trial phase three and in this conventional tablet used in the strength of 1:2 dosing ratio of RANO and MET, respectively. Coadministration of RANO and MET was well tolerated in these T2DM subjects, with no serious adverse event.[4]

RANO is N- (2, 6-dimethylphenyl)-2-{4- [2-hydroxy-3-(2-methoxyphenoxy) propyl] piperazin-1-yl} acetamide, which is class of antianginal drug. MET is 3- (diaminomethylidene) 1, dimethylguanidine; hydrochloride is a hypoglycaemic agent. The chemical structures of RANO and MET are shown in Figure 1.[5]

The literature review reveals that MET is official in IP,[6] USP,[7] JP,[8] and BP[9] and RANO is not official in any pharmacopoeia. First derivative UV spectrophotometric and reverse-phase

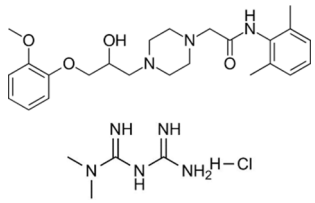

Figure 1: (a) Chemical structure of Ranolazine and (b) Chemical structure of Metformin hydrochloride

high-performance liquid chromatography (RP-HPLC) methods are available for simultaneous estimation of both drugs.[10,11] Several analytical methods were reported such as UV Spectrophotometry,[12-19] HPLC,[20-27] stability study,[28-31] and UPLC[32] for the estimation of RANO and MET individually and their combination with other drugs. The reported RP-HPLC[11] method lacks the sensitivity, and the recovery study is also not performed as per International Council on Harmonization (ICH) guidelines. In addition to that, method is not developed in the actual ratio mentioned in clinical phase three,[33] so it may not be applied. Thus, it is worthwhile to develop a method that is sensitive, scientifically, and appropriately developed and can be applied for a fixed-dose combination. The developed method can be used in routine analysis by the pharmaceutical industry or research laboratories.

MATERIALS AND METHODS

Materials and Software

RANO and MET were provided by B. K. mody government pharmacy college, Rajkot. HPLC grade methanol (Merck Pvt Ltd, India), acetonitrile (Merck Pvt Ltd, India), and water (Merck Pvt Ltd, India) were used; AR grade ammonium formate (Loba Chemie Pvt Ltd, Mumbai), microcrystalline cellulose (S. D. Fine Chem Ltd, India), lactose (Molychem, India), sodium starch glycolate (Molychem, India), and starch (S. D. Fine Chem Ltd, India) were used. LC-20 (Shimadzu, Japan), HPLC, was used and data were processed by laboratory solution software. There is no marketed formulation available as the fixed-dose combination is under phase three clinical study in USFDA. Thus, method was developed on a synthetic mixture of 10 mg of RANO and 20 mg of MET.

Chromatographic Condition

LC-20, Shimadzu HPLC, was used and data were processed by laboratory solution software. Chromatographic separation was performed using isocratic elution mode with ACE CN C_{18} (250 mm, 4.5 mm, 5 µm) column, in mobile phase used the composition of methanol: acetonitrile:ammonium formate (pH: 6.0) (45:45:10% v/v/v). Injection volume was set to 10 µl with a flow rate of 1.0 ml/min. The detection wavelength for the photodiode array detector was selected as 230 nm for both drugs.

Preparation of Standard Stock Solution

About 20 mg of MET and 10 mg of RANO were weighed accurately and transferred into a 100 ml volumetric flask. About 50 ml of methanol was added and sonicated to dissolve completely and made up to the mark with methanol to get a concentration of 200 µg/ml and 100 µg/ml for MET and RANO, respectively.

Preparation of Synthetic Mixture

The synthetic mixture was prepared by mixing 20 mg of MET and 10 mg of RANO with the spiking of common tablet excipients such as microcrystalline cellulose, lactose, sodium starch glycolate, and starch as a gliding agent, diluent, disintegrating agent, and binder, respectively, in 100 ml volumetric flask, about 50 ml of methanol was added and sonicated to dissolve completely and made up to the mark to get 200 µg/ml and 100 µg/ml for MET and RANO, respectively. From the above solution pipette out 2 ml appropriately, then dilute with methanol to get a final concentration of 40 μg/ml and 20 μg/ml for MET and RANO, respectively

System Suitability Parameter

The system suitability of the chromatographic system was tested before each stage of validation. Six replicate injection of standard preparation was injected into the system and retention time, tailing factor, numbers of theoretical plates, and relative standard deviation of each were determined.

METHOD VALIDATION

Linearity

A standard stock solution was, further, diluted with methanol to get a concentration in series of 1–50 µg/ml of RANO and 2–100 µg/ml of MET, respectively.

Accuracy

Accuracy was performed using a drug to drug spiking at three different amounts of analytes at levels of 50%, 100%, and 150%. Concisely, a recovery study was performed by spiking of 10 µg/ml, 20 µg/ml, 30 µg/ml of RANO, and 20 µg/ml, 40 µg/ml, 60 µg/ml of MET to the prepared mixture containing 20 µg/ml of RANO and 40 µg/ml of MET.

Precision

Repeatability was performed under six replicates at a concentration of 20 µg/ml of RANO and 40 µg/ml of MET. Intraday and interday variations of both drugs were performed in triplicate at three different concentration levels 50%, 100%, and 150% (20 µg/ml, 40 µg/ml, and 60 µg/ml for MET and 10 µg/ml, 20 µg/ml, and 35 µg/ml for RANO). Results from the determination of repeatability and intermediate precision are expressed in the form of RSD.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were computed to establish method sensitivity. LOD and LOQ were determined from the standard deviation of intercept and the slope of the calibration curve using the equation LOD = 3.3*σ/C and LOQ = 10*σ/C, respectively.[34]

Specificity

Specificity was performed under six replicates at a concentration of 20 µg/ml of RANO and 40 µg/ml of MET, with and without the addition of excipients to check the

interference of excipients. The specificity of the method was evaluated by calculating percentage interference.

Robustness

The robustness of the method was evaluated by deliberate variation in the method parameter such as flow rate variation by ±0.1 ml/min, mobile phase ratio by ±2.0 ml organic solvent, and pH of Mobile Phase ±0.5.

RESULTS AND DISCUSSION

Optimize Chromatographic Condition

The optimal composition of the mobile phase was methanol: acetonitrile:ammonium formate (pH: 6.0) (45:45:10% v/v/v) using isocratic elution mode with ACE CN C_{18} (250 mm, 4.5 mm, 5 µm) column. The flow rate was set to 1 ml/min and UV detection was carried out at 230 nm. The mobile phase was filtered through a nylon 0.22 mm membrane filter and was degassed before use. Chromatogram of optimizing condition is shown in Figure 2 and system suitability parameters are expressed in Table 1.

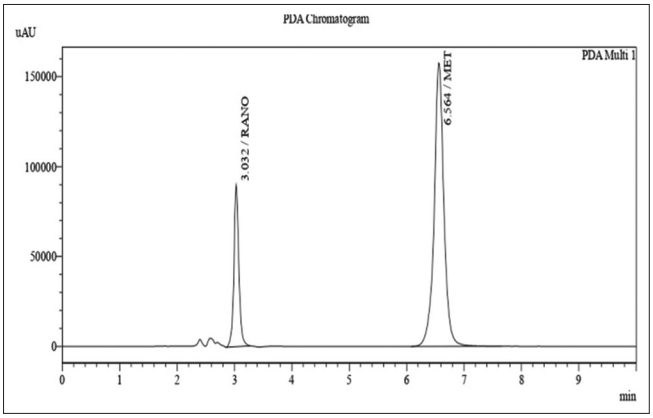

Figure 2: Chromatogram of optimized condition

Analytical Method Validation

Linearity

Linearity for RANO and MET was found in the range of 1–50 µg/ml and 2–100 µg/ml, respectively. The correlation coefficient of RANO and MET was found to be 0.9994 and 0.9998, respectively. Linearity overlay and a calibration curve are given in Figures 3 and 4, respectively.

Specificity

Percentage interference was calculated, and it was should be found <0.5%. Thus, the method is specific.

Accuracy

Percentage recovery was found in the range 99.07–101.53% and 99.59–100.45% for RANO and MET, respectively. Thus, the percentage recoveries of drugs are acceptable and data for accuracy study are shown in Table 2.

Precision

RSD was found to be <2 that shows satisfactory precision of the method. Data for both are given in Tables 3 and 4.

LOD and LOQ

LOD and LOQ were determined using the formula given in ICH Q2 (R1) guideline. LOD and LOQ were found to be 0.136 µg/ml and 0.413 µg/ml for RANO and 0.0091 µg/ml and 0.0276 µg/ml for MET, respectively.

Robustness

This study revealed that the method was remaining unaffected by the deliberate changes in flow rate, mobile phase composition, and pH of the mobile phase. RSD was found to be <2, which shows that the proposed method was robust.

Table 1: System suitability parameter of optimized condition

Drug	Retention time±SD	Theoretical plate±SD	Tailing factor±SD	Resolution±SD
RANO	3.06±0.1	5005±100	1.0±0.1	-
MET	6.56±0.1	7574±110	1.0±0.1	14.93±0.1

RANO: Ranolazine, MET: Metformin hydrochloride

Table 2: Recovery data of metformin hydrochloride and ranolazine analyzed by the developed RP-HPLC method

% Recovery level	Target Conc. (µg/ml)		Spiked Conc. (µg/ml)		% Recovery range (*n*=3)	
	RANO	MET	RANO	MET	RANO	MET
50	20	40	10	20	101.28–101.53	100.34–100.45
100	20	40	20	40	99.70–99.83	99.59–100.11
150	20	40	30	60	99.07–99.28	99.63–99.94

RANO: Ranolazine, MET: Metformin hydrochloride

Table 3: Repeatability study data Metformin hydrochloride and Ranolazine

Drug	Concentration (µg/ml) (*n*=6)	Concentration found (µg/ml) Mean±SD	RSD
RANO	20	19.96±0.10	0.513
MET	40	39.95±0.08	0.223

RANO: Ranolazine, MET: Metformin hydrochloride

Table 4: Intraday and interday precisions of Metformin hydrochloride and Ranolazine

Precision	Interday (*n*=3)		Intraday (*n*=3)	
	Drug			
Level (%)	Area (Mean±SD)	RSD	Area (Mean±SD)	RSD
RANO				
50	27717.7±89.80	0.3240	27678.8±75.85	0.2740
100	541142±3734.16	0.6901	540824±1051	0.1943
150	782261±1946.9	0.2489	780943±4132.06	0.5291
MET				
50	916952±2199.29	0.2398	920196±1603.41	0.1742
100	1844284±4230.56	0.2294	1843165±5875.03	0.3187
150	2754548±17645.5	0.6406	2732908±11973	0.4381

RANO: Ranolazine, MET: Metformin hydrochloride

Table 5: Results of robustness study of Metformin hydrochloride and Ranolazine

Conc. (µg/ml)	Different Flow rate (RANO)			Different Flow Rate (MET)		
RANO : MET	0.9 ml/min	1 ml/min	1.1 ml/min	0.9 ml/min	1 ml/min	1.1 ml/min
(20 µg/ml: 40 µg/ml)						
Mean Area±SD	531544±519.336	532614±546.654	531187±634.278	1864391±2516.22	1864387±1952.26	1863561±1795.54
RSD	0.0977	0.10264	0.11941	0.13496	0.10471	0.09635
	Different pH (RANO)			Different pH (MET)		
	5.9	6	6.1	5.9	6	6.1
Mean Area±SD	534364±1333.01	532364±1477.87	534623±1462.65	1863472±2487.01	1863771±2419.49	1864231±2051.42
RSD	0.24946	0.2776	0.27358	0.13346	0.12982	0.11004
	Different Mobile phase composition (RANO)			Different Mobile phase Composition (MET)		
	(88:12)	(90:10)	(92:8)	(88:12)	(90:10)	(92:8)
Mean Area±SD	534472±556.311	534234±713.593	534781±751.951	1863571±1057.62	1863640±2101.39	1863452±1901.52
RSD	0.10409	0.13357	0.14061	0.0568	0.1128	0.1020

RANO: Ranolazine, MET: Metformin hydrochloride

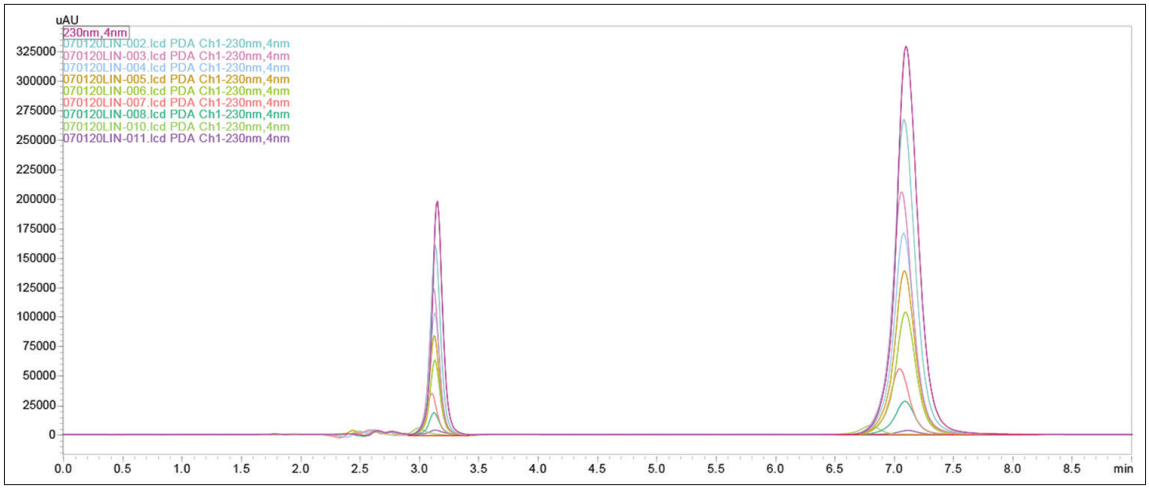

Figure 3: Linearity overlay chromatogram of Metformin hydrochloride and Ranolazine

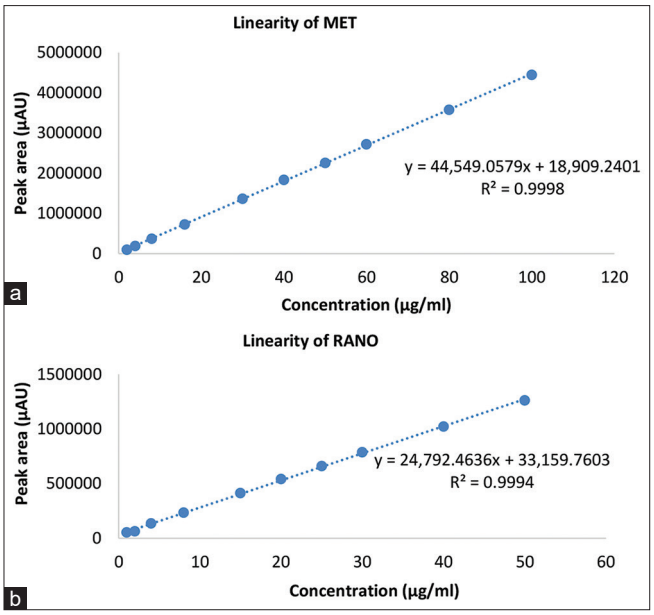

Figure 4: (a) Calibration curve of Metformin hydrochloride and (b) Calibration curve of Ranolazine

Table 6: Result of assay of the synthetic mixture

Drug name	Label claim (mg)	Amount found (mg/tablet)	% Assay (n=6)±SD
RANO	20 mg	20.17 mg	100.86±0.02
MET	40 mg	40.31 mg	100.77±0.33

RANO: Ranolazine, MET: Metformin hydrochloride

Data for robustness are given in Table 5.

Assay

Percentage assay of the synthetic mixture was calculated, and the result was found satisfactory. The result is expressed in Table 6.

CONCLUSION

A simple and economical RP-HPLC method has been developed for simultaneous estimation of RANO and Metformin HCl in a synthetic mixture. Linearity was found to be 1–50 µg/ml and 2–100 µg/ml for RANO and MET, respectively. Percentage recovery was found in the range 99.07–101.53% and 99.59–100.45% for RANO and MET, respectively, RSD was found <1 for precision and repeatability study. Hence, this method is less time-consuming, simple, specific, economical, robust, and accurate and can be successfully applied for the analysis of dosage forms with no interference by the pharmaceutical industry or research laboratories.

REFERENCES

1. Belardinelli L, Shryock JC, Fraser H. Inhibition of the late sodium current as a potential cardio protective principle: Effects of the late sodium current inhibitor ranolazine. Heart 2006;92:iv6-14.
2. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferramnini E, Nauck M, *et al*. Management of hyperglycemia in Type 2 diabetes: A patient-centered approach: Position statement of the American diabetes association (ADA) and the European association for the study of diabetes (EASD). Diabetes care 2012;35:1364-79.
3. Rumsfeld JS, MaWhinney S, McCarthy M, Shroyer AL, VillaNueva CB, O'Brien M, *et al*. Health related quality of life as a predictor of mortality following coronary artery bypass graft surgery. Participants of the department of veterans affairs cooperative study group on processes, structures, and outcomes of care in cardiac surgery. JAMA 1999;281:1298-303.
4. Zack J, Berg J, Juan A, Pannacciulli N, Allard M, Gottwald M, *et al.* Pharmacokinetic drug-drug interaction study of ranolazine and metformin in subjects with Type 2 diabetes mellitus. Clin Pharmacol 2015;4:121-9.
5. Oneil MJ. The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals. 8th ed. Cambridge, UK: Royal Society of Chemistry; 2013. p. 6103-8347.
6. Indian Pharmacopoeia, Government of India, Ministry of Health and Family Welfare. 8th ed., Vol. 2. Ghaziabad: Indian Pharmacopoeia Commission; 2018. p. 2544-8.
7. The United States Pharmacopoeial Convention Inc. US Pharmacopeia 39 NF 34. Vol. 3. Rockville: The United States Pharmacopoeial Convention Inc.; 2016. p. 4764-6.
8. Japanese Pharmacopoeia, The Ministry of Health. Labourand Welfare. 17th ed. Pharmaceuticals and Medical Devices Agency; 2016. p. 1222-3.
9. British Pharmacopoeia. Medicines and Healthcare Products Regulatory Agency. 9th ed., Vol. 2. London: British Pharmacopoeia Commission; 2018. p. 235-7.
10. Vyas AJ, Raval DH, Patel AI, Patel AB, Patel NK, Chudasama A. First order derivative spectrophotometric estimation of ranolazine and metformin hydrochloride in synthetic mixture. Chem Methodol 2020;4:605-13.
11. Patel J, Patel P, Mashru R. Novel UV-spectrophotometric and RP-HPLC method development and validation of simultaneous estimation of ranolazine and metformin HCl: A statistical analysis. Pharm Innov J 2020;9:50-7.
12. Dange YD, Honmane SM, Bhinge SD, Salunkhe VR, Jadge DR. Development and validation of UV-spectrophotometric method for estimation of metformin in bulk and tablet dosage form. Indian J Pharm Educ Res 2017;51:754-60.
13. Shukla R, Singh S, Patel JR, Kare S, Yadav R. Zero order and first order derivative spectrophotometric determination of metformin HCL in bulk dosage form. Asian J Pharm Pharmacol 2016;2:6-9.
14. Sulakshana S, Sankar R, Rao A, Harithanandini T, Reddy Y, Pushpalatha E. Spectrophotometric method development and comparative study of metformin HCl In API and solid dosage form using UV-spectroscopy. Int J Pharm Biol Sci 2015;5:1-8.
15. Vetrichelvi S, Aruna A, Niraimathi V, Suresh AJ. Spectrophotometric estimation of ranolazine in bulk drug and tablet formulation. Int J Chem Sci 2009;7:1889-92.
16. Sharma A, Prakash D, Singh S. Development and validation of UV spectrophotometric method for the estimation of ranolazine in bulk drug and pharmaceutical formulation. Int J Chem Tech Res 2010;2:1945-8.
17. Ramesh J, Rajasekhar K, Gandhi K. Method development and validation for the estimation of ranolazine in bulk and in pharmaceutical dosage form by UV-spectrophotometry. Ann Pharm Res 2013;1:4-7.
18. Ambadekar S, Keni S. Fast and economic spectrophotometric method for metformin and glipizide in combination tablet. Int J Adv Sci Eng Technol 2018;6:31-5.
19. Jani B, Shah KV, Kapupara P. Development and validation of UV spectroscopic method for simultaneous estimation of dapagliflozin and metformin hydrochloride in synthetic mixture. Int J Res Dev Pharm Life Sci 2015;5:1569-76.
20. Vani R, Kumar BV, Mohan G. Development and validation of RP-HPLC method for simultaneous estimation of metformin and telmisartan in bulk and its tablets. Indo Am J Pharm Res 2015;5:893-900.

21. Godasu SK, Sreenivas SA. A new validated RP-HPLC method for the determination of metformin HCl and empagliflozin in its bulk and pharmaceutical dosage forms. Int J Pharm Sci Res 2017;8:2223-32.
22. Triveni D, Kumar GV, Puranik SB. Development and validation of RP-HPLC method for simultaneous estimation of glipizide and metformin in bulk drugs and tablets dosage form. Int Res J Pharm 2012;3:260-3.
23. Nirupa G, Tripathi UM. RP-HPLC analytical method development and validation for simultaneous estimation of three drugs: Glimepiride, pioglitazone, and metformin and its pharmaceutical dosage forms. J Chem 2013;2013:726235.
24. Nirupama D, Rao PV, Thangabalan B, Babu SM. Development and validation of RP-HPLC method for simultaneous estimation of gliclazide and metformin in pure and tablet dosage form. Int J Pharm Anal Res 2014;3:326-33.
25. Khan B, Shakoor A, Nawaz S, Altaf A. Development and validation of Type-2 diabetes drug in combination metformin and glibenclamide by HPLC. Asian J Chem 2011;23:3347-9.
26. Kumar V, Sunandamma Y. New RP HPLC method development and validation for analysis of ranolazine in tablet dosage form. J Pharm Res 2011;4:3681-3.
27. Poornima K, Channabasavaraj K. Development and validation of RP-HPLC method for estimation of metoprolol and ranolazine in bulk and in formulation. J Chronot Drug Deliv 2015;1:41-8.
28. Rao AS, Baby NR, Natraj SK. Stability indicating RP-HPLC method development and validation of metformin hydrochloride and glimepiride in bulk and pharmaceutical dosage form. World J Pharm Sci 2016;4:433-11.
29. Patel V, Pandya S. Spectrophotometric and stability indicating RP-HPLC method for estimation of ranolazine in tablet dosage form. Int J Pharm Sci Res 2018;9:1218-25.
30. Lakshmi P, Pavani H, Agarwal N. Development and validation of stability indicating RP-HPLC method for estimation of ranolazine in bulk and tablet dosage form. Int Res J Pharm App Sci 2012;2:217-23.
31. Khedkar A, Veer S, Rakh M, Rao JR. Stability indicating method development and validation of ranolazine hydrochloride in bulk and tablet dosage form by HPTLC. Int J Pharm Clin Res 2015;7:77-83.
32. Malati V, Reddy A, Mukkanti K, Suryanarayana MV. A novel reverse phase stability indicating RP-UPLC method for the quantitative determination of fifteen related substance in Ranolazine drug substance and drug product. Talanta 2012;97:563-73.
33. United States Food and Drug Administration. Clinical Trials. https://www.clinicaltrials.gov/ct2/show/NCT01555164?term=Ranolazine+and+Metformin+Hydrochloride&cond=Diabetes+Mellitus%2C+Type+2&draw=2&rank=32020 [Last accessed on 2020 Nov 10].
34. International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, Topic Q2 (R1) Validation of Analytical Procedure: Text and Methodology. Geneva, Switzerland: International Conference on Harmonization (ICH); 2005.