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DEVELOPMENT AND VALIDATION OF CILNIDIPINE AND METOPROLOL SUCCINATE IN BULK AND MARKETED FORMULATIONS BY UV SPECTROSCOPIC METHOD

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ABSTRACT

A specific, accurate, precise, robust, and cost-effective UV method was developed and validated for quantitative analysis of Cilnidipine (CLD) and Metoprolol Succinate (MTS) in alone and marketed dosage forms using Medium I (Methanol: Phosphate buffer pH 6.8 (3:1) as solvent blend. The λ max for CLD and MTS was found to be 242 nm and 223 nm respectively. Linearity was observed in the concentration range of 1-10 µg/ml for Cilnidipine and 1-10 µg/ml for Metoprolol Succinate. The accuracy of method was assessed by recovery studies and was found to be within acceptable range for both CLD and MTS. The developed Medium I was validated with respect to linearity, accuracy (recovery) and precision. The results were validated statistically as per ICH guidelines and were found to be satisfactory. The Medium I can be successfully applied for the determination of CLD and MTS in alone and marketed samples.

KEYWORDS: Cilnidipine, Metoprolol Succinate, Q-analysis, UV spectrophotometry, Validation, Precision.

INTRODUCTION

Hypertension is a condition in which blood pressure is elevated to an extent where benefit is obtained from blood pressure lowering. Calcium channel blockers (CCB) are first-line drugs in the treatment of hypertension. However, CCB alone was insufficient in lowering blood pressure. Hence, CCBs have been widely co-administered with beta-selective adrenoceptor blocking agents to treat hypertension. These drugs act by inhibiting calcium (Ca)-channels in the myocardium and vascular smooth muscle cells, which lowers the myocardial contractions, decrease pulse conduction, and causes vasodilation and selectively blocks cardiac β1-adrenergic receptors with little activity against β2-adrenergic receptors in the lungs and vascular smooth muscle. Thus, they are found to be effective in the treatment of essential hypertension. Furthermore, among the three main classes of CCBs, 1,4-dihydropyridines (DHP) have contributed to a widely used hypotensive drug class. Among various 1,4-dihydropyridine CCBs, Cilnidipine (CLD) shows unique action on sympathetic N-type Ca-channels, besides acting on L-type Ca-channels, as with most Ca-channel antagonists. Their action is performed through vasodilatation, decreased heart rate, and increased renal blood flow. Metoprolol succinate act by mechanism of competitive antagonism of catecholamines at peripheral (especially cardiac) adrenergic neuron sites, leading to decreased cardiac output and central effect leading to reduced sympathetic outflow to the periphery suppression of renin activity.

Cilnidipine (CLD) is chemically described as 2-Methoxyethyl (2E)-3-Phenyl- 2-propen-1-yl-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydro-3,5- pyridine dicarboxylate as shown in figure 1(a). CLD is a light yellow-coloured crystalline powder, insoluble in water has the molecular weight of CLD is 492.528 g/mol. Japanese Pharmacopeia and Indian Pharmacopeia approved CLD in 2016 and 2018 respectively. The identification method for CLD includes Ultraviolet (UV)/ Visible (Vis)-spectrophotometry and infrared spectrophotometry. A thorough literature survey reveals that there were few analytical methods reported for the determination of CLD in bulk, pharmaceutical preparations and in biological fluids which include, Visible spectrophotometric method,[1] spectrophotometric methods.[2-5] RP-HPLC,[6-8] HPTLC,[9] HPLC with tandem mass spectrometry.[10] LCMS.[11] Metoprolol succinate (MTS) is a beta-selective adrenoceptor blocking agent. Use for the treatment of broad-spectrum cardiovascular disorders1.[12] MTS is official in united states (USP) and British pharmacopoeia.[13,14] Chemically MTS is a 1-(isopropyl amino)-3-[4-(2-methoxyethyl) phenoxyl]-2-

propanol succinate.[15] as shown in figure 1(b). It is white crystalline powder with a molecular weight 652, freely soluble in water, methanol and sparingly soluble in 2-propanol.[16] Literature survey reveals that a few HPLC methods, UV spectroscopy, and LCMS method has been used.[17,19]

In present study simple, rapid, cost effective and reproducible UV spectroscopic method was developed for the quantification of CLD and MTS in API and marketed tablets in alone and combination. The developed methods were optimized and validated as per the guidelines of International Conference on Harmonization (ICH) and demonstrated excellent specificity, linearity, precision and accuracy for CLD and MTS.

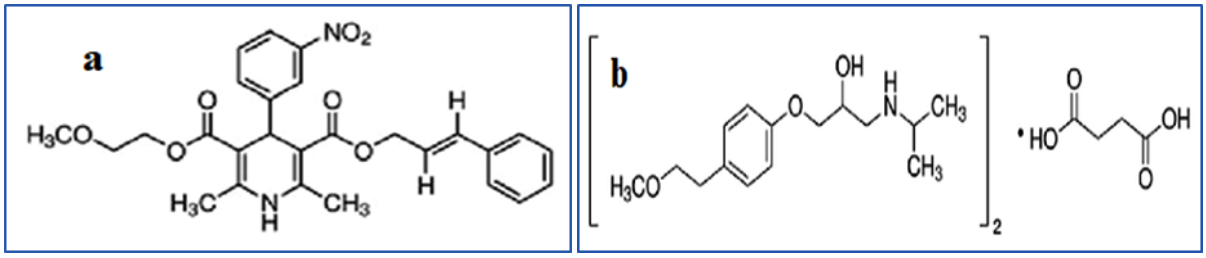

Figure 1: (a) Chemical structure of Cilnidipine (b) Chemical structure of Metoprolol succinate.

MATERIALS

Cilnidipine (CLD) and Metoprolol Succinate (MTS) obtained as gift sample Magnus Pharma Ltd, Birgunj, Nepal. Nescital 10 mg (Alkems Drugs and Pharmaceuticals Ltd., Uttarakhand, India) and Cilnidipine tablets (Cilindia 10; Cilizex 10) and Metoprolol succinate tablets (MTXL 50; Starpress XL 25) procured from community pharmacy. All reagents, solvents used were of analytical grade (SD Fine-Chemicals, Bangalore, India). Spectrophotometric measurements were performed by using a double beam UV–visible Spectrophotometer (SHIMADZU, Japan Model, UV-1900 Pharma Spec), double beam UV spectrophotometer connected to a compatible computer and supported with UV Probe software.

METHODS

Solvent blend (Medium I) viz., Methanol: Phosphate buffer pH 6.8 (3:1) was chosen for the quantification of cilnidipine and metoprolol succinate in marketed tablet dosage forms.

Preparation of CLD and MTS standard stock solution

Transfer accurately weighed 50 mg of CLD into a 50 ml volumetric flask to this add 40 ml of Medium I, shake for 5 min and sonicate for 10 min to dissolve completely, then make the volume to 50 ml with Medium I, similarly prepare MTS standard stock solution using Medium I to obtain 1mg/ml (1000 µg/ml) solution respectively.

Preparation of CLD and MTS working standard solution

Pipette out 5 ml of both standard stock solutions separately into a two 50 ml volumetric flask, make up the volume with the Medium I to obtain 0.1 mg/ml (100 µg/ml) solution of CLD and MTS respectively.

Method development

Determination of absorption maxima

Appropriately dilute the CLD and MTS working standard solution separately in 10 ml volumetric flask to get 10 µg/ml solution with Medium I. Scan both the solutions in the range of 200 to 400 nm using double beam UV-Visible Spectrophotometer (SHIMADZU, Japan Model, UV-1900 Pharma Spec) and observe the characteristic peak at standard wavelength (nm). The absorption maxima were observed at 242 nm for CLD and 223 nm for MTS and same were used for determine the range and linearity.

Range

In order to describe Beers law, appropriately dilute the CLD and MTS working standard solution with Medium I in a series of 10 ml volumetric flask to obtain 1- 40 µg/ml concentrations. Measure the absorbance of these solutions respectively at 242 nm for CLD and 223 nm for MTS to find out the range.

Linearity

Appropriately dilute CLD and MTS working standard solutions separately with Medium I in a series of 10 ml volumetric flask to obtain 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 µg/ml concentrations of CLD and MTS respectively. Measure the absorbance of these solutions at 242 nm for CLD and 223 nm for MTS keeping Medium I as blank. Plot the concentration vs absorbance curve for CLD and MTS separately and analyzed statistically.

Validation

The validation of proposed methods carried out as per ICH guideline.[20-22]

Limit of detection (LOD) and Limit of quantitation (LOQ)

Appropriately dilute the working standard solutions of CLD and MTS in Medium I separately in a series of 10 ml volumetric flask to obtain 0.1 to 1 µg/ml. measure the

absorbance of these solutions to find lowest amount of drug can be detected and quantified. The LOD and LOQ were calculated using appropriate equations with a suitable precision and accuracy. Both LOD and LOQ for CLD and MTS in Medium I are determined based on standard deviation (SD) of response and slope (S) by using the following equations.

(LoD = 3.3 x SD/S); (LoQ = 10 x SD/S)

Precision

Precision of Medium I was carried out by diluting appropriately working standard solutions of CLD and MTS separately in 10 ml volumetric flask with medium I to get 8 µg/ml concentration. Measure the absorbance of solutions and the results were expressed in terms of percentage recovery and % RSD. Further interday and intraday precision studies carried out for the same concentrations and express the results in terms of recovery and % RSD. In each six replicates were studied.

Accuracy

The accuracy of analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or as an accepted true value. The most common technique for determining accuracy in analytical method development studies is the recovery method, recovery defined as the ratio of the observed result to the expected result expressed as a percentage recovery and % RSD. From the sample preparation recovery studies are carried out for marketed tablets to find the drug content of CLD and MTS using Medium I.

For marketed CLD tablets (Cilindia 10; Cilizex 10): 5 Mkt tablets were weighed and ground into fine powder. Powder equivalent to 25 mg of CLD was transferred into a 25 ml volumetric flask, add 20 ml of Medium I and shake it on rotary shaker for 2 hr followed by sonication for 20 min, make up the volume to 25 ml with Medium I filter through 0.45µ filter. Transfer approximately 2.5 ml of filtrate into 25 ml volumetric flask and make up the volume with Medium I and used for analysis.

For marketed MTS tablets (MTXL 50; Starpress XL 25): 5 Mkt tablets were weighed and ground into fine powder. Powder equivalent to 25 mg of MTS was transferred into a 25 ml volumetric flask, add 20 ml of Medium I and shake it on rotary shaker for 2 hr followed by sonication for 20 min, make up the volume to 25 ml with Medium I filter through 0.45µ filter. Transfer approximately 2.5 ml of filtrate into 25 ml volumetric flask and make up the volume with Medium I and used for further analysis.

Further Accuracy studies performed at three different levels (40%, 80% and 120%) by standard addition method and the samples were analyzed in triplicate by the Medium I. In both the case known amount of standard CLD and MTS at 40%, 80% and 120% of predetermined samples was added to a prequantified tablet samples. The result expressed as a percentage recovery and % RSD.

Robustness

Robustness study performs to check the influence of method parameters varied intentionally on the proposed method results. Robustness of Medium I was carried out by diluting appropriately working standard solutions of CLD and MTS separately in 10 ml volumetric flask with medium I to get 8 µg/ml concentration. Change in the experimental parameter viz., varied wavelength ± 5 nm and determine the recovery and interpret the results in terms of % RSD.

Ruggedness

A ruggedness study performs to check the influence of process parameters varied intentionally on the Medium I viz., different analyst and different UV instrument. Ruggedness of Medium I was carried out by diluting appropriately working standard solutions of CLD and MTS separately in 10 ml volumetric flask with medium I to get 8 µg/ml concentration, determine the recovery and interpret the results in terms of % RSD.

Solution stability

The stability of stock solutions of respective CLD and MTS in Medium I studied at different temperature (45°C) and refrigerated temperature (2-8°C). Stuides on Medium I was carried out by diluting appropriately working standard solutions of CLD and MTS separately in 10 ml volumetric flask with medium I to get 8 µg/ml concentration. The samples were stored in tightly sealed glass containers at stated temperature, absorbances of solutions were measured at 24 and 48 hr time interval and the results in terms of % RSD.

RESULTS AND DISCUSSION

Method development

The absorption maxima were found to be 242 nm for CLD and 223 nm for MTS with characteristic peak as shown in figure 2 (a) for CLD and (b) for MTS. The beers range was found to be 1-40 µg/ml range, within range linearity curves for CLD and MTS were constructed at the concentrations of 1-10 µg/ml as shown in figure 3 (a) for CLD (b) for MTS, data was given in table 1 relative data viz., Beer's law range, molar absorptivity, best fit values, regression model fit equation relative statistical data was given in table 2. The linearity is the ability of analytical procedure to produce test results, which are proportional to the concentration (amount) of analyte in samples within a given concentration range. A linear relationship found in the concentration range of 1-10 µg/ml for Medium I. The goodness of fit study suggests good correlation coefficient (R^2 - 0.9997 and 0.9999 for CLD and MTS) shows the validity of Beer's law with intercept response < 2% calculated by the least square method indicating functional linearity between the concentration of analyte and the absorbance. Based on standard deviation of the response and slope, the LOD values for CLD and MTS

for Medium I found to be 0.0363 ± 0.00133 µg/ml, 0.0495 ± 0.03675 µg/ml, and limit of quantitation values found to be 0.011 ± 0.000133 µg/ml, 0.015 ± 0.03675 µg/ml with % RSD values less than 2.

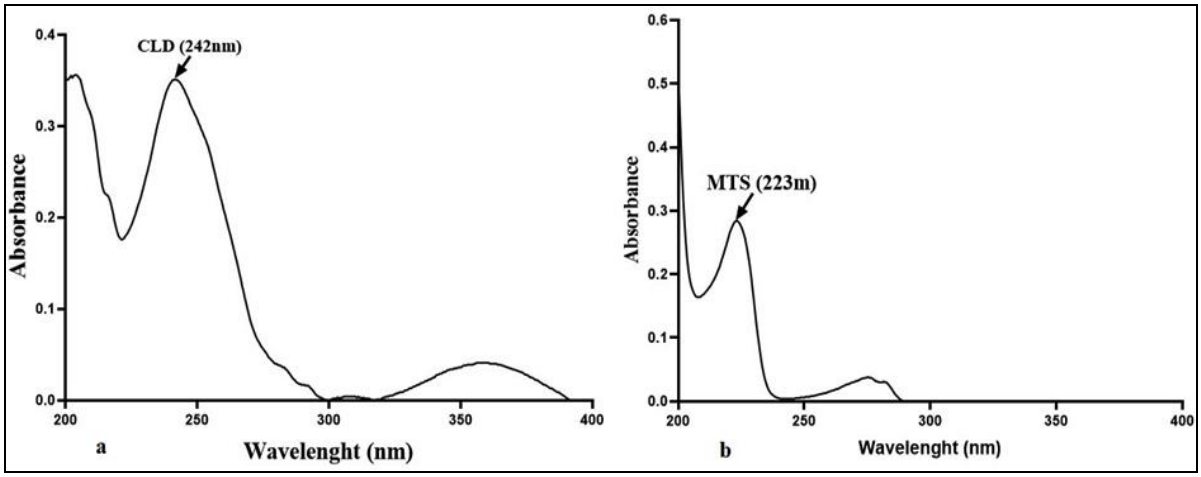

Figure 2: Absorption maxima of (a) CLD and (b) MTS.

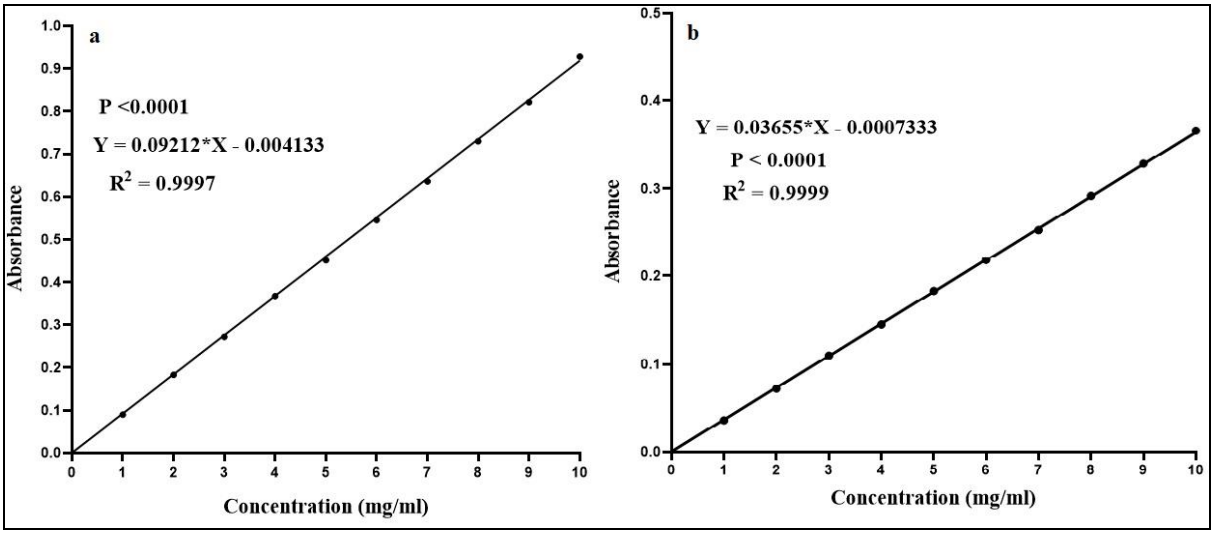

Figure 3: Linearity curve of (a) CLD and (b) MTS

Table 1: Linearity curve data for cilnidipine and metoprolol succinate.

Concentration (µg/ml)	Absorbance			
	CLD		MTS	
	Mean	SD	Mean	SD
1	0.090	0.001	0.036	0.0122
2	0.183	0.0011	0.072	0.0060
3	0.272	0.0034	0.110	0.0125
4	0.367	0.0032	0.145	0.0087
5	0.452	0.0032	0.183	0.0070
6	0.546	0.0032	0.218	0.0058
7	0.636	0.0035	0.253	0.0055
8	0.722	0.0025	0.292	0.0034
9	0.821	0.0015	0.323	0.0018
10	0.928	0.0010	0.366	0.0010

Table 2: Statistical data of linearity curve.

Parameters	CLD	MTS
Absorption maxima(λmax)	242	223
Beer's range (µg/ml)	1-10	1-10
Molar absorptivity (ε)	9.12×10^2 /(m^{-cm})	3.636×10^2 /(m^{-cm})
Best fit values		
Slope	0.09235	0.03675
Y-intercept	-0.004500	-0.001900
X-intercept	0.04873	0.05170
1/slope	10.83	27.21
95% confidence interval		
Slope	0.08812 to 0.09658	0.03651 to 0.03699
Y-intercept	-0.03258 to 0.02358	-0.003512 to -0.0002877
X-intercept	-0.2664 to 0.3388	0.007876 to 0.09501

Validation

Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the Medium I was justified from the recovery of CLD and MTS separately in repeatability studies (n=6) for labelled claim and recovery of CLD and MTS separately in intraday and interday study for labelled claim (in both case n=6). The mean % recovery, and % RSD were calculated and computed in table 3. The percentage RSD values for repeatability studies, intraday and interday studies is less than 2 % indicate Medium I was precise and reproducible.

Table 3: Repeatability, Intraday and Inter day precision data in Medium I.

Precision parameters	Labelled claim (µg/ml)	CLD			MTS		
		Amount recovered	% Recovered Mean*±SD	% RSD	Amount recovered	% Recovered Mean*±SD	% RSD
Repeatability	8	7.933	99.12±0.326	0.3288	7.97	99.45±0.6891	0.6929
Intra day	8	8.005	100.1±0.7197	0.7192	7.983	99.8±0.8811	0.8829
Inter day	8	7.977	99.71±0.3827	0.3838	7.947	99.34±0.5474	0.5511
* In each case six replicates were studied n=6							

Accuracy: The Medium I is analyzed for assay in two CLD (Cilizex 10; Cilindia 10) and MTS (MTXL 50; Starpress XL 25) marketed formulations and data were given in table 4. Further the accuracy was determined by standard addition method to find the known amount of analyte added and expressed as percent recovery with % RSD values, and data was given in table 5. The percentage recovery was within the permissible limit with RSD values less than 2%. The accuracy performed for the Medium I by standard addition method and the % recovery found within the permissible limits with RSD values less than 2% indicate non-interference of the excipients in the formulations. The CLD and MTS content of two marketed products determined by the Medium I was in good agreement with the label claim with % RSD values less than 2.

Table 4: Assay data of CLD and MTS marketed tablets.

Brand name	Labelled claim	Amount recovered (µg/ml)	% Recovery Mean ± SD	%RSD
CLD				
Cilizex – 10	6	6.05	100.9±0.207	0.205
	8	7.81	97.67±0.481	0.492
Cilindia – 10	6	6.01	100.3±0.279	0.217
	8	7.92	99.09±0.410	0.413
MTS				
MTXL 50	6	5.93	98.92±0.531	0.536
	8	8.11	101.4±0.600	0.591
Starpress XL 25	6	5.99	99.54±0.963	0.964
	8	8.16	102.4±0.920	0.898

Table 5: Accuracy data of marketed CLD and MTS tablets by standard addition method.

Brand name	Prequantified Sample (µg)	% of pure drug added	Pure drug Added (µg)	Amount Recovered (µg)	% Recovery * Mean ±SD	% RSD
CLD						
Cilindia 10	6	40	2.4	8.27	98.5±0.300	0.304
	6	80	4.8	10.7	99.07±0.20	0.202
	6	120	7.2	13.1	99.24±1.00	1.01
	8	40	3.2	11.12	99.28±0.057	0.057
	8	80	6.4	14.28	99.16±0.28	0.28
	8	120	9.6	17.51	99.48±0.15	0.10
Cilizex 10	6	40	2.4	8.3	99.7±0.34	0.34
	6	80	4.8	10.7	99.8±0.37	0.37
	6	120	7.2	13.2	100.1±0.100	0.099
	8	40	3.2	11.2	100.1±0.416	0.415
	8	80	6.4	14.3	99.8±0.300	0.300
	8	120	9.6	17.6	100±0.200	0.2
MTS						
MTXL 50	6	40	2.4	8.4	100.1±0.950	0.949
	6	80	4.8	10.6	98.13±0.750	0.764
	6	120	7.2	13.1	99.43±0.461	0.463
	8	40	3.2	11.2	100.1±0.873	0.872
	8	80	6.4	14.3	99.50±0.592	0.531
	8	120	9.6	17.5	99.53±0.450	0.452
Starpress XL 24	6	40	2.4	8.31	99.64±0.504	0.505
	6	80	4.8	10.6	99.18±0.575	0.579
	6	120	7.2	13.1	99.60±0.320	0.321
	8	40	3.2	11.3	100.7±0.691	0.686
	8	80	6.4	14.3	99.47±0.590	0.593
	8	120	9.6	17.6	100.2±0.318	0.317
* In each case average of six determinations						

Robustness: The robustness of the Medium I was done and data was given in table 6. The results suggest change in λ max of ± 5nm shows significant difference in the absorbance values when compared to actual λ max indicates Medium I was robust.

Ruggedness: In ruggedness analysis Medium I analyzed by different analyst and different instrument and data was given table 7. The result indicates the Medium I was significantly rugged.

Solution stability: The results of stability study of CLD and MTS in proposed methods were within the acceptable limit and indicate solutions in Medium I stable over the period of 24 hr.

Table 6: Robustness data for Medium I.

λmax	Concentration (µg/ml)	Absorbance nm Mean ±SD
CLD		
242 nm	6	0.546±0.0022
	8	0.722±0.0035
247 (+5 nm)	6	0.526±0.0014
	8	0.702±0.0041
237 (-5 nm)	6	0.536±0.0055
	8	0.700±0.00625
MTS		
223 nm	6	0.218±0.0033
	8	0.272±0.0042
228(+5 nm)	6	0.201±0.0055
	8	0.271±0.0024
218(-5 nm)	6	0.200±0.0061
	8	0.270±0.0029

Table 7: Ruggedness data for Medium I.

Parameter	Concentration (µg/ml)	Amount Recovered (µg)	% Recovery Mean ± SD	% RSD
CLD				
Analyst 1	6	6.01	99.95±0.653	0.653
	8	7.93	99.14±0.080	0.080
Analyst 2	6	5.9	99.77±0.31	0.317
	8	8.01	100.1±0.311	0.310
MTS				
Analyst 1	6	5.96	99.38±1.751	1.761
	8	8.20	102.5±0.732	0.705
Analyst 2	6	5.94	99.07±0.930	0.938
	8	8.13	101.7±0.600	0.589

CONCLUSION

The results and the statistical parameters demonstrate that the proposed mediums for spectrophotometric methods are simple, rapid, specific, accurate and precise. Therefore, this method can use for the quantification of Cilnidipine and Metoprolol succinate in tablet dosage formulations without interference with commonly used excipients and related substances.

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CONFLICT OF INTERESTS

No conflict of interest.

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