



Original Article

Dualwavelength & Q-Absorption Ratio Spectrophotometry Methods Development and Validation for Simultaneous Estimation of Metoprolol Succinate and Chlorthalidone in Bulk and Dosage Form

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A B S T R A C T

To develop and validate a simple & accurate Spectrophotometry methods for simultaneous estimation of Metoprolol Succinate and Chlorthalidone in their combined pharmaceutical dosage form. Two simple, accurate, precise U.V Spectroscopy methods have been developed. First method was based on Dual wavelength method. Here 297 nm & 267.22 nm selected for the estimation of Metoprolol Succinate where Chlorthalidone show same absorbance. Other 281 nm & 268.96nm selected for estimation of Chlorthalidone where Metoprolol Succinate show same absorbance. The second method was the Q-Absorption method, where 263.51 nm (Isoabsorptive point) was selected as λ_1 and λ_2 was selected any one form other two components (275nm Metoprolol Succinate). Metoprolol Succinate and Chlorthalidone showed linearity in the range of 20-100 μ g/ml and 5- 25 μ g/ml respectively in both methods. Both methods were validated by validation parameters and it show result where lie within its acceptance criteria as per ICH Q2 (R1) guideline. Hence, it can be successfully used for the routine analysis of Metoprolol Succinate and Chlorthalidone in their combined pharmaceutical dosage forms.

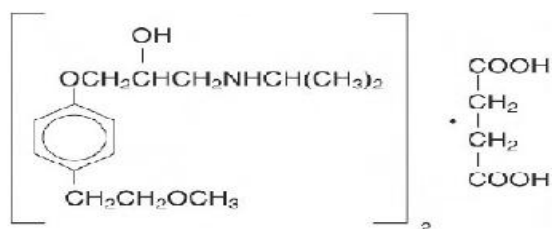
Keywords: Metoprolol Succinate, Chlorthalidone, Methanol and Validation parameter

1. INTRODUCTION

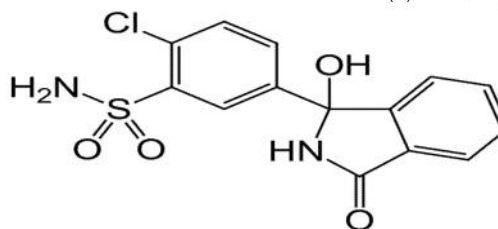
Metoprolol succinate is a β_1 -selective (cardio selective) adrenoceptor blocking agent, for oral

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administration, available as extended-release tablets. TOPROL-XL has been formulated to provide a controlled and predictable release of Metoprolol for once-daily administration. The tablets comprise a multiple unit system containing Metoprolol succinate in a multitude of controlled release pellets. Each pellet acts as a separate drug delivery unit and is designed to deliver Metoprolol continuously over the dosage interval. Its chemical name is (±)1(isopropyl amino)-3-[p-(2-methoxyethyl) phenoxy]-2-propanol succinate (2:1) (salt). Its Molecular formula and Molecular weight are $(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_4$ and 652.8 respectively. With structural formula is:



Metoprolol succinate is a white crystalline powder. It is freely soluble in water, soluble in methanol, sparingly soluble in ethanol, slightly soluble in dichloromethane and 2-propanol, practically insoluble in ethyl-acetate, acetone, diethyl ether and heptane. It is indicated for the treatment of hypertension, to lower blood pressure. Lowering blood pressure lowers the risk of fatal and non-fatal cardiovascular events, primarily strokes and myocardial infarctions. These benefits have been seen in controlled trials of antihypertensive drugs from a wide variety of pharmacologic classes including Metoprolol. Chlorthalidone is an antihypertensive/diuretic tablets for oral use. It is a monosulfamyl diuretic that differs chemically from thiazide diuretics in that a double ring system is incorporated in its structure. It is a racemic mixture of 2-chloro-5-(1-hydroxy-3-oxo-1-isoindolinyl) benzene sulfonamide, with the following structural formula:



Its Molecular formula and Molecular weight are $C_{14}H_{11}ClN_2O_4S$ & 338.766 respectively.¹ Chlorthalidone is practically insoluble in water, in ether and in chloroform, soluble in methanol, slightly soluble in alcohol. Chlorthalidone is a long-acting oral diuretic with antihypertensive activity. Its diuretic action commences a mean of 2.6 hours after dosing and continues for up to 72 hours. The drug produces diuresis with increased excretion of sodium and chloride. The diuretic effects of Chlorthalidone and the Benzothiadiazine (thiazide) diuretics appear to arise from similar mechanisms and the maximal effect of Chlorthalidone and the thiazides appear to be similar. The site of the action appears to be the distal convoluted tubule of the nephron. The diuretic effects of Chlorthalidone lead to decreased extracellular fluid volume, plasma volume, cardiac output, total exchangeable sodium, glomerular filtration rate, and renal plasma flow. Still now some methods publish on Metoprolol succinate and Chlorthalidone¹⁻⁶ but no Dual wavelength and Q-absorption method was publish. So here its aim to be publish these methods and to validated them as per the ICH guideline.²

2. MATERIALS AND METHODS

Instrumentation, Reagents and Material

Jasco UV-1800 UV spectrophotometer, Metoprolol Succinate, Chlorthalidone, Methanol

Dual wavelength method

Determination of wavelength for measurement

4 ml of working standard solution of Metoprolol Succinate (100 µg/ml) and 1ml of working standard of Chlorthalidone (100 µg/ml) was diluted to 10 ml with Methanol to get 40 µg/ml of Metoprolol Succinate and

10 µg/ml of Chlorthalidone. Each solution was scanned between 200-400 nm. The spectra of each solution were obtained. Here 297 nm & 267.22 nm selected for the estimation of Metoprolol Succinate where Chlorthalidone show same absorbance. Other 281 nm & 268.96nm selected for estimation of Chlorthalidone where Metoprolol Succinate show same absorbance. Absorption differences were calculated which shown in figure no. 1.

Preparation of Calibration Curve:

Calibration STD curve for Metoprolol Succinate (20-100 µg/ml)

Calibration curve for Metoprolol Succinate consisted of different concentrations of standard Metoprolol Succinate solution ranging from 20-100 µg/ml. The solutions were prepared by pipetting out 2,4,6,8, & 10 ml of the working standard solution of Metoprolol Succinate (100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with Methanol. Here 297 nm & 267.22 nm selected for the estimation of Metoprolol Succinate where Chlorthalidone show same absorbance. The straight-line equation was determined by putting graph con VS abs. And data was recorded in table no. 1 and figure no. 2- 3.

Calibration STD curve for Chlorthalidone (20-100µg/ml)

Calibration curve for Chlorthalidone consisted of different concentrations of standard Chlorthalidone solution ranging from 5-25 µg/ml. The solutions were prepared by pipetting 0.5, 1, 1.5, 2 & 2.5ml of the working standard solution of Chlorthalidone (100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with Methanol. Here 281 nm & 268.96nm selected for estimation of Chlorthalidone where Metoprolol Succinate show same absorbance The straight-line equation was determined

by putting graph con VS abs. And data was recorded in table no. 1 and figure no. 4- 5.

Validation of proposed method

Linearity

The linearity response was determined by analyzing independent levels of concentrations in the range of 20-100 and 5-25µg/ml for Metoprolol Succinate and Chlorthalidone respectively six times. Absorbance of each solution was measured at selected wavelength. The correlation coefficient and regression line equations for Metoprolol Succinate and Chlorthalidone were determined. Linearity of 6 concentrations was measured six times.

Precision:

Repeatability

6 replicates of 40 µg/ml concentrations of Metoprolol Succinate and 10 µg/ml of Chlorthalidone were prepared and absorbance was measured at selected wavelength. SD and RSD were calculated and recorded in table no. 1.

Intraday Precision

Standard solutions containing 40, 60 & 80 µg/ml Metoprolol Succinate and 10, 15 & 20 µg/ml Chlorthalidone were analyzed 3 times on the same day. Absorbance was measured at selected wavelength. SD and RSD were calculated and recorded in table no. 1.

Interday Precision

Standard solutions containing 40, 60 & 80 µg/ml Metoprolol Succinate and 10, 15 & 20 µg/ml Chlorthalidone were analyzed 3 times on the three different days. Absorbance was measured at selected wavelength. SD and RSD were calculated and recorded in table no. 1.

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. Recovery studies were carried out by addition of standard drug to the pre analysed sample at 3 different concentration levels (80,

100 and 120 %) taking into consideration percentage purity of added bulk drug samples. It was determined by calculating the recovery of Metoprolol Succinate and Chlorthalidone Sodium by standard addition method. Absorbance of spiked samples was measured and total amount of drug was calculated and from which % recovery was calculated and recorded in table no. 1.

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

The LOD & LOQ are estimated from the set of 6 calibration curves used to determine method linearity.

$$\text{LOD} = 3.3 \times (\text{SD} / \text{Slope})$$

$$\text{LOQ} = 10 \times (\text{SD} / \text{Slope})$$

Where, SD = the standard deviation of Y- intercept of 6 calibration curves.

Slope = the mean slope of the 6 calibration curves.

Which are shown in table no 1

Analysis of marketed formulation:

The absorbance of the sample solution was measured at selected wavelength. The concentration of each drug was calculated. This is shown in table no. 2.

Q-ABSORPTION RATIO METHOD

Determination of wavelength for measurement

4 ml of working standard solution of Metoprolol Succinate (100 µg/ml) and 1 ml of working standard of Chlorthalidone (100 µg/ml) was diluted to 10 ml with Methanol to get 40 µg/ml of Metoprolol Succinate and 10 µg/ml of Chlorthalidone. Each solution was scanned between 200-400 nm. From the overlay spectra two wavelengths 263.51 nm & 275 nm was selected as λ_1 and λ_2 for measurement. Where 263.51 nm Isoabsorptive point of two components. Shown in Figure no.6

Preparation of Calibration Curve

Calibration curve for Metoprolol Succinate at 263.51nm and 275 nm (20-100µg/ml)

Calibration curve for Metoprolol Succinate consisted of different concentrations of standard Metoprolol Succinate solution ranging from 20-100µg/ml. The solutions were prepared by pipetting out 2,4,6,8, & 10 ml of the working standard solution of Metoprolol Succinate (100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with Methanol. Then spectra were measured at 263.51 nm & 275nm which show in Figure 7& figure 8 show respectively.

Calibration curve for Chlorthalidone 263.51nm and 275 nm (5-25 µg/ml)

Calibration curve for Chlorthalidone consisted of different concentrations of standard Chlorthalidone solution ranging from 5-25 µg/ml. The solutions were prepared by pipetting 0.5, 1, 1.5,2 & 2.5ml of the working standard solution of Chlorthalidone (100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with Methanol. Then spectra were measured at 263.51 nm & 275nm which show in Figure 9 & figure 10 show respectively.³

Validation of proposed method

Linearity

The linearity response was determined by analyzing independent levels of concentrations in the range of 20-100 and 5-25µg/ml for Metoprolol Succinate and Chlorthalidone respectively six times. Absorbance of solution was measured at 275nm and 263.51nm for Metoprolol Succinate and Chlorthalidone. The correlation coefficient and regression line equations for Metoprolol Succinate and Chlorthalidone were determined. Linearity of 6 concentrations was measured six times.

Precision:

Repeatability

6 replicates of 40 µg/ml concentrations of Metoprolol Succinate and 10 µg/ml of Chlorthalidone were prepared and absorbance was measured at 275 nm &

263.51 nm. SD and RSD were calculated and recorded in table no. 3.

Intraday Precision

Standard solutions containing 20, 40 & 60µg/ml Metoprolol Succinate and 5, 10 and 15µg/ml Chlorthalidone were analyzed 3 times on the same day. Absorbance was measured at 275 nm & 263.51. SD and RSD were calculated and recorded in table no. 3.

Interday Precision

Standard solutions containing 20, 40 & 60µg/ml Metoprolol Succinate and 5, 10 and 15µg/ml Chlorthalidone were analyzed 3 times on the three different days. Absorbance was measured at 275 nm & 263.51 nm. SD and RSD were calculated and recorded in table no. 3.^{4,5}

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

Which are shown in table no 3

Analysis of marketed formulation:

The absorbances of the sample solution i.e. A1 and A2 were recorded at 275 nm (-max of Chlorthalidone) and 263.51nm (isoabsorptive point) respectively. The concentration of each drug was calculated using equation below. This is shown in table no. 4.⁶

The concentration of two drugs in the mixture can be calculated using following equations.

$$CX = [(QM - QY) / (QX - QY)] \times A1/ax1 \dots \dots \dots (1)$$

$$CY = [(QM - QX) / (QY - QX)] \times A1/ay1 \dots \dots \dots (2)$$

Where, A1 and A2 are absorbance of mixture at 275 nm and 263.51 nm; ax1 and ay1 are absorptivity of METOPROLOL SUCCINATE and CHLORTHALIDONE at 275 nm, ax2 and ay2 are absorptivity of METOPROLOL SUCCINATE and CHLORTHALIDONE respectively at 263.51 nm; QM = A2 / A1, QX = ax2 / ax1 and QY = ay2 / ay1.

3. RESULT AND DISCUSSION

DUAL WAVELENGTH

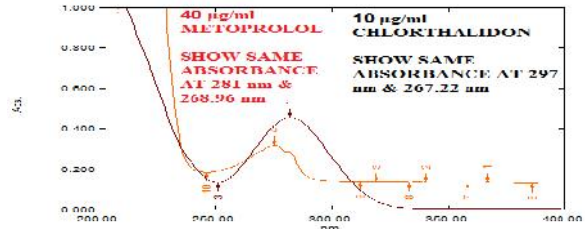


Fig 1: Determination of wavelength

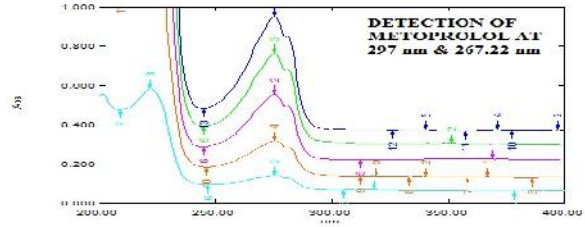


Fig 2: STD curve for Metoprolol Succinate at 297nm & 267.22 nm

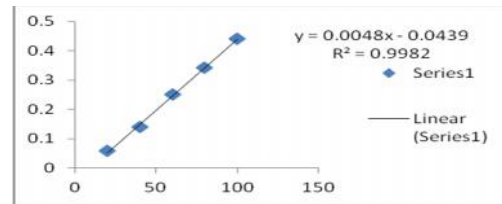


Fig 3: STD curve for Metoprolol Succinate

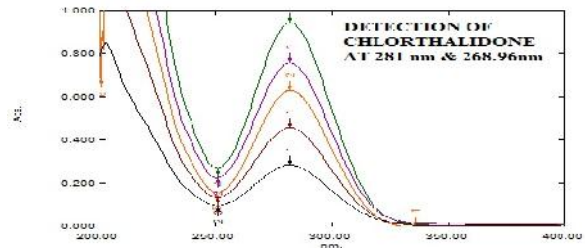


Fig 4: STD curve for Chlorthalidone at 281nm & 268.96 nm

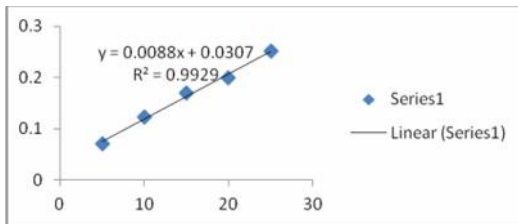


Fig 5: STD curve for Chlorthalidone

Table 1: Summary for Metoprolol Succinate and Chlorthalidone for dual wavelength

PARAMETER	METOPROLOL SUCCINATE	CHLORTHALIDONE
Wavelength (nm)	Absorption diff measure at 297nm & 267.22 nm	Absorption diff measure at 281nm & 268.96 nm
Bear's law limit (µg/ml)	20-100	5-25
STD CURVE		
Regression equation	y = 0.0048x - 0.0439	y = 0.0088x + 0.0307
r ²	0.9982	0.9929
Slope (m)	0.004817	0.00875

Intercept (c)	0.0006919	0.0007581
PRECISION	INTRA DAY	INTRA DAY
	0.139±0.001732	0.122±0.001155
	0.252±0.001	0.172±0.001155
	0.340±0.002	0.202±0.001528
INTERDAY	INTER DAY	INTER DAY
	0.136±0.002	0.122±0.001732
	0.248±0.003055	0.173±0.002
	0.339±0.004041	0.203±0.002517
Repeatability	40 µg/ml	10 µg/ml
LOD	0.139±0.001472	0.122±0.001211
LOQ	0.474	0.2859
	1.43	0.8664

Analysis of marketed formulation

Table 9: Analysis of marketed formulation

Drugs	Label Claim (mg)	Amount Found (mg)	% Label Claim*
Metoprolol Succinate	50 mg	50.10	100%
Chlorthalidone	12.5 mg	12.40	99.2%

Q-ABSORPTION RATIO METHOD

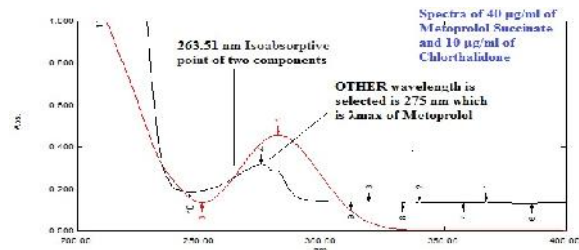


Fig 6: Determination of Iso absorptive point

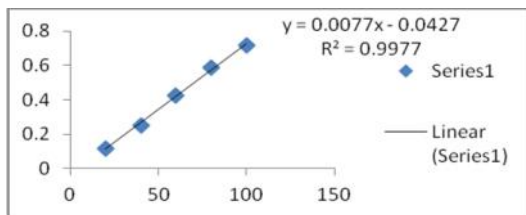


Fig 7: Graph for Metoprolol Succinate at Iso absorptive point 263.51 nm

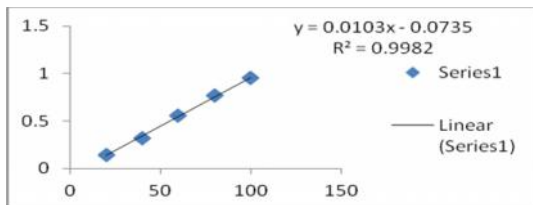


Fig 8: Graph for Metoprolol Succinate at 275 nm

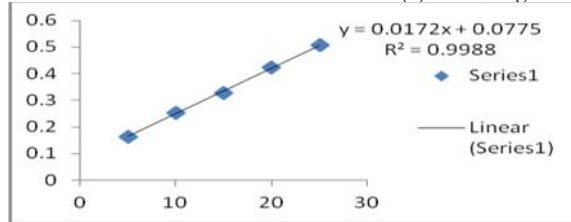


Fig 9: Graph for Chlorthalidone at Iso absorptive point 263.51 nm

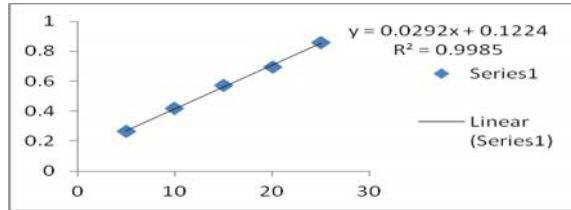


Fig 10: Graph for Chlorthalidone at 275 nm

Table 3: Summary of Q-absorption ratio method

Parameter	Metoprolol succinate	Chlorthalidone	Metoprolol succinate	Chlorthalidone
Wavelength (nm)	275 nm	275 nm	263.51 nm	263.51 nm
Beer's law limit (µg/ml)	20-100	5-25	20-100	5-25
STD CURVE				
Regression equation	y = 0.0103x - 0.0735	y = 0.0292x + 0.1224	y = 0.0077x - 0.0427	y = 0.0172x + 0.0775
r²	0.9982	0.9985	0.9977	0.9988
Slope (m)	0.010317	0.029133	0.0077	0.017233
Intercept (c)	0.001769746	0.001034247	0.000816	0.00188326
PRECISION	INTRA DAY	INTRA DAY	INTRA DAY	INTRA DAY
	0.144±0.001	0.267±0.00153	0.119±0.0015	0.164±0.001528
	0.321±0.0015	0.418±0.00115	28	0.255±0.001155
	3	0.572±0.001	0.251±0.001	0.328±0.001528
	0.555±0.0020		0.424±0.0011	
	8	INTER DAY	5	INTER DAY
		0.262±0.00404		0.162±0.003055
	INTERDAY	0.413±0.00451	INTER DAY	0.250±0.002517
	0.140±0.0025	0.564±0.00503	0.117±0.002	0.323±0.003512
	2		0.245±0.004	
	0.315±0.004		0.418±0.0040	
	0.551±0.0066		4	
	6			
Repeatability	40µg/ml	10 µg/ml	40 µg/ml	10 µg/ml
y	0.318±0.00210	0.4170.0018619	0.247±0.0013	0.253±0.002137
	3		78	
LOD	0.5659	0.1171	0.3414	0.3606
LOQ	1.71	0.3550	1.06	1.092

Table 4: Analysis of marketed formulation

Drugs	Label Claim (mg)	Amount Found (mg)	%Label Claim*
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Metoprolol Succinate	50 mg	49.8	99.6%
Chlorthalidone	12.5 mg	12.68	101.44%

4. CONCLUSION

Two simple & precise UV Spectrophotometric methods have been developed and validated for the estimation of Metoprolol Succinate and Chlorthalidone pharmaceutical dosage form. All method validation parameters lie within its acceptance criteria as per ICH Q2 (R1) guideline so we can conclude that methods are specific, linear, accurate and precise. Hence, it can be successfully used for the routine analysis of Metoprolol Succinate and Chlorthalidone pharmaceutical dosage forms.

5. ACKNOWLEDGEMENT

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6. REFERENCES

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