Simultaneous Estimation of Metoprolol Succinate and Lacidipine in Binary Combination using High Performance Liquid Chromatographic Method

Nilesh Jain¹, Deepak Kumar Jain²*, Ruchi Jain¹, Preeti Patel², Vijay Kumar Patel² and Surendra Kumar Jain¹

¹ Sagar Institute of Research & Technology-Pharmacy, Ayodhya Bypass Road, Bhopal-462041, (MP) India.
² Medicinal Chemistry Research Laboratory, SLT Institute of Pharmaceutical Sciences, Guru Ghasidas Central University, Bilaspur-495009, (CG) India

ABSTRACT

A simple, reliable, rapid, precise, sensitive, rugged and validated RP-HPLC method has been developed to determine Metoprolol succinate and Lacidipine in synthetic mixture form. Chromatographic separation achieved isocratically on Prontosil C18 column (5µm, 250mm x 4.60mm) and acetonitrile: 20mM Phosphate buffer (pH 3.6) in the ratio of 60:40 (v/v) as the mobile phase, at a flow rate of 1 mL/min. Detection was carried out at 278 nm. The mean retention times for metoprolol succinate and lacidipine was found to be 5.25±0.5 and 6.72±0.5 min, respectively. No interference was found by the excipients in the synthetic mixture. Linearity for metoprolol succinate and lacidipine was in the range of 5-25μg/mL and 4-20μg/mL, respectively. The mean recoveries obtained for metoprolol succinate and lacidipine were 100.03 % and 99.67 % respectively and RSD was less than 2. The correlation coefficients for all components are close to 1. The proposed method was validated in terms of linearity, range, accuracy, precision, specificity, robustness and the method is successfully applies to the estimation of metoprolol succinate and lacidipine in bulk and in a synthetic mixture.

Keywords: Metoprolol succinate, Lacidipine, RP-HPLC, Validation.

1. INTRODUCTION

Metoprolol succinate (MTS, Fig. 1A) Chemically (RS) -1- (isopropylamino)-3-(4-(2-methoxyethyl) phenoxy) propan-2-ol succinate (2:1) (salt) is a cardioselective β1-adrenergic blocking agent used for acute myocardial infarction (MI), heart failure, angina pectoris and mild to moderate hypertension. It may also be used for supraventricular and tachyarrhythmias and prophylaxis for migraine headaches. The β1-selectivity of these agents is thought to be due in part to the large substituents in the para position. At low doses, metoprolol selectively blocks cardiac β1-adrenergic receptors with little activity against β2- adrenergic receptors of the lungs and vascular smooth muscle. Receptor selectivity decreases with higher doses. Unlike propranolol and pindolol, metoprolol does not exhibit membrane-stabilizing or intrinsic sympathomimetic activity. Membrane-stabilizing effects are only observed at doses much higher than those needed for β-adrenergic blocking activity. Metoprolol possesses a single chiral centre and is administered as a racemic mixture. It may be used alone or in combination with other antihypertensive agents. It is official in IP, BP and USP which describe potentiometric method for its estimation. Several analytical methods that have been reported for the estimation of metoprolol succinate alone

* jaindeepak2022@gmail.com
Received on 12/4/2016 and Accepted for Publication on 25/9/2016
or combination with other drugs in pharmaceutical formulations and/or biological fluids include spectrophotometric\(^{(6-12)}\), spectrofluorometry\(^{(13)}\), high-performance liquid chromatography (HPLC)\(^{(14-19)}\), stability indicating\(^{(20-22)}\), HPTLC\(^{(23-25)}\), LC-MS\(^{(26)}\), LC-MS/MS\(^{(27)}\), GC-MS\(^{(28, 29)}\), UPLC\(^{(30)}\). Lacidipine (LCP, Fig. 1B) chemically (E)-diethyl 4-(2-(3-(tert-butoxy)-3-oxoprop-1-en-1-yl) phenyl)-2, 6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate, is a calcium channel blocker developed for oral administration and widely used in therapy since the early 1990s\(^{(31)}\). In addition to its antihypertensive effect, lacidipine has also shown anti-atherosclerotic and antioxidant effects as well as antibacterial activity with respect to 389 Gram-positive and Gram-negative bacterial strains. It is one of the most vascular selective of the dihydropyridines. Lacidipine has a high degree of lipophilicity, which deposits on the lipid moiety and continuously releases into the binding site during the cleanup phase, so it has long duration of action. The active trans form is used in therapy\(^{(32-36)}\). Some analytical methods for the determination of lacidipine alone or/and combination with other drug in pharmaceutical dosage form and biological fluids have been reported, including some spectrophotometric method\(^{(37,38)}\), HPLC\(^{(39-43)}\), Stability-Indicating LC Method\(^{(44,45)}\) high performance thin layer chromatography (HPTLC)\(^{(46)}\), LC–MS/MS methods\(^{(47-50)}\), ultra performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS) method\(^{(51)}\) and SFC–MS/MS\(^{(52)}\). Monotherapy with various antihypertensive agents is not always sufficient to control the blood pressure and concomitant use of two or more drugs is necessary in 50% of the hypertensive patients\(^{(19)}\). The primary goal of any antihypertensive therapy is therefore achievement of normotension without addition of intolerable side effects, which can be accomplished by combination of drugs with different mechanism of action. The therapeutic importance of these two compounds justifies establishing analytical methods for its determination in bulk and laboratory mixture. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of MTS and LCP in their combined synthetic mixture. In the present work we are therefore focused on to achieve the optimum chromatographic conditions for the simultaneous determination of MTS and LCP in a synthetic mixture. We describe in this paper a simple, sensitive and validated HPLC method with total run time less than 9 minutes for the simultaneous determination of MTS and LCP. The developed method can be applied successfully to quality control and for other analytical purposes. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines\(^{(53)}\), which is mandatory also.

![Figure 1: Chemical structure of (A) Metoprolol succinate (B) Lacidipine](image-url)
2. Materials and Methods

2.1. Instrumentation

A high performance liquid chromatographic system from Young Lin 9100 comprising of manual injector, YL 9111 quaternary pump for constant flow and constant pressure delivery and Photodiode array detector (YL 9160 detector) connected to software YL clarity for controlling the instrumentation as well as processing the data generated was used.

2.2. Chemicals and reagents

Analytically pure sample of MTS (98.8%) was a generous gift from Unichem Laboratories Ltd. Mumbai, India and LCP (99.0%) was obtained from Cipla Health Care, Ahmadabad. Potassium dihydrogen phosphate and acetonitrile (HPLC Grade) was purchased from E. Merck Ltd. Worli, Mumbai, India. The 0.45µm nylon filters were purchased from Advanced Micro Devices Pvt. Ltd. Chandigarh, India. All excipients used were of pharmaceutical grade. Triple distilled water was generated in house.

2.3. Chromatographic conditions

The isocratic mobile phase consisted of acetonitrile: 20mM Phosphate buffer (pH 3.6) in the ratio of 60:40 (v/v), flowing through the column at a constant flow rate of 1ml/min. These were filtered through 0.45µ membrane filter and degassed by sonication before use. A Prontosil C18 column (5 µm, 250mm × 4.60mm) was used as the stationary phase. By considering the chromatographic parameter, sensitivity and selectivity of method for two drugs, 278 nm was selected as the detection wavelength for UV-PDA detector. The HPLC system was operated at room temperature 25°C.

2.4. Standard preparation

1.4.1. Standard stock solution

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 100 ml of diluent which was a mixture of acetonitrile and phosphate buffer in the ratio of 60:40 (pH 3.6) to get concentration of 1000µg/mL.

1.4.2. Standard substock solution

10 ml of solution was taken from standard stock solutions and transferred into 100 ml volumetric flask separately and diluted up to 100 ml with diluent to get the concentration of 100 µg/ml. This standard sub stock solution is used to prepare working standard solution.

1.4.3. Working standard solution

For MTS 0.5ml, 1.0 ml, 1.5ml, 2.0ml and 2.5ml of stock-B was taken separately in 10 ml volumetric flask and volume was made up to 10ml with diluent. This gives the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml for MTS. For LCP 0.4ml, 0.8 ml, 1.2 ml, 1.6 ml and 2.0ml of substock solution was taken separately in 10 ml volumetric flask and volume was made up to 10ml with diluent. This will give the dilutions ranging from 4-20 µg/mL for LCP.

1.4.4. Sample preparation

The content of twenty tablets where taken and weighed. Powdered equivalent to 25 mg MTS and 4mg LCP was dissolved in 100ml diluents and then sonicated for 15min. and filtered through whatman paper no. 41. Then different concentration of solution were prepared by serial dilution technique, as per standard and each dilution was analysed.

3. Results and Discussion

3.1. Chromatography

The mobile phase was chosen after several trials with methanol, isopropyl alcohol, acetonitrile, water and buffer solutions in various proportions and at different pH values. A mobile phase consisting of acetonitrile: 20mM Phosphate buffer (pH 3.6) in the ratio of 60:40 (v/v) was selected to achieve maximum separation and sensitivity. Flow rates between 0.5 and 1.5 mL/ml were studied. A flow rate of 1 mL/ml gave an optimal signal to noise ratio with a reasonable separation time. Using a reversed-phase C18 column, the retention times for MTS and LCP were observed to be 5.25±0.5 and 6.72±0.5 min respectively.
Total time of analysis was less than 9 min. The maximum absorption of MTS and LCP together was detected at 278 nm and this wavelength was chosen for the analysis. Fig. 2.

![HPLC chromatogram of placebo.](image-a)

**Figure 2:** (A) HPLC chromatogram of placebo. (B) Chromatograms of MTS (10 µg/ml) and LCP (8 µg/ml) reference substance at 278 nm

### 3.2. System suitability

System suitability parameters such as number of theoretical plates, HETP and peak tailing are determined. The results obtained are shown in Table 1. The number of theoretical plates for MTS and LCP were 31580 and 2957, respectively.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MTS</th>
<th>LCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time*</td>
<td>5.25±0.5</td>
<td>6.72±0.5</td>
</tr>
<tr>
<td>No. of theoretical plates*</td>
<td>31580.83±4.51</td>
<td>2957.166±6.27</td>
</tr>
<tr>
<td>Tailing factor*</td>
<td>1.047±0.019</td>
<td>1.233±0.019</td>
</tr>
<tr>
<td>HETP*</td>
<td>0.0785±0.003</td>
<td>0.0533±0.009</td>
</tr>
<tr>
<td>Calibration range</td>
<td>5-25 µg/ml</td>
<td>4-20 µg/ml</td>
</tr>
</tbody>
</table>

* Each value is the Mean ± S.D of six determinations

- 196 -
3.3. Linearity

The calibration curve was linear over the concentration range of 5-25μg/ml for MTS and 4-20μg/ml for LCP.

The linearity was represented by a linear regression equation as follows. Y (MTS) = 249.2conc. + 42.52 \( (r^2=0.998) \)

Y (LCP) = 253.8conc. + 7.787 \( (r^2=0.999) \)

Where Y is area under curve and \( r^2 \) is correlation coefficient.

3.4. Limit of detection and quantitation

Estimation of DL and QL considered the acceptable signal-to noise ratios 3:1 and 10:1 respectively. The limit of detection and quantitation to be determined 0.205 and 0.615μg/mL for MTS, 0.145 and 0.458μg/mL for LCP respectively.

3.5. Accuracy

Method accuracy was performed by adding known amounts of MTS and LCP to the pre-analysed synthetic mixture solution and then comparing the added concentration with the found concentration. Three levels of solutions were made which correspond to 80, 100 and 120 % of the nominal analytical concentration (15μg/mL for MTS and 8μg/mL for LCP). Each level was made in triplicate Table 2. The mean percentage recoveries obtained for MTS and LCP were 100.03 and 99.67% respectively and RSD was less than 2.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Conc. of drug in preanalyzed samples (μg/ml)</th>
<th>Std. drug sol. Added (μg/ml)</th>
<th>Recovered amount* (μg/ml)</th>
<th>% Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTS</td>
<td>LCP</td>
<td>MTS</td>
<td>LCP</td>
<td>MTS</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>8</td>
<td>12</td>
<td>6.4</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>8</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>8</td>
<td>18</td>
<td>9.6</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% R.S.D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean of Nine determinations (3 replicates at 3 concentration level)

3.6. Precision and Robustness

Precision of the methods was studied at three levels as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility. Mean ± SD and % relative standard deviation (RSD) values were used to express precision. As per ICH norms, small, but deliberate variations, by altering the pH or concentration of the mobile phase were made to check the method’s capacity to remain unaffected. The ratio of mobile phase was change from, ACN: phosphate buffer pH- 3.6 (60:40% V/V), to (55:45% V/V). Results of precision and robustness are reported in Table 3.

3.7. Stability of sample solution

The stability of the standard solution was to test for an interval 24 and 48h at room temperature. There were no significant changes observed in the system suitable parameters like theoretical plates, tailing factors, retention time and resolution. Hence the standard solution is stable up to 48 h of room temperature. Results are shown in Table 4.
Table 3. Result of precision and robustness

<table>
<thead>
<tr>
<th>Validation Parameter</th>
<th>Percentage Mean ± S.D.* (n=15) MTS</th>
<th>Percentage Mean ± S.D.* (n=15) LCP</th>
<th>Percentage RSD* MTS</th>
<th>Percentage RSD* LCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repeatability</td>
<td>97.69±0.68</td>
<td>98.60±1.10</td>
<td>0.69</td>
<td>1.11</td>
</tr>
<tr>
<td>Reproducibility</td>
<td>100.37±0.15</td>
<td>99.60±0.14</td>
<td>0.14</td>
<td>0.14</td>
</tr>
<tr>
<td>Intermediate precision Day to Day</td>
<td>100.62 ±0.98</td>
<td>99.72±0.27</td>
<td>0.97</td>
<td>0.27</td>
</tr>
<tr>
<td>Analyst to Analyst</td>
<td>100.22±1.30</td>
<td>100.33±0.61</td>
<td>1.29</td>
<td>0.60</td>
</tr>
<tr>
<td>Robustness*</td>
<td>100.62±0.10</td>
<td>98.40±1.15</td>
<td>0.09</td>
<td>1.16</td>
</tr>
</tbody>
</table>

* Mean of fifteen determinations (3 replicates at 5 concentration level)

Table 4. Stability data of MTS and LCP

<table>
<thead>
<tr>
<th>Hours</th>
<th>MTS</th>
<th>LCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3830.258</td>
<td>2025.569</td>
</tr>
<tr>
<td>24</td>
<td>3840.456</td>
<td>2021.458</td>
</tr>
<tr>
<td>48</td>
<td>3838.478</td>
<td>2019.985</td>
</tr>
</tbody>
</table>

3.8. Specificity and selectivity

A representative chromatogram (Fig. 3) was generated to show that other components, which could be present in the sample matrix, are resolved from the parent analyte. No significant changes in retention times of the drugs in the presence and the absence of excipients clearly indicated the specificity of the method.

![HPLC Chromatograms of MTS (15µg/ml) and LCP (12µg/ml) in a synthetic mixture at 278 nm](image)

3.9. Mobile phase stability

The stability of the mobile phase was to test for an interval 24 and 48 h at room temperature. There were no significant changes observed in peak areas, theoretical plates, tailing factors, retention time and resolution. Hence the mobile phase is stable up to 48 h of room temperature.

3.10. Synthetic mixture analysis

The concentration of MTS and LCP in the synthetic mixture was found to be 100% and 98.61% respectively. The low values of R.S.D. indicate that the method is
precise and accurate in Table 5.

Table 5. Statistical evaluation of synthetic mixture analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean % estimated</td>
<td>MTS</td>
</tr>
<tr>
<td>Mean % estimated</td>
<td>LCP</td>
</tr>
<tr>
<td>Standard deviation(S.D.)</td>
<td>1.05</td>
</tr>
<tr>
<td>Standard deviation(S.D.)</td>
<td>0.45</td>
</tr>
<tr>
<td>% Coefficient of variation</td>
<td>0.96</td>
</tr>
<tr>
<td>% Coefficient of variation</td>
<td>0.46</td>
</tr>
<tr>
<td>*Standard error (SE)</td>
<td>0.17</td>
</tr>
<tr>
<td>*Mean of Nine determinations (3 replicates at 3 concentration level)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

4. Conclusion

A simple precise, reliable, rapid, sensitive and accurate reverse phase HPLC method has been developed for the simultaneous determination of metoprolol succinate and lacidipine. The developed method is suitable for the identification and quantification of binary combination of metoprolol succinate and lacidipine. A high percentage of recovery and the run time of less than nine minutes allow its application for the routine determination of metoprolol succinate and lacidipine in pharmaceutical dosage form.

REFERENCES

(2) http://www.drugbank.ca/drugs/DB00264.
(5) Indian Pharmacopoeia, Vol. II, the Controller of Publication, Govt of India, New Delhi, 2010, pp. 1681.
(16) Pokala M., Rani P.J., Pranathi G., Haque M.A.,...


(32) Tcherdakoff P. French large-scale study evaluating the


تقييم في وقت واحد للجمع بين ثنائي السكسيات ميتوبرولول وlasideبين باستعمال

طريقة الكروماثوجرافى السائلة عالية الأداء

نيليش جينام،1 دييبار كومار جايین،2 درتشي جانام1،2، درسيتي باتنیب 1،2، فيرجاي كومار باتنیب 2، وسوریندا كومار جاین1

1 معهد أبحاث الكيمياء الطبية، معهد لامبار، الهند. 2 مختبر أبحاث الكيمياء الطبية، معهد SLT (الموكل من المركز الجامعي للبحوث الجامعي، الهند).

ملخص

لقد تم تطوير طريقة RP-HPLC بسيطة وموثوقة وسريعة ودقيقة وحساسة، وقوية متحفظة من صحتها لتحديد السكسيات ميتوبرولول وlasideبين في شكل مزيج مصنوع. حقق الطرفي الكروماثوجرافى على عمود بروتونسييل (5 μm) (الاسم: MM20(الرقم 180 μm، C18 × 250) مزدوج الفرامل، عالیة الدقة، (ال⏰ تكرل) مصلحة (التوالي) 60% شموع في 4.60 mm، C18 × 250MM، C18 × 250MM) مع بدقة 1 مل لكل دقيق. وجرى الكشف عن المستخرج في 278 نانومتر. تم العثور على استيقاع السكسيات ميتوبرولول وlasideبين في الأقراط 5.25 ± 0.5 و 6.72 ± 0.5 دقيقة على التوالي. ولم يعثر على أي تدخل من قبل سواغ في مزيج الاصطناعية. وكان المحتوى الحطي للسكسيات ميتوبرولول وlasideبين في نطاق 5–25 μg/ملم و 4–20 μg/ملم على التوالي. المستقر من السكسيات ميتوبرولول وlasideبين وكان 100.03% و 99.7% على التوالي. وكان RSD من 2. ومعنیات الارتباط لجميع المكونات هي قريبة إلى 1. تم التحقق من صحة الطريقة المفترضة من حيث المحتوى الحطي، مدى الدقة، والدقة، والدقة، و هي طريقة ناجحة لتطبيق على تقدير السكسيات ميتوبرولول وlasideبين بمكانيات كبيرة وفي خليط الاصطناعية.

المصطلحات: ميتوبرولول السكسيات، lasideبين، RP-HPLC، التحقق من صحة.


- 202 -