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SIMULTANEOUS ESTIMATION OF METOPROLOL SUCCINATE, TELMISARTAN AND CILNIDIPINE IN BULK PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A high-performance liquid chromatographic and UV spectrophotometric method were developed and validated for the quantitative determination of three anti-hypertensive drugs viz. Metoprolol succinate, Telmisartan and Cilnidipine in pure bulk and tablet dosage form. The different validation parameters such as linearity, precision, accuracy and specificity, limit of detection (LOD) and limit of Quantification (LOQ) were determined according to the International Conference on Harmonization ICH Q2 guidelines. The UV spectrophotometric determinations were performed at 224 nm, 299 nm, and 242 nm for Metoprolol succinate, Telmisartan, Cilnidipine respectively. Chromatography was carried out by isocratic technique on a reversed-phase Luna C_{18} phenomenix column (250 mm × 4.6 mm × 5 µm) thermo stated HPLC column with Acetonitrile and water (90:10) as mobile phase based and optimized depending on the polarity of the molecules. The linearity of the calibration curves for each analyte in the desired concentration range is good (r^2>0.999) by both the HPLC and UV methods. Moreover, the accuracy and precision obtained with HPLC correlated well with the UV method which implied that UV spectroscopy can be a cheap, reliable and less time consuming alternative for chromatographic analysis. The proposed methods are highly sensitive, precise and accurate and hence were successfully applied for the reliable quantification of API content in the commercial formulations of Metoprolol succinate, Telmisartan and Cilnidipine.

Key words-*Cilnidipine, Metoprolol succinate, Reversed-phase liquid chromatography, Telmisartan*

INTRODUCTION

Validation of an analytical procedure is defined as a process by which it is established that the performance characteristics of the procedure meet the requirements for the intended analytical applications in laboratory studies. The main objectives of any analytical measurement are to obtain consistent, reliable and accurate data. The results from method validation can be used to judge the quality, reliability and consistency of analytical results, which is an integral part of any good analytical practice. The main parameters used for validation purpose are linearity and range, accuracy and precision, recovery, limit of detection and limit of quantitation.

The 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. The primary goal of any antihypertensive therapy is therefore achievement of norm tension without addition of intolerable side effects, which can be accomplished by combination of drugs with

different mechanism of action. Metoprolol succinate is a chemically (RS)-1-(isopropylamino)-3-[4-(2-Methoxyethyl)Phenoxy)propan-2-ol.Succinate is an anti-hypertensive drug belongs to cardio selective β1 adrenergic receptor antagonist used in hypertension, angina pectoris, cardiac arrhythmia, congestive heart failure and myocardial infraction[1]. Telmisartan is chemically 4-[1,4'-dimethyl-2-propyl [2,6-bi-benzimidazole]-1-yl] methyl 1,1-biphenyl 2-carboxylic acid. It is an angiotensin receptor blocker (ARB) that shows high affinity for the angiotensin II type 1 (ATI) receptors[2]. Cilnidipine is chemically 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine carboxylic acid 2-methoxyethyl (2E)-3-phenyl-2-propenyl ester. Cilnidipine is an N-type calcium channels in sympathetic nerve terminals that supply blood vessels and dual blocker of L-type voltage gated calcium channel in vascular smooth muscles[3] (Figures 1-3).

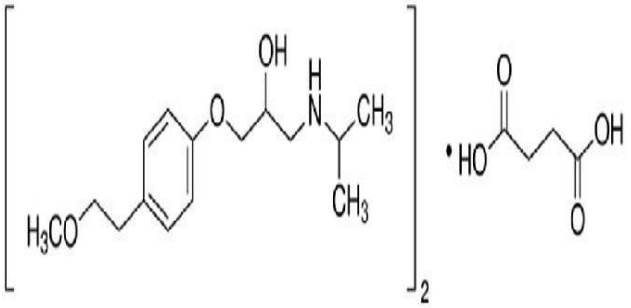

Fig. 1: Structure of metoprolol

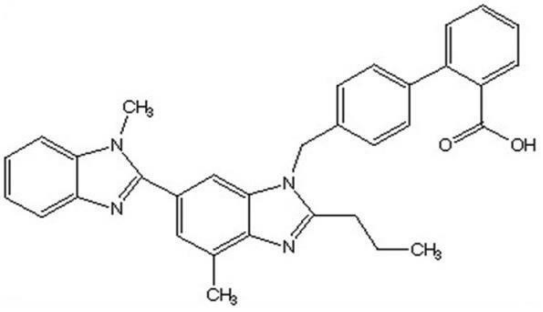

Fig. 2: Structure of telmisartan

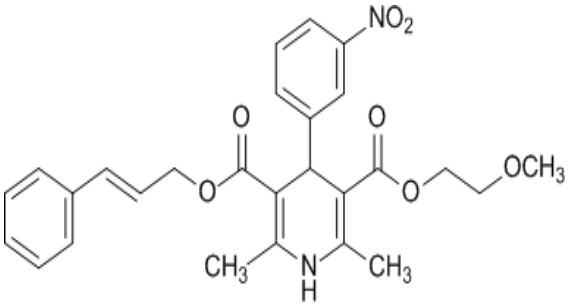

Fig. 3: Structure of cilnidipine

Literature survey revealed that methods were reported for the estimation of cilnidipine[4-10], Metoprolol succinate[11-18], and for Telmisartan[19-22] on these either single drug or combination with other drug analytical method was reported. To the best of our knowledge no spectrophotometric and RP-HPLC methods of analysis has yet been reported for analysis of Metoprolol succinate, Telmisartan and Cilnidipine. Present study describes a simple, accurate, and validated chromatographic and RP-HPLC method for the quantification of these compounds as a bulk drug and in tablet dosage form.

MATERIALS AND METHODS

Chemicals

The bulk drugs of Metoprolol succinate, Telmisartan and Cilnidipine were obtain as gift sample from Ajanta Pharma Ltd. All solvents and reagents used were of HPLC and Spectrophotometric grade, respectively. HPLC grade acetonitrile were obtained from SD fine Chem. Pvt. Ltd, Mumbai. Tablets Met XL Trio 50 of Metoprolol succinate (50 mg).Telmisartan (40 mg) and Cilnidipine (10 mg) from Ajanta Pharma were purchase. Ultra-pure water obtained from (reverse osmosis of demineralized water) was used in all experiments. All the solutions for analysis were prepared freshly and analysed.

Instrumentation

Chromatography was performed using a JASCO PU 2080 HPLC 200047. The JASCO Model PU-2080 is a full function HPLC pump designed to meet the all modern laboratory demands. The simple design consists of only 2 plungers and 2 check valves for maximum reliability and ease of maintenance. Purospher® STAR RP -18 end capped HPLC columns are designed for universal use. A Lasco double beam UV-spectrophotometer UV-2070/2075 was used for this study. UV-Vis Detector with a fixed bandwidth 2 nm and 1 cm quartz cell is used for all spectrophotometric determinations.

UV-visible spectrophotometry

Preparation of standard stock solution: Metoprolol succinate 10 mg was weighed by using electronic balance (model).and dissolved in small amount of diluents and then completely dissolved. After this the volume was adjusted to 100 mL in volumetric flask, to get 100 µg/mL solution of metoprolol. The stock solution of telmisartan and cilnidipine was also prepared by following same procedure.

Preparation of standard solution: From the above stock solution of Metoprolol succinate (MET), Telmisartan (TEL) and Cilnidipine (CIL) the standard solution of 10 µg/ml was prepared separately for each drug, scan this solution in entire UV region of 200-400 nm using double-beam UV-visible spectrophotometer and absorbance maxima for each drug was recorded which is used for further analysis. From the stock solution of Metoprolol succinate, Telmisartan and Cilnidipine the standard solutions of various concentrations were prepared for each drug and measure their absorbance at absorbance maxima of each drug and a calibration curve were prepared (Figures 4-7).

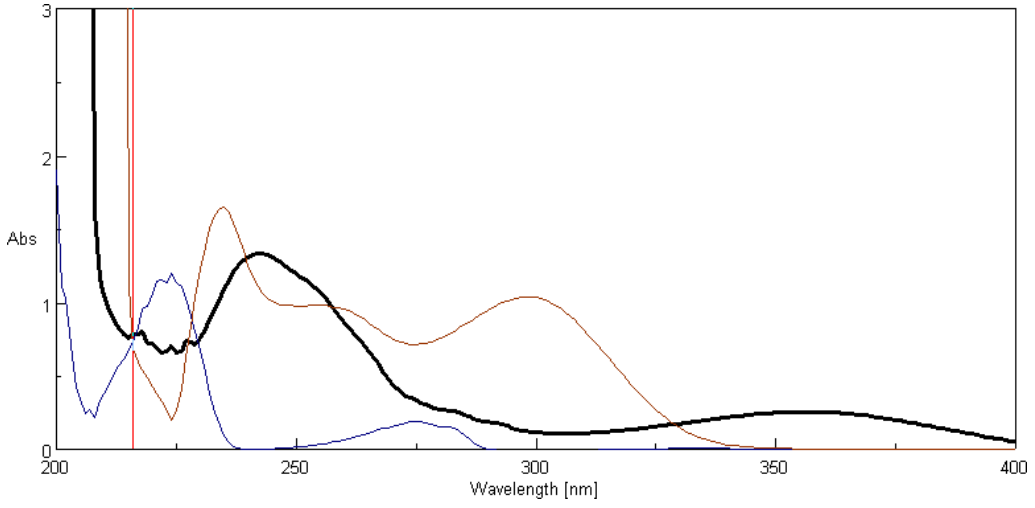

Fig. 4: Overlain absorption spectra of Metoprolol succinate, Telmisartan and Cilnidipine shows isoabsorptive point at 216 nm in Acetonitrile and water (50:50)

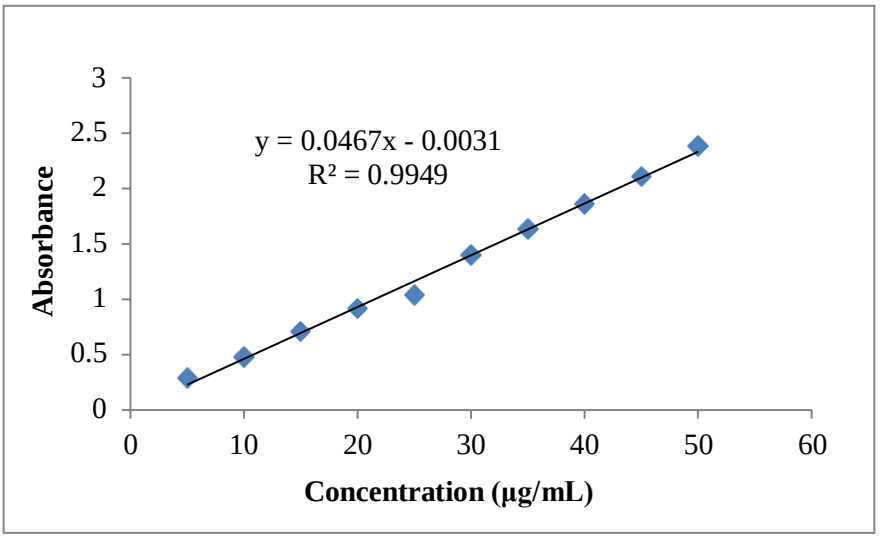

Fig. 5: Standard calibration plot of metoprolol succinate

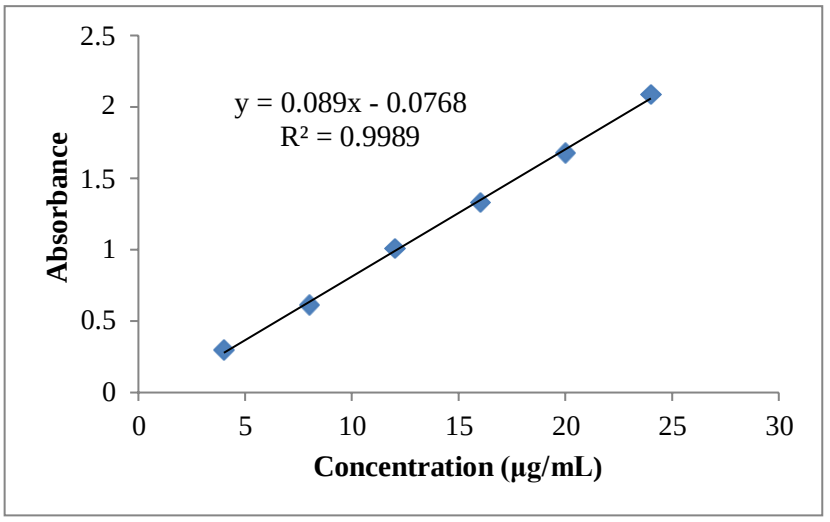

Fig. 6: Standard calibration plot of telmisartan

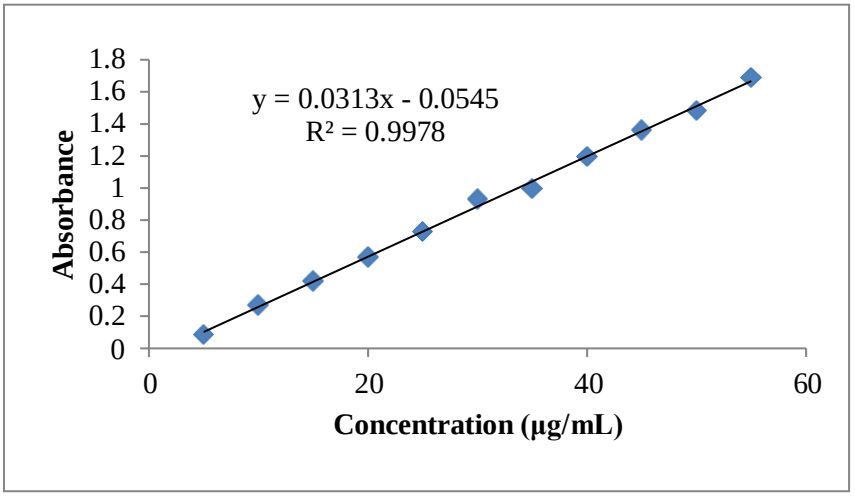

Fig. 7: Standard calibration plot of cilnidipine

Preparation of sample solution: From the above stock solution of Metoprolol succinate, Telmisartan and Cilnidipine 1 ml solution of each drug was taken and transfer it in a 10 mL volumetric flask, mix well and volume was made up to mark using sa me solvent. Then absorbance of this mixed sample solution was measured at respective λ_{max} of each drug.

Analysis of pharmaceutical formulation: Twenty tablets were taken and their average weight was determined and then they were crushed to fine powder. The powder equivalent to 10 mg of MET was taken in a 100 mL volumetric flask, dissolved in respective selected diluent with vigorous shaking for 5-10 min. and volume was made up to mark with same solvent. The solution was then filtered through Whatman filter paper no. 41, this solution contains 100 µg/mL of MET, 80 µg/mL of TEL and 20 µg/mL of CIL. Then 2 mL of this sample stock solution was taken in a 10 mL volumetric flask. Then 0.4 mL of standard solution of TEL (100 µg/mL) and 1.6 mL of standard solution of CIL (100 µg/mL) was added to it and final volume was made up with solvent used. This sample solution was then analysed at 224, 299 and 242 nm wavelengths and values of the absorbance were substituted in respective equations to obtain the content of MET, TEL and CIL respectively (Table1).

Table 1: Analysis of marketed formulation

Drug	Label Claim (mg/tablet)	Amount of drug estimated (mg/tablet)	% Label claim estimated ± S.D
MET	50	49.28	99.76
TEL	40	38.99	98.33
CIL	10	8.89	98.88

UV method development

Simultaneous estimation method: From the overlain spectra of the drugs Metoprolol succinate, Telmisartan and Cilnidipine shows absorbance maxima at 224 nm, 299 nm and 242 nm which is the respectively. Working standard solution is analysed in concentration range 5-55 µg/mL for Metoprolol succinate, and for Telmisartan 5.50 µg/mL and 4-24 µg/mL for Cilnidipine respectively. 1 Ml each from stock solution of all drugs was taken in a 10 mL volumetric flask, mix well, diluted up to mark using solvent and absorbance was measured at selected wavelengths.

HPLC method

Preparation of mobile phase: The Mobile phase was prepared by using HPLC grade acetonitrile and water (90:10). It was filtered through 0.45 µ membrane filter. The degassing of mobile phase was done by sonication for 15 min. The column temperature was maintained at 30℃.

Preparation of stock solution: Standard stock solution containing Metoprolol succinate, Telmisartan and Cilnidipine were prepared individually by dissolving 10 mg of each drug in 100 mL volumetric flask by using diluent and volume was made up to the mark to get the 100 µg/mL of solution. From this stock solution, 2 mL solution was pipette out and transferred in 10 mL volumetric flask and dilute up to the mark using mobile phase to get 20 µg/mL solutions respectively for each drug which was used for sample injection.

HPLC method development

Selection of appropriate UV wavelength: Appropriate wavelength for the detection of each drug was determined by scanning solution of each drug over the range of 200-400 nm.

Chromatographic condition: Mobile phase was prepared using acetonitrile and water (9:1).The degassing of mobile phase was done by sonication for 40 min. The flow rate was set to 1 mL/min. All drugs show good absorbance at 254 nm, which was selected as wavelength for further analysis. The column temperature was maintained at room temperature (Figures 8-11 and Table 2).

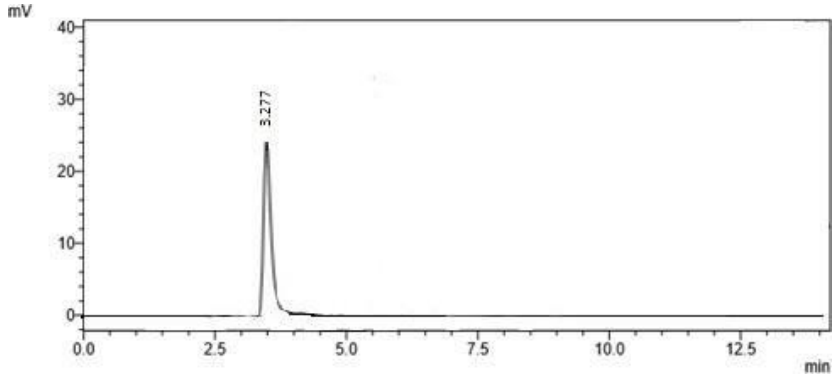

Fig. 8: Chromatogram of metoprolol succinate sample at λ_{max} (254 nm)

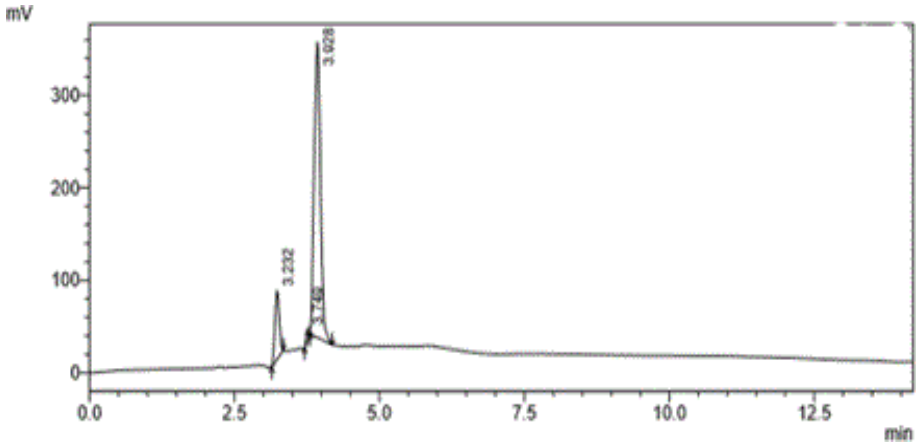

Fig. 9: Chromatogram of telmisartan sample at λ_{max} (254 nm)

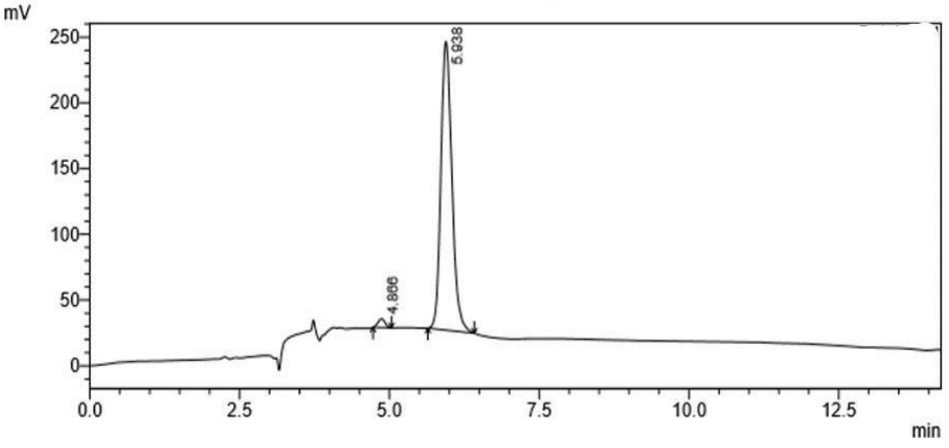

Fig.10: Chromatogram of cilnidipine sample at λ_{max} (254 nm)

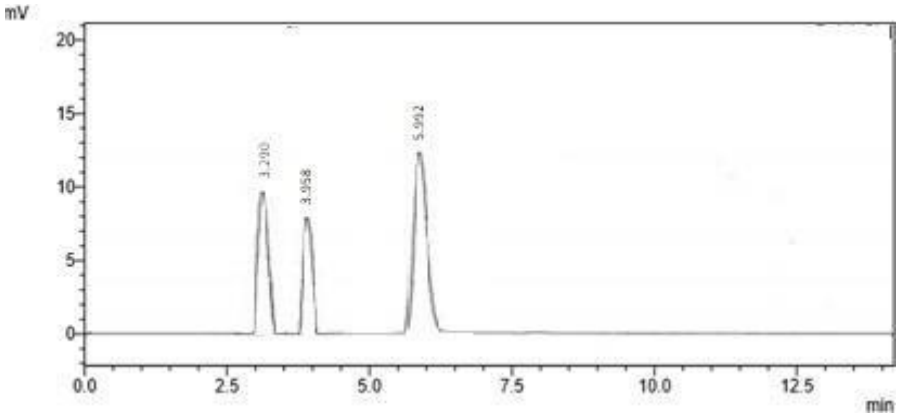

Fig. 11: Chromatogram of metoprolol succinate, telmisartan and cilnidipine combined mixture

Table 2: Chromatographic data for metoprolol succinate, telmisartan, and cilnidipine mixture

Name	Retention time (min)	Area (µV. Sec)_	Resolution	Plates	Capacity	Asymmetry
Metoprolol succinate	3.27	95811	0.00	2092	0.36	1.22
Telmisartan	3.928	2389639	0.00	9437	0.21	1.04
Cilnidipine	5.938	2804475	7.14	5154	0.22	1.30

Method validation

Linearity: The methods were validated according to International Conference on Harmonisation guidelines[23] for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for each analyte. Six point calibration curves were generated with appropriate volumes of working standard solutions for both UV and HPLC methods. In case of UV the range was optimized at for Metoprolol succinate 5-55 µg/mL, for Telmisartan 5-50 µg/mL and for Cilnidipine 4-24 µg/mL respectively.

Precision and accuracy: Both precision and accuracy were determined with standard quality control samples (in addition to calibration standards) prepared in triplicates at different concentration levels covering the entire linearity range. Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day) and reported as % RSD for a statistically significant number of replicate measurements.

Specificity: The method specificity was assessed by comparing the chromatograms (HPLC) and scans (UV) obtained from the drug and the most commonly used excipients mixture with those obtained from blank (excipients solution in water without drug). The excipients chosen are the ones used commonly in tablet formulation, which included lactose, starch, microcrystalline cellulose, PVP, and magnesium stearate. The drug to excipient ratio used was similar to that in the commercial formulations.

LOD and LOQ: The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision and variability.

RESULTS AND DISCUSSION

Linearity

The calibration curves were taken for Metoprolol succinate, Telmisartan and Cilnidipine at 224 nm, 299 nm and 242 nm. All drugs show linearity and obey Beer's Law in the concentration range of 5-35 µg/mL. The correlation coefficient of calibration curves were 0.994, 0.998 and 0.991 (Table 3)

Table 3: Regression characteristics

Parameter	MET	TEL	CIL
Wavelength (λ_{max})	224 nm	299 nm	242 nm
Beers Law Range	5-55 µg/mL	5-50 µg/mL	4-24 µg/mL
Regression Equation (y=mx+c)	y=0.031x-0.054 R^2=0.997	y=0.046x-0.003 R^2=0.994	y=0.089x-0.076 R^2=0.998
Slope (m)	0.031	0.046	0.089
Intercept (c)	0.054	0.003	0.076
Correlation Coefficient (R^2)	0.997	0.994	998

Precision

Relative standard deviations (% RSD) for intraday and inter day were calculated as precision study (Table 4).

Table 4: Intraday and Interday precision studies

	Conc. of drug (µg/mL)	Absorbance			SD			%RSD		
		MET	TEL	CIL	MET	TEL	CIL	MET	TEL	CIL
Intra-day	20	0.5677 0.5430 0.5599	0.9154 0.8967 0.9089	1.6790 1.6356 1.6808	0.01262	0.0099	0.02559	0.05631	0.0497	0.1275
Inter-day	20	0.5578 0.5635 0.5489	0.9202 0.9007 0.9177	1.6689 1.6425 1.6759	0.00735	0.0106	0.01761	0.03675	0.0505	0.08805

Accuracy

The validity and reliability of proposed methods were assessed by recovery studies by standard addition method (Table 5).

Table 5: Recovery study

Recovery level	% Recovery ± RSD		
	MET	**TEL**	**CIL**
80%	99.99 ± 0.0456	99.08 ± 0.60	98.11 ± 0.45
100%	100.11 ± 0.55	100.61 ± 0.47	101.12 ± 0.55
120%	99.99 ± 0.22	99.23 ± 0.11	101.55 ± 0.42

Limit of detection

LOD was found to be 1.3434 for Metoprolol succinate, 0.713086 for Telmisartan and 0.94550 for Cilnidipine at the wavelength 224 nm, 299 nm, and 242 nm.

Limit of quantification

LOQ was found to be 4.0709 for Metoprolol succinate, 2.16086 for Telmisartan and 2.86516 for Cilnidipine and at the wavelength 224 nm, 299 nm and 242 nm (Table 6).

Table 6: Limit of detection and Limit of quantification

Parameter	**MET (224 nm)**	**TEL (299 nm)**	**CIL (242 nm)**
LOD (µg/mL)	1.34341	0.71308	094550
LOQ (µg/mL)	4.0709	2.16086	2.86516

CONCLUSION

The combination of Metoprolol succinate, Telmisartan, Cilnidipine it plays a vital role in the treatment of the patient suffering from Hypertension. Hence the present work provides a very simple and accurate method for simultaneous estimation of Metoprolol succinate, Telmisartan, Cilnidipine. The proposed RP-HPLC and UV methods are simple, reliable and selective providing satisfactory accuracy and precision with lower limits of detection and quantification. Moreover the shorter duration of analysis for Metoprolol succinate, Telmisartan and Cilnidipine make these reported methods suitable for routine quantitative analysis in pharmaceutical dosage forms.

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CONFLICTS OF INTEREST

There is no conflict of interest.

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