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Validated UV spectrophotometric method for simultaneous estimation of metoprolol succinate and benidipine hydrochloride in their combined tablet dosage form

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ABSTRACT

Simple, sensitive and economic spectrophotometric method was developed and validated for simultaneous estimation of Metoprolol succinate and Benidipine hydrochloride in tablet dosage form. The analysis was carried out in JASCO V 560 double beam UV Spectrophotometer. In simultaneous equation method, Metoprolol succinate and Benidipine hydrochloride were quantified using their absorptivity values of at selected wavelengths, 223nm and 237nm respectively. Methanol was used as solvent for both drugs. The developed method was validated as per ICH guidelines. The linearity range was found to be 3-21 µg/mL for both drugs with correlation coefficient of 0.9979 and 0.9978 for Metoprolol succinate and Benidipine hydrochloride respectively.Recovery studies were conducted at three different concentration levels within the linearity limits and the average percentage in tablet dosage form was determined and found to be 99.23% for Metoprolol succinate and 99.20 % for Benidipine hydrochloride. So the developed simultaneous equation method permits simple, rapid and direct determination of Metoprolol succinate and Benidipine hydrochloride in commercially available tablet dosage form without previous separation and can therefore be used for routine analysis of both drugs in quality control laboratories.

INTRODUCTION

Metoprolol succinate is a selective adrenergic beta-1 blocking agent that is commonly used to treat angina pectoris, hypertension and cardiac arrhythmias. Its molecular formula is C□□$H_{56}N_2O_{10}$[1].Metoprolol blocks β1 adrenergic receptors in heart muscle cells, thereby decreasing the slope of phase 4 in the nodal action potential (reducing Na^+ uptake) and prolonging repolarization of phase 3 (slowing down K^+ release)[2].

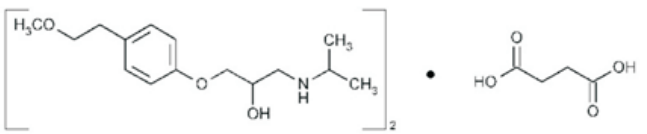

Figure 1 : Structure of Metoprolol succinate

Benidipine also known as Benidipinum or Benidipine hydrochloride is a calcium channel blocker for the treatment of high blood pressure (hypertension). It is a triple L-, T- and N-type calcium channel blocker. Its molecular formula is $C_{28}H_{32}ClN_3O_6$[3,4]. The vasorelaxant effect of Benidipine is due to its affinity towards dihydropyridine binding sites in calcium channels. Binding of Benidipine with calcium channels inhibits calcium current. The onset of action is slow, which results in minimal tachycardia or palpitation[5,6].

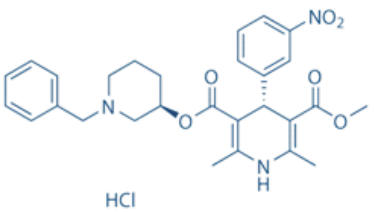

Figure 2 : Structure of Benidipine hydrochloride

Analytical method development and validation play important roles in the discovery, development and manufacture of pharmaceuticals[7]. As per the literature review, there are no analytical methods are reported for the simultaneous estimation of Metoprolol succinate and Benidipine hydrochloride in combined pharmaceutical dosage form. Various publications are available regarding the UV simultaneous estimation, HPTLC and RP-HPLC method development of Metoprololsuccinate[8-12], either alone or in combination with other drugs in pharmaceutical dosage form. But there are a few methods developed for the estimation of Benidipinehydrochloride[13-16] in its single dosage form and in combination with other drugs.

Objective was to develop and validate UV spectrophotometric method for the estimation of Benidipine hydrochloride and Metoprolol succinate in the combined tablet dosage form by UVSimultaneous equation method

MATERIALS AND METHODS

Instrumentation

- JASCO V 560 double beam UV Spectrophotometer.
- Shimadzu analytical balance.
- GT sonic, professional ultrasonic cleaner.

Chemicals and reagents

- Benidipine hydrochloride RS from Pure Chem Pvt. Ltd.
- Metoprolol succinate RS from Unichem Laboratories.
- Methanol HPLC grade from Merck specialties (P) Ltd Mumbai.
- BENIDIN M 25 (containing Benidipine hydrochloride 4mg and Metoprolol succinate 25mg), manufactured by Mascot Health Series Pvt. Ltd.

Preparation of standard solution

Accurately weighed 10mg of Benidipine hydrochloride RS and Metoprolol succinate RS and quantitatively transferred into 100mL standard flask. It was then dissolved in methanol and the solution was made up to the mark using methanol to obtain a concentration of 100µg/mL of both Benidipineand Metoprolol(solution A). From the solution A 1 mLwas pipetted out to a 10mL standard flask and made up the volume with methanol. The solution had a concentration of 10µg/mL of Benidipine and Metoprolol (solution B).

Preparation of sample solution

Twenty tablets of BENIDIN M 25 (containing 25mg of Metoprolol succinate and 4mg of Benidipine hydrochloride) were weighed; average weight of one tablet was calculated and finely powdered with the help of a mortar and pestle. A quantity of powder equivalent to 100mg of Metoprolol succinate (containing 16mg of Benidipine hydrochloride) was weighed accurately and transferred to a glass stoppered flask. The powder was extracted initially with 15mL methanol by sonication for 10 minutes and filtered through Whatmann No.1 filter paper to a 100mL standard flask. The residue was further extracted twice with 10mL of methanol and transferred to the same standard flask through the same filter paper. The volume was finally made up to the mark using methanol. The resulting solution had a concentration of 1000µg/mL of Metoprolol succinate and 160µg/mL of Benidipine hydrochloride. From the above solution, accurately pipetted out 2mL and transferred to 100mL standard flask. Then the volume was made up to the mark using methanol to obtain a concentration of 3.2µg/mL of Benidipine hydrochloride and 20µg/mL of Metoprolol succinate.

Simultaneous Equation Method[17-25]

Two wavelengths selected for the method are 223 nm and 237 nm that are absorption maxima's of Metoprolol succinate and Benidipine hydrochloride respectively in methanol. The stock solutions of both drugs were further diluted separately with methanol to get a series of standard solution of 3-21µg/ml of Metoprolol succinate and Benidipine hydrochloride. The absorbance were measured at the selected wavelengths and absorptivity's (A1%, 1cm) for both the drugs at both wavelengths were determined as mean of six independent determinations. Concentrations in the sample were obtained by using following equations[15].

$$C_x = \frac{A_2 a_{y1} - A1 a y2}{a_{x2} a_{y1} - a x1 a y2} \ldots\text{Eq. (i)}$$

$$C_y = \frac{A_1 a_{x2} - A2 a x1}{a_{x2} a_{y1} - a x1 a y2} \quad \ldots\text{Eq. (ii)}$$

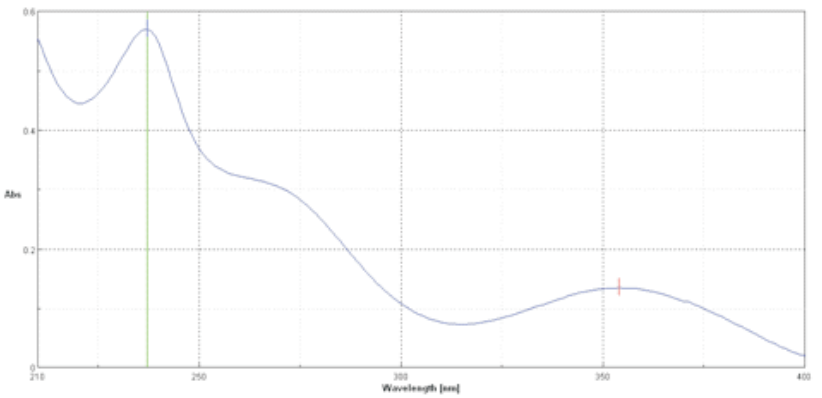

Figure 3 : UV absorption spectrum of Benidipine hydrochloride RS in methanol with absorption maximum at 237nm

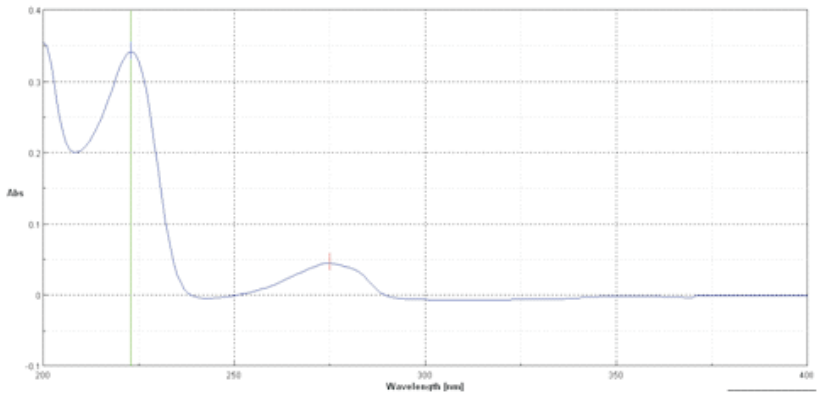

Figure 4 : UV absorption spectrum of Metoprolol succinate RS in methanol with absorption maximum at 223nm

WhereA_1 and $A_2$are absorbance's of sample at 223nm and 237nm respectively, ax_1 and $ax_2$are absorptivity's of Metoprolol succinate at 223nm and 237nm respectively, ay_1 and $ay_2$are absorptivity's of Benidipine hydrochloride at 223nm and 237nm respectively. $C_x$and C_y are the concentrations of Metoprolol succinate and Benidipine hydrochloride respectively in the diluted sample.

RESULTS

After stabilizing the instrument initially for 30 minutes, blank correction was done using methanol. Then 10µg/mL solutions of both Benidipine hydrochloride and Metoprolol succinate were scanned separately in UV region ranging from 200nm to 400nm. The absorption spectra were observed with maximum absorption at 237nm and 223nm for Benidipine hydrochloride and Metoprolol succinate respectively. The spectra obtained are given below.

METHOD VALIDATION

The developed method was validated as per ICH guidelines for Linearity, Precision, Accuracy, Limit of detection and quantitation by the following procedure.

1. Linearity

Suitable dilutions were made from the standard stock solutions containing 100µg/mL of Benidipine hydrochloride and Metoprolol succinate to prepare standard seven different concentrations ranging from 3-21µg/mL for both drugs. Absorbance of each standard solution was measured. The absorbance was plotted against concentration to get calibration curve. The plots of absorbance v/s concentration of Benidipine were found to be linear in the range of 3-21µg/mL with a correlation coefficient r^2 of 0.9981 at 223nm and 0.9978 at 237nm. The plots of absorbance v/s concentration of Metoprolol were found to be linear in the range of 3-21µg/mL with a correlation coefficient r^2 of 0.9979 at 223nm and 0.9954 at 237nm.

Table 1 : Absorbance of Benidipineand Metoprololstandard solution

Sl. No.	Concentration (µg/mL)	Absorbance BENIDIPINE		Absorbance METOPROLOL	
		223nm	237nm	223 nm	237 nm
1	3	0.1843	0.1950	0.1140	0.0125
2	6	0.2852	0.3356	0.2202	0.0161
3	9	0.3761	0.4844	0.3122	0.0203
4	12	0.4956	0.6320	0.4131	0.0202
5	15	0.5921	0.8280	0.5093	0.0262
6	18	0.6708	0.9504	0.6284	0.0292
7	21	0.7702	1.0884	0.6930	0.0321

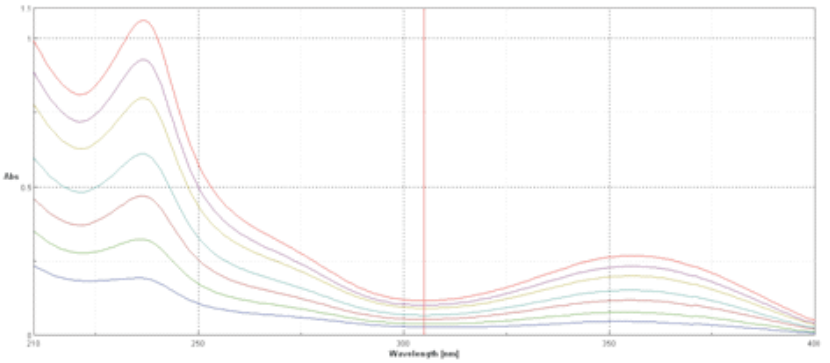

Figure 5 : Zero order spectra of Benidipine hydrochloride overlaid

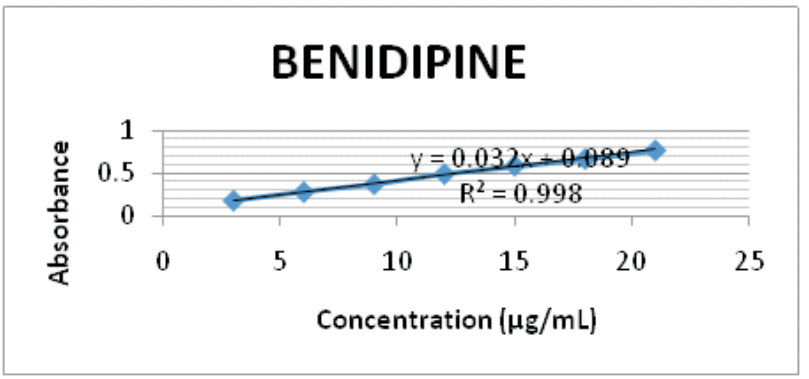

Figure 6 : Calibration plot of Benidipine hydrochloride at 223nm

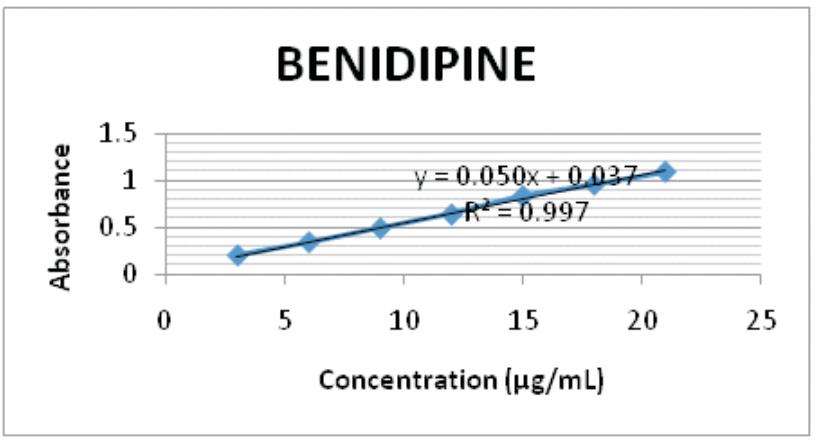

Figure 7 : Calibration plot of Benidipine hydrochloride at 237nm

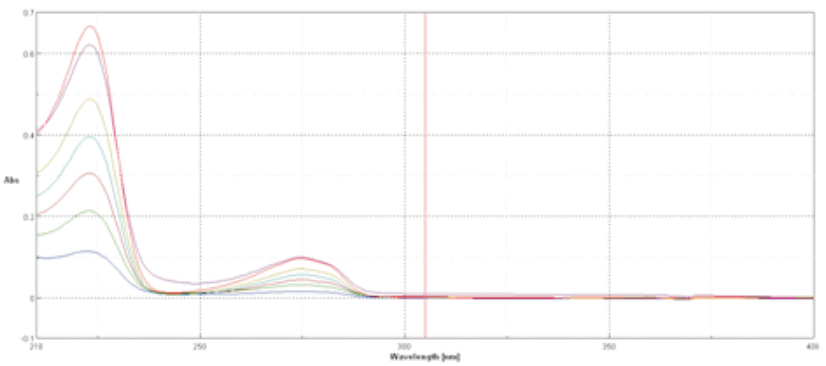

Figure 8 : Zero order spectra of Metoprolol succinate overlaid

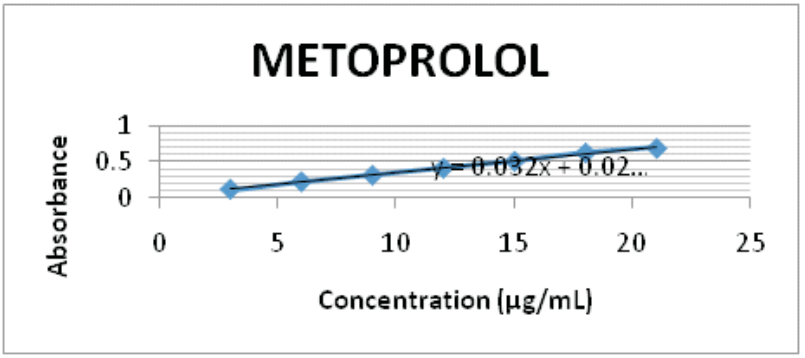

Figure 9 : Calibration plot of Metoprolol succinate at 223nm

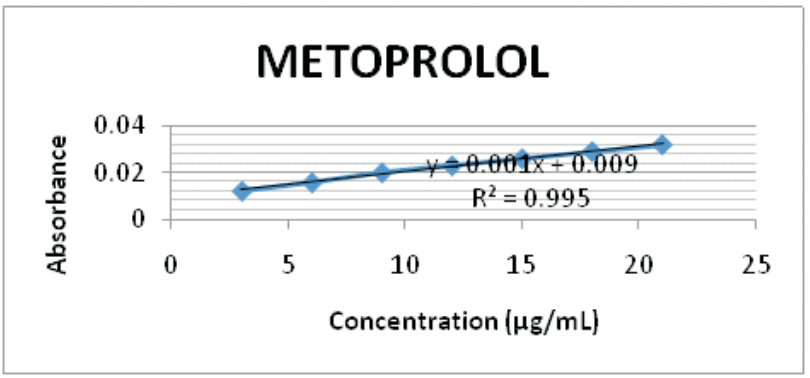

Figure 10 : Calibration plot of Metoprolol succinate at 237nm

2. Precision

Precision was determined in two levels- repeatability and intermediate precision

Repeatability of the method was checked by taking replicate samples of tablet solution for six times on the same day as intraday precision. The inter day precision was carried out by estimating the corresponding responses in triplicate for three days. (Table 2, 3 & 4)

3. Accuracy

Accuracy of the method was determined by recovery studies. To the formulation, the reference standards of the drugs were added at the level of 15%, 20% and 25%. The recovery studies were carried out three times and the percentage recovery were calculated. (Table 5)

4. Limit of Detection and Quantitation (LOD and LOQ)

LOD and LOQ were determined by linearity curve method and by using the equations.

LOD= 3.3(σ/S)

LOQ=10(σ/S)

Where σ is the standard deviation of the y-intercept and S is the slope of the linearity curve. (Table 6)

DISCUSSION

This research work was done to develop simple, accurate and economic UV method for the simultaneous estimation of Benidipine hydrochloride and Metoprolol succinate in tablet

Table 2 : Result of Repeatability study Statistical validation data

Components	Mean of % label claim	Standard deviation (SD)	Relative standard deviation (%RSD)	Coefficient of variation (CV)
Benidipine	99.23%	0.1127	0.1136	0.0011
Metoprolol	99.20%	0.0754	0.0760	0.0007

Table 3 : Result of Intermediate precision study - Statistical validation

Components	Mean of % Label claim (n=1)	Standard deviation (SD)	Relative standard deviation (%RSD)	Coefficient of variation
Benidipine	99.15	0.1152	0.1162	0.0012
Metoprolol	99.21	0.0696	0.0702	0.0007

Table 4 : Assay results of tablet

Sl. No.	Amount present (Label Claim) mg/tablet		Amount obtained mg/tablet		Percentage label claim	
	Benidipine	Metoprolol	Benidipine	Metoprolol	Benidipine	Metoprolol
1	4	25	3.9615	24.8082	99.04	99.23
2	4	25	3.9709	24.7925	99.27	99.17
3	4	25	3.9666	24.7910	99.17	99.16
4	4	25	3.9691	24.8206	99.23	99.28
5	4	25	3.9765	24.7717	99.41	99.08
6	4	25	3.9734	24.8260	99.28	99.30

Table 5 : Result of Recovery study-statistical validation data

Level of % recovery	Mean % recovery		Standard deviation		%RSD		Coefficient of variation	
	BENI	METO	BENI	METO	BENI	METO	BENI	METO
15%	100.3	99.58	0.0816	0.3429	0.0813	0.3443	0.0008	0.0034
20%	99.75	99.37	0.0816	0.2531	0.0818	0.2547	0.0008	0.0025
25%	99.80	101.25	0.0326	0.2041	0.0326	0.2015	0.0003	0.0020

Table 6 : LOD and LOQ results

Method parameters	Benidipine		Metoprolol	
	223nm	237nm	223nm	237nm
LOD (µg/mL)	0.0101	0.0046	0.0010	0.0282
LOQ (µg/mL)	0.0306	0.0138	0.0300	0.0855

Table 7 : SUMMARY OF RESULTS

PARAMETERS	RESULT	
	BENIDIPINE	METOPROLOL
λ_{max}	237nm	223nm
Linearity range (µg/mL)	3-21	3-21
Regression equation ✓ 237nm ✓ 223nm	 Y=0.0506x+0.0372 Y=0.0327x+0.0899	 Y=0.0011x+0.0098 Y=0.0327x+0.02
Correlation coefficient ✓ 237nm ✓ 223nm	 0.9978 0.9981	 0.9954 0.9979
Accuracy (%RSD) ✓ 15% ✓ 20% ✓ 25%	 0.3443 0.2547 0.2015	 0.0813 0.0818 0.0326
Precision (%RSD) ✓ Repeatability ✓ Inter day	 0.1136 0.1162	 0.0760 0.0702
% Label claim	99.23	99.20
LOD (µg/mL)	0.004	0.0010
LOQ (µg/mL)	0.0138	0.0300

dosage form. The two drugs were soluble in methanol and also gave excellent UV detection in methanol. So methanol was chosen as the desirable solvent for simultaneous estimation of these drugs. Benidipine hydrochloride in methanol showed a sharp peak at 237nm and Metoprolol succinate gave a single sharp peak at 223nm. Both absorption maxima wavelengths were employed for further spectrophotometric measurements. In the quantitative assay of two components (Benidipine and Metoprolol) in the mixture by Simultaneous equation method, absorbance are measured at two wavelength, one being the wavelength at which Benidipine shows maximum absorbance, that is at 237nm, one being the wavelength at which Metoprolol shows maximum absorbance, that is at 223nm. Calibration graphs were plotted for each drug. For both Metoprolol and Benidipine a linear relationship between concentration and absorbance was observed for 3-21 µg/mL.BNIDIM M 25, a marketed formulation (containing Benidipine hydrochloride 4mg and Metoprolol succinate 25mg) was analyzed by the proposed method and the result obtained was:

- Amount of Benidipine hydrochloride= 0.0039g.
- Amount of Metoprolol succinate= 0.0248g.
- Percentage label claim of Benidipine hydrochloride= 99.23% W/W.
- Percentage label claim of Metoprolol succinate= 99.20% W/W.

The validation of the propose method was performed in accordance with IH guidelines (Q2B Validation for analytical

procedures: Methodology).The accuracy of the method was checked by recovery studies at three levels i.e., at 15%, 20% and 25%.The precision of the proposed method was done by repeatability and intermediate precision study. The %RSD of the method was found to be <2.The linearity was obtained in the concentration range of 3-21µg/mL for both Benidipine and Metoprolol.The proposed method was found to be accurate and precise.

CONCLUSION

The proposed UV spectrophotometric method enable the simultaneous determination of Benidiine hydrochlorideand Metoprolol succinate in their binary mixture with good accuracy and precision, either in laboratory prepared samples or in combined dosage form.The method was successfully applied for determination of Benidipine and Metoprolol in its pharmaceutical formulations, as no interference was observed due to excipients or other components present. Hence, this method can be conveniently used for routine quality control analysis of Benidipine and Metoprolol in their pharmaceutical formulations.

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