A NEW RP-UPLC METHOD FOR SIMULTANEOUS QUANTIFICATION OF IVABRADINE AND METOPROLOL IN COMBINED DOSAGE FORMS

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Abstract

An accurate and yet fast, easy-to-use, affordable, sensitive, single method for measuring Ivabradine and Metoprolol in bulk and pharmaceutical products using Reversed-Phase Ultra-Performance Liquid Chromatography (RP-UPLC) was developed. A Hibar C18 column was used with a running phase composed of Water: ACN (60:40 v/v) at a flow rate of 0.5 ml/min. UV detection was used at a wavelength of 260 nm. The correlation parameters for Ivabradine and Metoprolol were found to be of the order of 99.9% and % MSD <2. The validation results agreed with what's acceptable and had good limits. This method, proposed as a regular analysis and quality control tool for medications that contain these active drugs either individually or in combination, was evident to be a suitable one.

Keywords: *UPLC, Ivabradine, Metoprolol, Development, Validation.*

1. Introduction

Ivabradine, [1] sold under the brand name Procoralan among others, is a medication, which is an I_f inhibitor, used for the symptomatic management of stable heart-related chest pain and heart failure [2, 3]. Patients who qualify for use of Ivabradine for coronary heart failure are patients who have symptomatic heart failure, with reduced ejection volume and heart rate at least 70 bpm, which condition cannot be fully managed by beta blockers.[4-6]

Ivabradine acts by allowing negative chronotropy in the sinoatrial structure thus reducing the heart rate via specific inhibition of the pacemaker current, a mechanism different from that of beta blockers and calcium channel blockers[7,8], two commonly

prescribed antianginal classes of cardiac pharmaceuticals. Ivabradine has no apparent inotropic properties and may be a cardiotonic agent.

Metoprolol[9,10], sold under the brand name Lopressor, among others, is a selective β_1 receptor blocker . It is used to treat high blood pressure, chest pain due to poor blood flow to the heart, and a number of conditions involving an abnormally fast heart rate. By working on the beta-1 receptor of the cardiac muscle cells, it yields both a chronotropic and inotropic effect. It is also used to prevent further heart problems after myocardial infarction [11, 12] and to prevent headaches in those with migraines.[13, 14]

For ascertaining quality, various analytical methods are used. Various methods used for evaluation of Ivabradine and Metoprolol were Titrimetric, UV, Visible spectroscopy, dissolution study using HPLC, IR, HPLC, HPLC coupled with MS (LC-MS), HPTLC, UPLC, UPLC coupled to MS, UPLC-MS/MS. Out of the available ethods, UPLC-MS method is the generally used for the analysis of biological samples, whereas the HPLC method is the used for the analysis of pharmaceutical samples. Ivabradine and Metoprolol in pharmaceutical or biological matrixes can be evaluated by different methods of analysis[15].

Effective and reliable results for Pharmaceutical analyses are essential as they have an impact on making analytical decisions besides, the method must be suitable for the intended investigation. Ivabradine is a newly approved drug, hence analytical methods are not available in any compendia, whereas analysis of Metoprolol is available in IP[16] and USP[17] for various salt forms.

However, simultaneous quantification of both Ivabradine and Metoprolol is not available to date. Hence, in this study a novel, quick, sensitive, and easy-to-use UPLC method was attempted for simultaneous estimation of Ivabradine and Metoprolol in API and pharmaceutical dosage types without having to separate the drugs beforehand.

2. Materials and Methods

I. Materials

a) Solutions, Reagents and Buffers

The reference standard specimens of Ivabradine and Metoprolol were obtained from Matrix Ltd. Acetonitrile and Methanol used was of HPLC grade, while Sodium hydroxide, hydrogen peroxide was of GR grade (Merck Ltd. Mumbai, India). Milli-Q water was utilized throughout the analysis. An easy, inexpensive, and appropriate buffer was chosen, such as Water.

b) Collection of instruments

Waters Acquity UPLC system with a quaternary pump and PDA detector (PDA lamp) was used. Data was done using Empower 2.0.

c) Step of mobility

For standard review, the mobile step was Water in a 60:40 (v/v) Acetonitrile mixture and was degassed beforehand. A mobile phase chosen to produce well-defined peaks

with a low tailing factor (2.0) and a plate count of over 2000 was selected and mobile process of diluent was set up.

d) Conditions of Chromatography

For the UPLC experiments, a Hibar C18 (100 x 2.1 mm, 2 µ) was used. The elution was conducted under isocratic conditions using Acetonitrile: Water (40:60 by volume) at a flow rate of 0.5 ml/min. The injection volume was 5µl, with the column temperature set to 30°C. Maximum absorbance was observed at this wavelength at a wave length of 260 nm

e) Standard Solution Preparation

5 mg of Ivabradine and 25 mg of Metoprolol were accurately weighed and transferred into a 100 mL clean dry volumetric flask. 50 mL of the diluent was added to it and sonicated for 5 min. The final volume was made up with the diluent. 5 mL from the above stock solutions was taken into a 50 mL volumetric flask and diluted to 50 mL to get desired concentration.

II. Methods: Validation Process

a) System Precision

The system's performance has been validated through assessment of device suitability parameters. Limits were found to be met for a variety of parameters, including plate count, tailing, and RSD percentage.

b) Specificity

Being able to identify and test a given analyte in the presence of other elements required to be combined in the Standard and the standard solution is known as specificity. Blank Standards and those with Ivabradine and Metoprolol will be tested using chromatograms.

c) Accuracy

Being close to the real meaning of the technique is what defines accuracy. Three concentrations will be used to test the recovery trials. The drug's quantity, percentage of recovery, and standard deviations were calculated after every injection at each level.

d) Precision

It is the level of agreement between the various test results that determines the precision of the analytical methodology. Researchers examined the effects of sampling a homogeneous population more than once. The current process was evaluated in terms of its ability to provide repeatable, intraday, and inter-day results. It was examined by sampling the materials on the same day and over the course of different days.

e) Linearity

Linearity is the feature of analytical process which allows for a direct proportion of analytical results in response to a certain concentration of the analyte in the Standard. A

total of seven series of standard solutions were selected for the assessment of the linearity spectrum. The calibration curve was drawn by comparing regular solution concentration with peak area. Using the least square method, the slope, intercept, and coefficient of correlation were calculated.

f) Robustness

Robustness refers to a procedure's resistance to small process parameter changes, as well as its reliability in normal operation. An organic solution was introduced into the UPLC system for a robustness analysis, and the chromatographic settings (such as flow rate and mobile-phase organic content) were modified. The separation factor, retention time, and peak asymmetry were determined by evaluating the effects of altered parameters.

3. Results and Discussion

In this study a single isocratic UPLC method for the simultaneous quantification of Ivabradine and Metoprolol in bulk and pharmaceutical dosage forms that is reliable, precise, and cost effective was developed. Based on the UV spectra of these compounds, an appropriate wavelength for simultaneous estimation of two drugs was chosen.

a) Optimization

Using buffer (Water) and Acetonitrile as mobile phase different trials were conducted in isocratic and gradient modes for optimizing the method. Various stationary phases including phenyl, biphenyl, amino, C4, and C8, were used to test the system. The resolution and retention times were improved by changing the mobile step composition at each trial. In the end, the separation was achieved using a Hibar C18 column (100mm x 2.1mm, 2µ) and a mobile phase of Water: Acetonitrile (60:40 v/v) with a flow rate of 0.5 ml/min and UV detection at a wavelength of 260 nm. The entire performance lasted five minutes. Conditions for optimized chromatography are provided in table 1.

b) System Suitability

To attain results, the following device suitability parameters were established after six consecutive injections of normal solution: theoretical plate number, time, peak area, tailing factor, and resolution.

Table 1. Method suitability conditions

Parameter	Suitable conditions
Column	Hibar C_{18} (100 x 2.1 mm, 2 µ)
Moving Phase	Water: Acetonitrile (60:40 v/v)
Volume of injection	5 µl
Stream rate	0.5 mL/min
Temperature of column	30°C
Wavelength	260 nm

Table 2. Results of system suitability

Parameter	Ivabradine	Metoprolol
Number of plates	3049	2884
Tailing	1.05	1.14
Resolution	-	3.6
Peak area	124826	1254926

c) Specificity

There was no participation from Ivabradine and Metoprolol at the elution time.

d) Linearity

By using a calibration curve to determine the linearity of the area of peak, its corresponding concentration was discovered. Linearity results given in Table 3 were demonstrated in Figures1&2 for Ivabradine and Metoprolol respectively

Table 3. Results of linearity

% of Linearity		Metoprolol
	Area	Area
25%	36421	361465
50%	66102	646021
75%	96865	962158
100%	129301	1241023
125%	161277	1559866
150%	187652	1836849

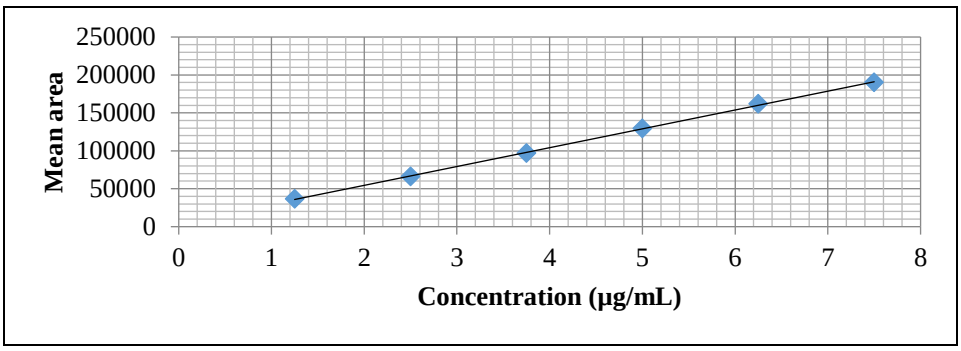

Figure1. Calibration plot of Ivabradine

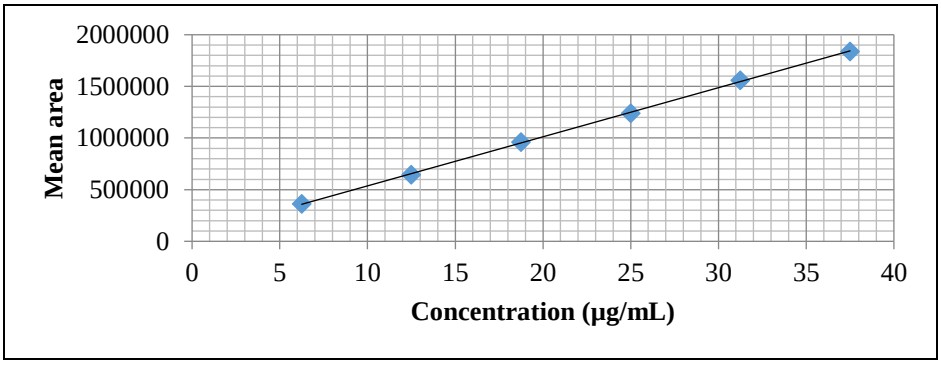

Figure 2. Calibration plot of Metoprolol

e) Precision & Ruggednes (Intermediate Precision)

Intraday and intermediate precision variances were assessed in relation to the accuracy of the procedure. The Standards were examined six times on the same day to obtain intraday results for Ivabradine and Metoprolol. The system's intermediate precision was explored by analyzing data in the same laboratory using a variety of examiners and tools. It is very accurate, with an RSD percentage of less than 2%. The process was precise, yielding the best drug recoveries at each additional concentration. Table 4 shows the method precision results. Ruggedness (Intermediate precision) results were shown in table 5.

Table 4. Outcome of method precision

S.No	Ivabradine	Metoprolol
1	122533	1240187
2	127142	1265656
3	125066	1253198
4	123947	1247504
5	126451	1261838
6	125028	1257612
Mean	125027	1254332
SD	1666.671	9416.156
% RSD	1.33	0.75

Table 5. Results of intermediate precision

S.No	Ivabradine		Metoprolol	
	Day-1	Day-2	Day-1	Day-2
1	127054	128869	1248544	1226481
2	127341	127425	1239710	1240352
3	126695	128633	1250198	1231694
4	128012	126780	1243765	1238475
5	125834	128197	1257223	1244023
6	128351	129371	1232162	1229356
Mean	127214	128212	1245267	1235063
SD	910.501	961.498	8750.734	6891.554
% RSD	0.72	0.75	0.70	0.56

f) Accuracy

By measuring the recovery experiments at three stages, the method's precision was reached (50 percent, 100 percent, and 150 percent). For each stage of the spike, the test solution was injected three times, and the assay was performed in accordance with the test process. In addition to being able to determine the percentage of recovered data, the mean and relative standard deviations have also been found. The strategy was effective because the recovery fell within the target range. Table 6 presents the accuracy results.

Table 6.Results of accuracy

Accuracy	% Recovery of Ivabradine	% Recovery of Metoprolol
50*****	99.42	99.78
100*	99.84	99.70
150*	99.76	99.51

* Results are mean recovery of three sample preparations

g) Robustness

To ensure the robustness of the chromatographic technique, the researchers evaluated flow rate and the composition of the mobile phase. By changing the flow rate and mobile phase ratio, the area of drugs changes. So, the percentage of relative standard deviation changes. Here in Table 7 (robustness results) the %RSD values are in within the acceptable limit.

Table 7. Outcomes of robustness

Parameter	% RSD of Ivabradine	% RSD of Metoprolol
Flow (0.4 mL/min)	0.89	0.54
Flow (0.6 mL/min)	1.02	0.71
Organic phase (50:50)	0.75	1.10
Organic phase (70:30)	1.54	0.99

4. Conclusion

A novel, quick, sensitive, and easy-to-use UPLC method was developed for simultaneous estimation of Ivabradine and Metoprolol in API and pharmaceutical dosage types. With no UPLC or HPLC methods published to-date, the approach developed is the most practical option with benefits of shorter run time, low cost and many the other characteristics are benefits. The suitability parameters were verified and were found to be within the acceptable range including linearity, accuracy, specificity, robustness, and process precision. According to the study, the RSD values for all the parameters found to be less than 2%, and the results are consistent, illustrating that the procedure is accurate. Therefore, it's recommended to use the current approach in QC laboratories for routine study for manufacturing pharmaceuticals containing Ivabradine and Metoprolol without having to separate the substances first.

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Conflicts of Interest

None

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5. References

1. *S. Sulfi, AD. Timmis, "Ivabradine – the first selective sinus node If channel inhibitor in the treatment of stable angina", Int. J. Clin. Pract.* ***(2006)****, 60 (2): 222–8.*
2. *SA. Fisher, C. Doree, A. Mathur, DP. Taggart, E. Martin-Rendon, "Stem cell therapy for chronic ischaemic heart disease and congestive heart failure", The Cochrane Database of Systematic Reviews.* ***2016*** *(12): CD007888.*
3. *S. Stewart, A. Jenkins, S. Buchan, A. McGuire, S. Capewell, JJ. McMurray, "The current cost of heart failure to the National Health Service in the UK", European Journal of Heart Failure.* ***(June 2002)****, 4 (3): 361–71.*
4. *N. Freemantle, J. Cleland, P. Young, J. Mason, J. Harrison, "beta Blockade after myocardial infarction: systematic review and meta regression analysis", BMJ.* ***(June 1999)****, 318 (7200): 1730–7.*
5. *HY. Kim, YJ. Jung, SH. Lee, HJ. Jung, K. Pak, "Is Beta-Blocker Use Beneficial in Breast Cancer? A Meta-Analysis", Oncology.* ***(2017)****, 92 (5): 264–268.*
6. *JM. Cruickshank, "Beta-blockers and heart failure", Indian Heart Journal.* ***(2010)****, 62 (2): 101–10.*
7. *Y. Tekol, "The medieval physician Avicenna used an herbal calcium channel blocker, Taxus baccata L", Phytotherapy Research.* ***(2007)****, 21 (7): 701–02.*
8. *LT. Iseri, JH. French. "Magnesium: Nature's Physiologic Calcium Blocker", American Heart Journal.* ***(1984)****, 108 (1): 188–93.*
9. *S. Albers, JP. Elshoff, C. Völker, A. Richter, S. Läer, "HPLC quantification of metoprolol with solid-phase extraction for the drug monitoring of pediatric patients", Biomedical Chromatography.* ***(2005)****, 19 (3): 202–207.*
10. *C. Blake, E. Kharasch, M. Schwab, P. Nagele, A meta-analysis of CYP2D6 metabolizer phenotype and metoprolol pharmacokinetics, Clinical Pharmacology and Therapeutics.* ***2013****: 94(3); 394-399.*
11. *K. Perry, KJ. Petrie, CJ. Ellis, R. Horne, R. Moss-Morris, "Symptom expectations and delay in acute myocardial infarction patients", Heart.* ***(July 2001)****, 86 (1): 91–3.*
12. *EM. Bucholz, S. Ma, SL. Normand, HM. Krumholz, "Race, Socioeconomic Status, and Life Expectancy After Acute Myocardial Infarction", Circulation.* ***(October 2015)****, 132 (14): 1338–46.*

13. *PC. Tfelt-Hansen, PJ. Koehler, "One hundred years of migraine research: major clinical and scientific observations from 1910 to 2010", Headache.* ***(May 2011)****, 51 (5): 752–78.*
14. *A. Hougaard, FM. Amin, M. Ashina, "Migraine and structural abnormalities in the brain", Current Opinion in Neurology.* ***(June 2014)****, 27 (3): 309–14.*
15. *Gandhi NK and Ezhava SB: "Ivabradine and metoprolol: a review of analytical methods for pharmaceutical quality control and monitoring". Int J Pharm Sci & Res 2021; 12(12): 6221-32. doi: 10.13040/IJPSR.0975-8232.12(12).6221-32.*
16. Indian Pharmacopoeia Government of India Ministry of Health and Family Welfare. The Indian Pharmacopoeia Commission, Ghaziabad 2018; 1,2(583), 2583-93
17. *United State pharmacopoeia and National formulary. Asian Edition. The United State Pharmacopeial Convention, Rockville; USP 40 NF 35 2017; 3: 5135-46*