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Method Development and Validation for Simultaneous Estimation of Benidipine Hydrochloride and Metoprolol Succinate in Tablet

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ABSTRACT

Present work focusing in developing and validating a new high performance liquid chromatography method for estimation of Metoprolol Succinate and Benidipine Hydrochloride in their combine tablet dosage form. The method was performed on Shimadzu LC-20AT instrument using C_{18} (250 mm x 4.6 mm, 5 µm) Hypersil BDS Column and Potassium Dihydrogen Phosphate Buffer (pH 4.0): Methanol (65: 35% v/v) as mobile phase at ambient temperature. Detection was carried out at 269 nm. Concentration range 4-12 µg/ml for Benidipine Hydrochloride and 25-75 µg/ml for Metoprolol Succinate . The Percentage recovery of Benidipine Hydrochloride and Metoprrolol succinate was found to be 99.59% and 99.39 respectively. Correlation coefficient for Metoprolol succinate and Benidipine Hydrochloride was found 0.9995 and 0.9997 respectively. The Rt values for Metoprolol succinate and Benidipine Hydrochloride were found to be 3.4 and 5.9 min respectively. The method was validated according to the guidelines of International Conference on Harmonisation (ICH) and was successfully employed in the estimation of commercial formulations.

Keywords: Metoprolol Succinate, Benidipine Hydrochoride, HPLC, Mobile Phase,

INTRODUCTION

Metoprolol succinate is Beta-Blocker class of drug and chemically it is bis 1-[4-(2-methoxyethyl) phenoxy]-3-(propan-2-yl) amino] propan-2-ol ; butanedioic acid. [1,2,3] Benidipine hydrochloride is a long acting dihydropyridine Ca++ channel blocker and chemically it is O5-methyl O3-[(3R)-1-(phenylmethyl)piperidin-3-yl] 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate, hydrochloride. [1,2,4] Structure of Metoprolol succinate is illustrated in figure I. Structure of Benidipine Hydrochloride is illustrated in figure II.

So far, to our present knowledge a literature survey revealed that various methods are reported for the analysis of individual drug and in combination with other drugs but any RP- HPLC method is not reported for these two drugs in combined dosage form.[5-27] Therefore, it was thought worthwhile to Develop and Validate RP-HPLC methods for analysis of Benidipine Hydrochloride and Metoprolol Succinate in its Pharmaceutical Dosage Form.
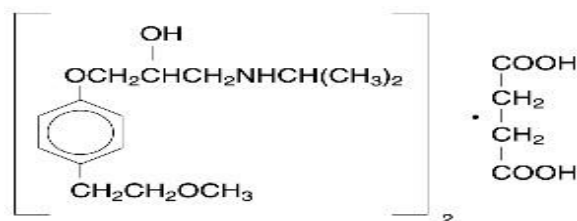

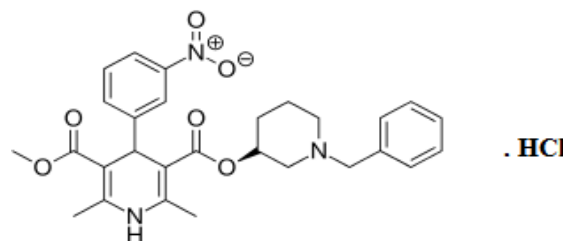Figure I : Structure of Metoprolol Succinate

Figure II : Structure of Benidipine Hydrochloride

MATERIALS AND METHODS

Reagents and Chemicals : Metoprolol succinate was received as a gift sample from Intas Pharmaceuticals Pvt. Ltd , Ahmedabad and Benidipine Hydrochoride was received as a gift sample from Prayosha Healthcare Pvt. Ltd, Ankleshwar. All chemicals and reagents used were a HPLC grade and purchased from Merck specialties Pvt., Ltd., Mumbai. Combined Tablet Formulation (Benipack- M) was procured from Local market.

Preparation of mobile phase:

Mobile phase was prepared by mixing 65 ml of Potassium Dihydrogen Phosphate Buffer (pH 4.0) and 35 ml of methanol and degas in ultrasonic water bath for 15 minutes. Filter through 0.2 μ filter under vacuum filtration before injection.

Chromatographic Conditions:

- Column : C18 (250 mm x 4.6 mm, 5 μm) Hypersil BDS

- Mode of Elution : Isocratic

- Mobile phase : Potassium Dihydrogen Phosphate Buffer (pH 4.0): Methanol (65: 35% v/v)

- Detection Wavelength : 269 nm

- Injection volume : 20 μl

- Flow rate : 1.0 ml/min

- Column Temperature: 25°C

- Run time : 07 minutes

Preparation of Standard stock solution Metoprolol Succinate (50 μg/ml) and Benidipine Hydrochloride (8μg/ml)

Accurately weigh and transfer 50 mg of weighed quantity of Metoprolol Succinate and 8 mg of Benidipine Hydrochloride and dilute it up to 100 ml with Methanol. Further from above solution take 1 ml and make the final volume up to 10 ml with the use of Methanol to produce 50 μg/ml of Metoprolol Succinate and 8μg/ml of Benidipine Hydrochloride .

Preparation of Standard solution of binary mixtures of Metoprolol Succinate (50 μg/mL) and Benidipine HCl (8 μg/mL)

Form standard stock solution take 1 ml from the Metoprolol Succinate Stock solution and 1ml from Benidipine Hydrochloride Stock solution respectively and transferred to 10 ml volumetric flask and volume was made up to the mark by mobile phase.

Selection of Wavelength:

Metoprolol Succinate (50μg/ml) and Benidipine Hydrochloride (8μg/ml) were prepared in Methanol as per

above mentioned procedure. After that these drug solutions were scanned in UV region of 200-400 nm and overlay spectrums were recorded. The observed UV Spectra is mentioned in figure no. III. From the spectra 269nm was selected as a wavelength of detection.
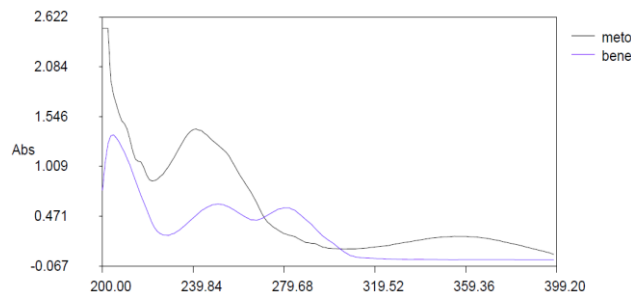

Figure III : Overlay Spectra of Metoprolol Succinate and Benidipine Hydrochloride

Preparation of Calibration Curve : Calibration curves were plotted over a concentration range of 4- 12 μg/ml for Benidipine Hydrochloride and 25-75 μg/ml for Metoprolol Succinate. Accurately measured standard stock solution Volume as 5,7.5,10,12.5,15 ml solutions were pipette out from the Stock solution of Metoprolol Succinate (500 μg/ml) and Benidipine Hydrochloride (80 μg/ml) and transfer to 100 ml volumetric flask and make up with mobile phase to obtain 25,37.5,50,62.5 and 75 μg/ml, and 4,6,8,10 and 12μg/ml for Metoprolol Succinate and Benidipine Hydrochloride respectively.

Preparation of Sample Stock Solution (Benidipine HCl 80 μg/mL, Metoprolol Succinate 500 μg/ml)

10 tablets were weighed and finely powdered. The average weight was calculated. A portion of powder equivalent to the weight of one tablet was accurately weighed and transferred to a 100 ml volumetric flask. (8mg of Benidipine HCl, and 50mg of Metoprolol Succinate). Add 60 ml Mobile phase and Shake for 15 min and make up volume with Mobile phase. The solution was filtered through Whatman filter paper no. 42.

RESULTS AND DISCUSSION:

Method Validation Parameters:

The method was validated by establishing linearity, accuracy, interday and intraday precision of measurement of sample application. The limit of detection and the limit of quantification were also determined. Chromatogram of Metoprolol succinate and Benidipine Hydrochloride in active pharmaceutical ingredients is shown in figure no. IV, V and VI. Chromatogram of Metoprolol succinate and Benidipine Hydrochloride in Tablet dosage form is shown in figure no. VII.
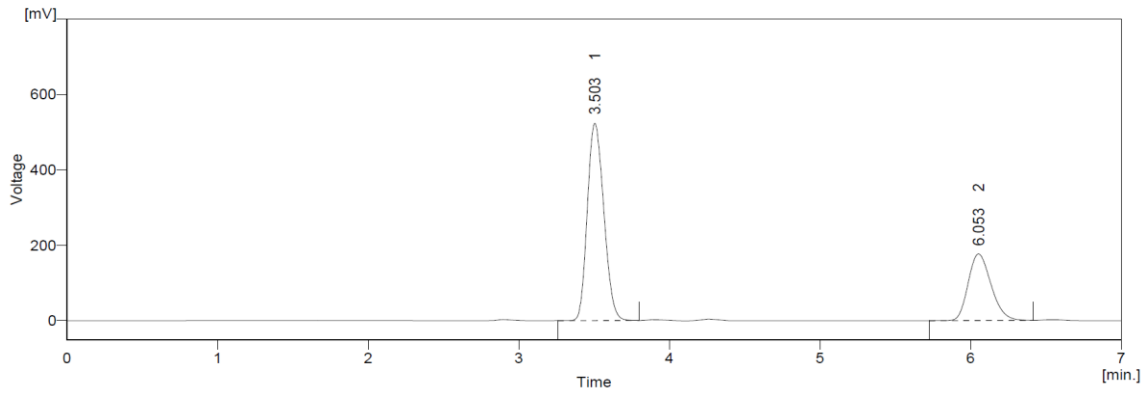
Figure IV : Chromatogram o f Benidipine HCl and Metoprolol Succinate in standard solution.
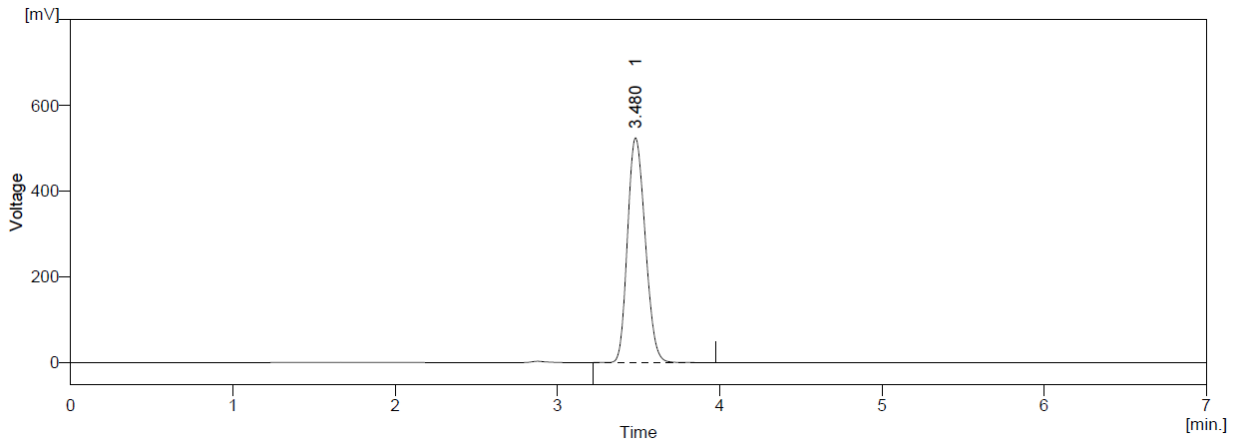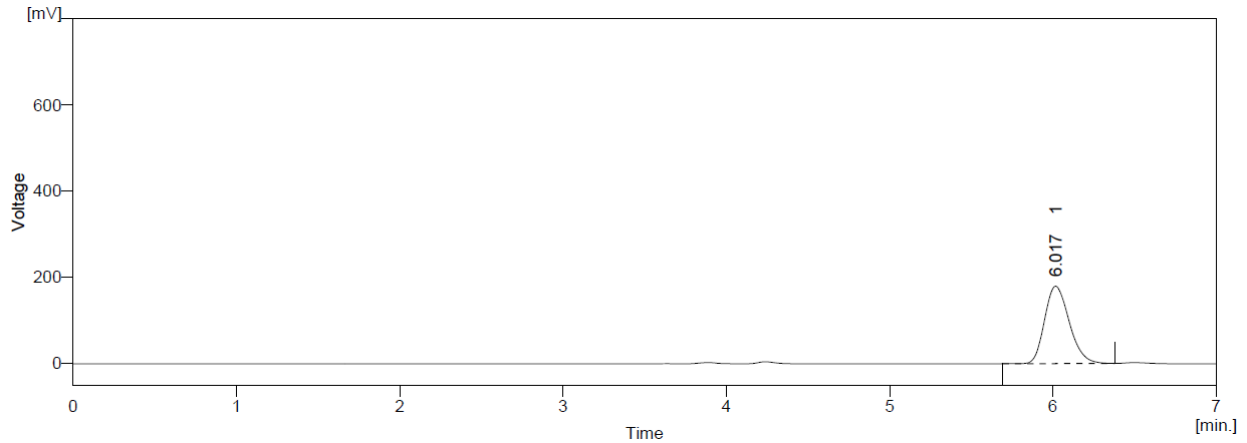

Figure V: Chromatogram of Metoprolol Succinate

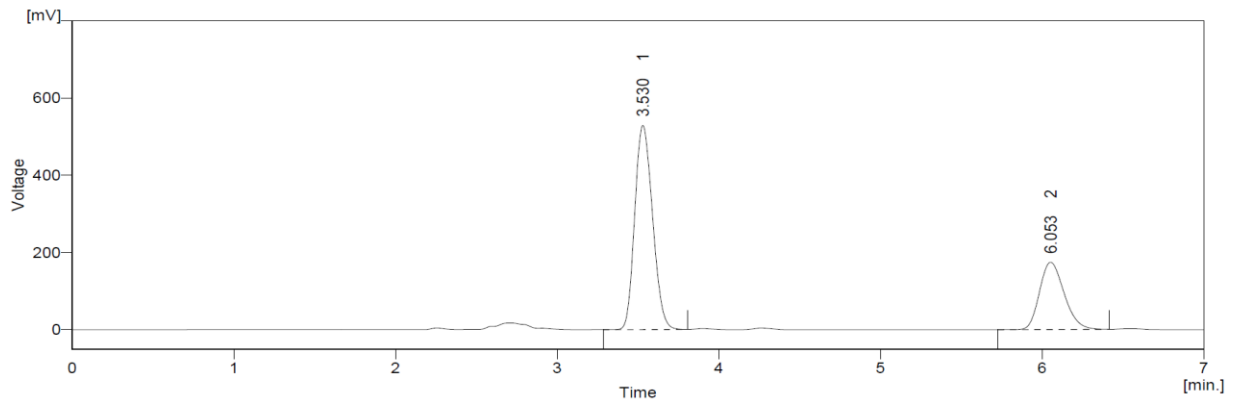Figure VI: Chromatogram of Benidipine HCl

Figure VII : Chromatogram of Benidipine HCl and Metoprolol Succinate in sample solution.

Method Validation Parameters: [28,29]

Linearity:

Calibration curve were found to be linear in the range of 25-75 µg/ml of Metoprolol Succinate and 4-12 µg/ml of Benidipine Hydrochloride .Five concentration points were assayed in triplicate. Both Metoprolol Succinate and Benidipine Hydrochloride showed good linearity in tested range. The regression coefficient (R2) value for Metoprolol Succinate and Benidipine Hydrochoride were found to be 0.9995 and 0.9997 respectively. Linear regression data for the calibration plots are illustrated in figure no VIII and IX respectively. Overlay chromatogram of different concentrations of mixtures of Metoprolol Succinate and Benidipine Hydrochloride is shown in figure no X.
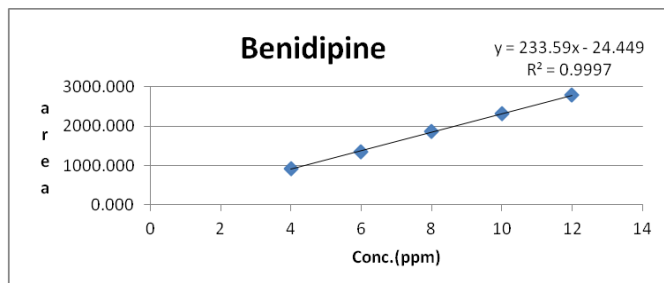

Figure VIII : Calibration Curve o f Benidipine HCl (4-12 Mg/Ml)
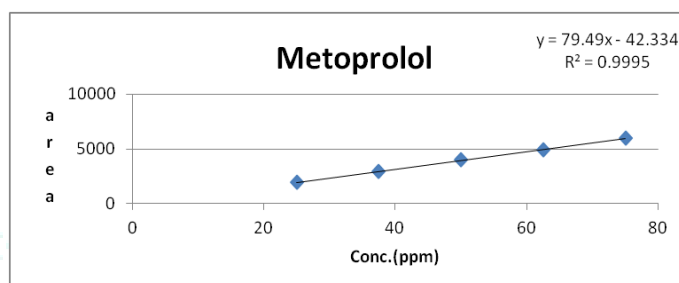
Figure IX : Calibration Curve of Metoprolol Succinate (25-75 MG/ML)
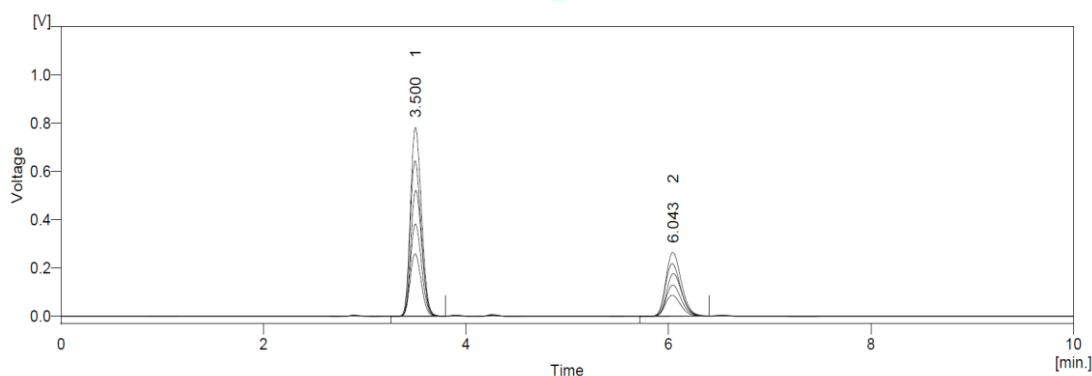
Figure X: Overlay Chromatogram of different concentrations of mixtures of Metoprolol Succinate and Benidipine Hydrochloride

Accuracy: Accuracy may often be expressed as % Recovery by the assay of known, added amount of analyte. It is measure of the exactness of the analytical method. The recovery experiments were carried out in triplicate by spiking previously analyzed samples of the tablets (Metoprolol succinate and Benidipine Hydrochloride) with three different concentrations of standards at 80 %, 100 % and 120% Metoprolol Succinate (20,25 30 µg/ml) and Benidipine Hydrochloride (3.2, 4 ,4.8 µg/ml). The % recovery were calculated and found to be within the limit as shown in table no I.

Table I : % Recovery of Metoprolol Succinate and Benidipine Hydrochloride

	Conc. Level (%)	Sample Amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery
Benidipine Hydrochloride	80%	4	3.2	3.229	100.89
	100%	4	4	4.011	99.59
	120%	4	4.8	4.577	99.105
Metoprolol Succinate	80%	25	20	19.89	99.43
	100%	25	25	24.09	99.39
	120%	25	30	29.89	99.43

Precision : Intraday Precision was found by analysis of standard drug at six times on the same day.While Interday Precision was carried out on six different day. The RSD was found to be less than 2 for both interday precision and intraday Precision. Result for the interday precision and intraday precision is shown in table II , III, IV ,V respectively.

Table II **:** Intraday Precision Data for estimation of Benidipine HCl

	Benidipine HCl		
SR. NO.	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	4	911.282 ± 7.622	0.836
2	8	1834.337 ± 21.617	1.178
3	12	2573.720± 25.122	0.912

Table III : Intraday Precision data for estimation of Metoprolol Succinate

	Metoprolol Succinate		
SR. NO.	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	25	1951.287 ± 13.155	0.674
2	50	3936.533 ± 25.460	0.647
3	75	5900.630 ± 37.377	0.633

Table IV : Interday Precision data for estimation of Benidipine HCl

	Benidipine HCl		
SR. NO.	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	4	908.023 ± 10.533	1.160
2	8	1835.168± 16.643	0.907
3	12	2754.057± 16.371	0.594

Table V : Interday Precision data for estimation of Metoprolol Succinate

	Metoprolol Succinate		
SR. NO.	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	25	1948.001 ± 13.549	0.695
2	50	3922.802± 42.249	1.077
3	75	5900.556 ± 21.806	0.369

LOD and LOQ : Limit of Detection and Limit of Quantitation are calculated based on calibration curve and the results are shown in table. VI

Table VI : Result oF LOD & LOQ for Benidipine HCl & Metoprolol Succinate

S.N.	Name of Drug	LOD	LOQ
1	Metoprolol Succinate	1.738 µg/ml	5.266 µg/ml
2	Benidipine HCl	0.204 µg/ml	0.618 µg/ml

Application of Proposed Method to the Pharmaceutical Dosage Form:

The Proposed method was applied successfully to the tablet dosage form and results obtained are shown in table VII .

Table VII : Result of analysis on marketed formulation

Tablet	Benipack - M	
Label claim	Benidipine HCl (4mg)	Metoprolol Succinate (50mg)
Assay (% of label claim) Mean ± S. D.	98.403±1.196	101.828±0.700

CONCLUSION:

A simple rapid, precise and reliable method was developed for the estimation of the Benidipine HCl and Metoprolol Succinate in combined dosage forms. The results obtained are within the specified limit by the ICH guidelines. Analytical column used and the mobile phase provide good separation and gives the sharp results. The retention time observed for both the drugs was good hence the method can be used for routine analysis in quality control laboratories.

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