IJPSR (2021), Volume 12, Issue 3 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 13 March 2020; received in revised form, 23 October 2020; accepted, 31 October 2020; published 01 March 2021

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METOPROLOL, TELMISARTAN AND CLINIDIPINE IN TABLET

M. S. Kalshetti * and S. S. Kankure

Department of Pharmaceutical Quality Assurance, D. S. T. S. Mandal's College of Pharmacy, Solapur - 413004, Maharashtra, India.

Keywords:

RP-HPLC, Metoprolol succinate, Telmisartan, Clinidipine, Method development and Validation

Correspondence to Author:
M. S. Kalshetti

Professor,
Department of Pharmaceutical Quality Assurance, D. S. T. S. Mandal's College of Pharmacy, Solapur - 413004, Maharashtra, India.

E-mail: mskalshetti@gmail.com

ABSTRACT: RP-HPLC method has been developed for separation and quantification of Metoprolol succinate, Telmisartan, and Clinidipine using Phenomenex Luna C18 column (150 mm × 4.6 mm, 5 µm) as a stationary phase with security guard cartridge C18 (4 × 3 mm) and acetonitrile: methanol: phosphate buffer pH 7.5 (45:30:25) as a mobile phase by isocratic elution at the flow rate of 1.0 ml/min. The detection was carried out at 229 nm. The drugs are eluted at retention times 2.0, 2.8, and 6.8min for telmisartan, metoprolol succinate, and clinidipine, respectively. The method is linear in the range of 10-80 µg / ml, 6.25-50 µg / ml, and 2.5-20 µg / ml with regression coefficients 0.997, 0.995, and 0.999 respectively for telmisartan, metoprolol succinate, and clinidipine. The method is precise within the acceptable limits (% RSD- 1.22). The proposed method showed linearity, accuracy, precision, specificity, robustness, LOD, LOQ, and system suitability results within the acceptance criteria of ICH. The method can be applied for the routine analysis of pharmaceutical formulations.

INTRODUCTION: Metoprolol succinate (MET), bis (1 - [4 - (2 - methoxyethyl) phenoxy] - 3 - [(propan – 2 - yl) amino] propan-2-ol); butanedioic acid [1] is a β1-selective receptor antagonist indicated for the treatment of hypertension [2]. Metoprolol has also been approved for use in treating angina pectoris and in therapy following myocardial infarction [3]. It is a competitive antagonist of catecholamines at peripheral adrenergic neuron sites, leading to decreased cardiac output [4]. Telmisartan (TEL), 2 - (4 - {[4 – methyl – 6 - (1 – methyl - 1H - 1, 3-benzodiazol-2-yl)-2-propyl-1H-1, 3 – benzodiazol - 1 - yl] methyl}phenyl) benzoic acid [5] is an orally active nonpeptide angiotensin II antagonist that acts on the angiotensin II receptor subtype [3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(3).1651-57

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(3).1651-57

It is currently approved for the treatment of hypertension and, along with ACE inhibitors, diuretics, β-blockers, and calcium channel blockers, have been designated as first-line agents either alone or in combination with other antihypertensive agents. Telmisartan has been approved to reduce hyper-tension, cardiovascular risk reduction of myo-cardial infarction, and stroke [6]. Clinidipine (CLN), 3-(2-methoxyethyl) 5-(2E)-3-phenylprop-2-en - 1 - yl 2, 6 – dimethyl - 4 - (3-nitrophenyl) -1, 4 - dihydropyridine-3, 5 - dicarboxylate [7] is dual blocker of L-type voltage-gated calcium channels in sympathetic nerve terminals that supply blood vessels. It also dilates efferent and afferent arterioles [8]. They exert their effects by binding to specific receptor sites located within the central α-1 subunit of L-type, potential-dependent channels.

Calcium channel blockers are used for hyper-tension, angina pectoris, subarachnoid haemorrhage, and specific types of arrhythmias. Calcium channel blockers cause vasodilation and decrease peripheral resistance [5].

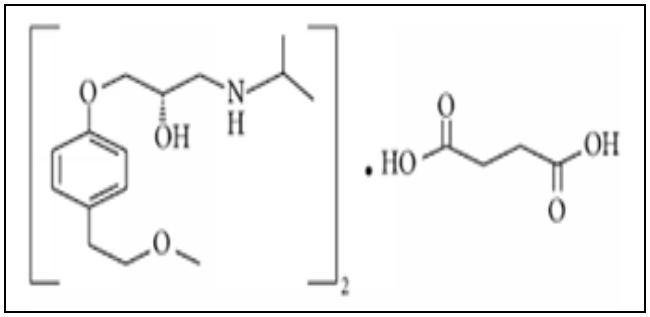

FIG. 1: METOPROLOL SUCCINATE

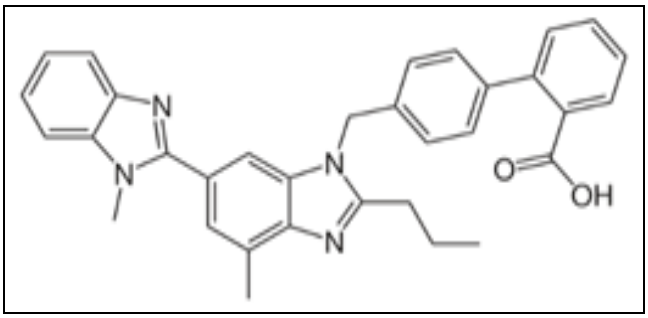

FIG. 2: TELMISARTAN

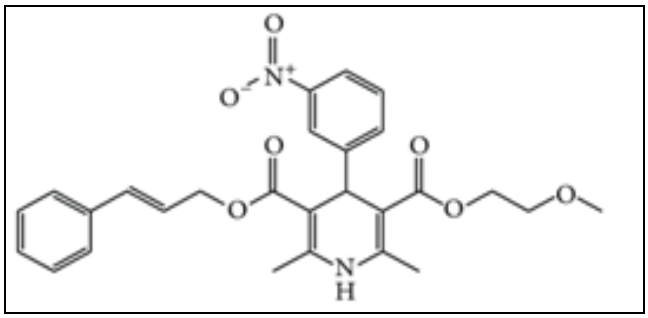

FIG. 3: CLINIDIPINE

Combination therapy is found to be more improvement than with either agent alone [4] therefore, a combination of Metoprolol succinate, Clinidipine, and Telmisartan is useful for the treatment of patients with uncontrolled essential hypertension and stable ischemic heart disease [9]. Reverse-phase high-performance liquid chromate-graphy (RP-HPLC) methods for the single drug-containing Metoprolol succinate, Telmisartanor Clinidipine combinations containing Metoprolol succinate, and Clinidipine, Metoprolol succinate, and Telmisartan or Clinidipine and Telmisartan are reported [10-15]. Also, these drugs in combination with other drugs are reported. However, no HPLC method is reported for simultaneous estimation of these three drugs in the combined dosage form. Hence the present study is to develop an HPLC method for simultaneous estimation of these three drugs in the tablet dosage form.

MATERIALS AND METHODS:

Materials:

Instrument used: HPLC system (Younglin Acme 9000) equipped with UV detector (UV730D), Column Luna C18 (150 mm × 4.6 mm, 5 µm) and Security Guard Cartridge C18 (4 × 3 mm) (Phenomenex, USA), RC membrane 0.45 µm, 15 mm Syringe Filters (Phenomenex, USA), Nylon membrane 0.45 µm, 47 mm D filters (Pall India Pvt. Ltd., Mumbai), Microanalytical balance (Shimadzu AY220), Ultrasonic cleaner (Oscar Microclean 103), pH meter (Equiptronics EQ-615), UV-VIS Double Beam Spectrophotometer 2201 (Systronics, Mumbai).

Chemicals: Metoprolol succinate and Telmisartan were obtained as gift samples from Aurobindo Pharma Ltd., Hyderabad, and Clinidipine from Micro Labs Limited, Bangalore. Marketed formu-lation of this combination MET XL Trio 25 was procured from the local market. Methanol (HPLC grade), Acetonitrile (HPLC grade), and Water (HPLC grade) were procured from Merck Life Sciences Pvt. Ltd., Mumbai. Potassium dihydrogen phosphate was procured from S D Fine-Chem Ltd., Mumbai. Dipotassium hydrogen phosphate and Sodium hydroxide pellets were procured from Merck Specialities Pvt. Ltd., Mumbai.

Methods:

Preparation of Buffer pH 7.5: Accurately weighed 0.136 gm of Potassium dihydrogen phosphate and 0.174 gm of Dipotassium hydrogen phosphate was transferred in the 100 ml volumetric flask and dissolved in HPLC water, then the volume was made up to the mark with HPLC water and adjusted the pH to 7.5 by using NaOH solution and pH meter.

Preparation of Mobile Phase: Acetonitrile (45%), Methanol (30%), and Phosphate buffer pH 7.5 (25%) was taken into a 1000 ml round bottom flask

and mixed properly, and then it is filtered through Nylon membrane filter having pore size 45 µm. Then, this filtered mobile phase is sonicated for 15 min and used as the mobile phase by isocratic elution method.

Preparation of Standard Stock Solution of MET: Accurately weighed 10 mg of metoprolol succinate and transferred into 10 ml volumetric flask and dissolved with methanol and adjusted the volume with methanol up to the mark (1000 µg/ml). From this again, 1ml was taken into 10 ml volumetric flask and diluted with methanol up to the mark (100 µg/ml).

Preparation of Standard Stock Solution of TEL: Accurately weighed 10 mg of telmisartan and transferred into 10 ml volumetric flask and dissolved with methanol and adjusted the volume with methanol up to the mark (1000 µg/ml). From this again, 1 ml taken into 10 ml volumetric flask and diluted with methanol up to the mark (100 µg/ml).

Preparation of Standard Stock Solution of CLN: Accurately weighed 10 mg of clinidipine and transferred into 10 ml volumetric flask and dissolved with methanol and adjusted the volume with methanol up to the mark (1000 µg/ml). From this again 1ml taken into 10ml volumetric flask and diluted with methanol up to the mark (100 µg/ml).

Standard Stock Solution of MET, TEL and CLN: 2.5 ml of 'Std MET' (1000 µg/ml), 4 ml of 'Std TEL' (1000 µg/ml), and 1ml of 'Std CLN' (1000 µg/ml) transferred to 10ml volumetric flask and diluted to 10 ml to with methanol to obtain a concentration of 250 µg/ml of MET, 400 µg/ml of TEL and 100 µg/ml of CLN.

Preparation of Sample Solution: 20 tablets were weighed and triturated in a glass mortar, and 592 mg of tablet powder (equivalent to 25 mg of Metoprolol succinate, 40 mg of Telmisartan, and 10 mg of Clinidipine) was transferred into 100 ml volumetric flask and diluted with methanol (LR), solution sonicated for 10 min and volume was made up to the mark with methanol. Then, it is filtered through Whatmann filter paper. 1ml from filtered solution was taken into 10 ml volumetric flask and diluted with the mobile phase.

Selection of Analytical Wavelength for UV (λ_{max}): Standard solutions of MET, TEL, and CLN were scanned in the UV range (200-400 nm), and the spectrums obtained were overlaid, and the overlain spectrum was recorded. From the overlain spectrum, 229 nm was selected as the detection wavelength for the present study **Fig. 4.**

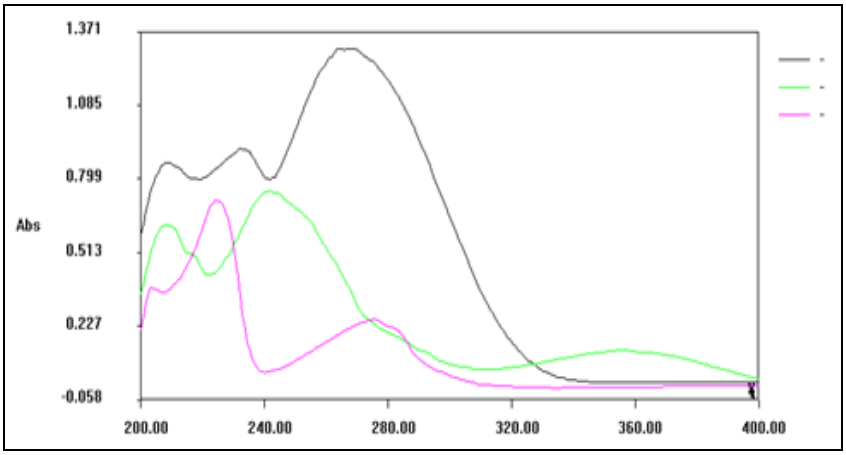

FIG. 4: OVERLAIN SPECTRUM OF MET, TEL AND CLN

Chromatographic Conditions: The instrument used was high-performance liquid chromatography equipped with UV detector, and column used was Phenomenex, Luna C18 column (150 mm × 4.6 mm, 5 µm). A various combination of mobile phase was screened with respect to resolution, theoretical plate, capacity factor and other system suitability parameters, finally, the separation was performed with freshly prepared mobile phase consisting of Acetonitrile: Methanol: Phosphate buffer pH 7.5 (45:30:25) at a flow rate of 1.0 ml/min and total run time was 10 min. The wavelength selected for analysis was 229 nm.

RESULTS AND DISCUSSION:

Method Development: RP-HPLC method has been developed for simultaneous estimation of Metoprolol succinate, Telmisartan, and Clinidipine in tablet dosage form. The separation has achieved by Phenomenex, Luna C18 column (150 mm × 4.6 mm, 5 µm) and mobile phase Acetonitrile: Methanol: Phosphate buffer pH 7.5 (45:30:25) at a flow rate of 1.0 ml/min. The detection has carried out at 229 nm. The retention time has been found to be 2.0, 2.8, and 6.8 min for telmisartan, metoprolol succinate, and clinidipine, respectively **Fig. 5.**

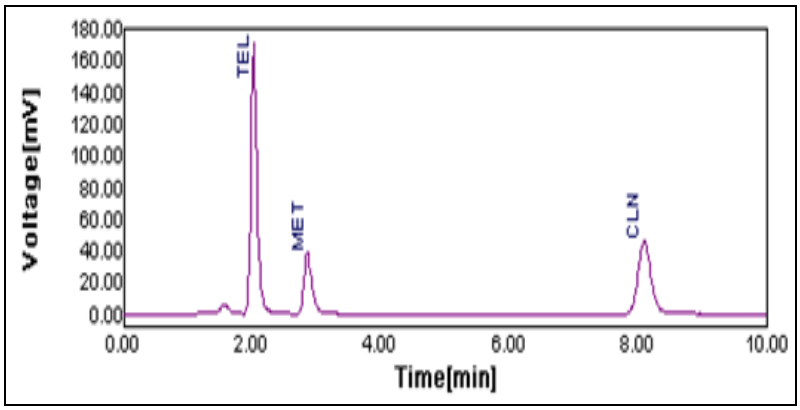

FIG. 5: CHROMATOGRAM OF COMBINATION OF TEL (10 µg/ml), MET (10 µg/ml) AND CLN (10 µg/ml)

Method Validation: The validation has been done according to ICH guidelines. The proposed method has validated with respect to specificity, linearity, the limit of detection (LOD), limit of quantitation (LOQ), precision, accuracy, and robustness.

Specificity: The specificity of the method has been determined by checking the interference of the tablet components against API and no interference has observed for any of the excipients of the drugs.

The retention time has observed at 2.0 min for telmisartan, 2.8 min for metoprolol succinate and 6.8 min for clinidipine permits a rapid assay, which is important for routine analysis.

Linearity: A series of dilutions has prepared such that 0.5 ml, 1 ml, 2 ml, 3 ml and 4 ml using combined standard stock solution to get 10, 20, 40, 60, 80 µg/ml for telmisartan 6.25, 12.5, 25, 37.5, 50 µg/ml for metoprolol succinate and 2.5, 5, 10, 15, 20 µg/ml for clinidipine. All the solutions have filtered through 0.22 µm syringe filter prior to use. A calibration curve was plotted between the areas vs. concentrations. The results are given in **Fig. 6, 7, 8, 9** and **Table 1, 2, 3.**

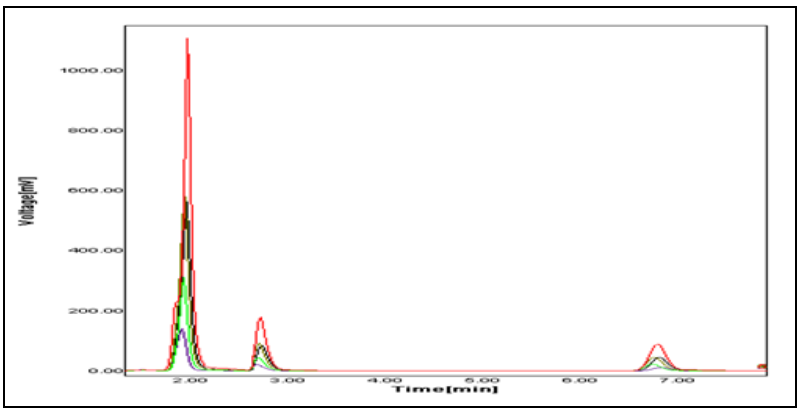

FIG. 6: OVERLAIN CHROMATOGRAM OF TEL, MET AND CLN

TABLE 1: LINEARITY STUDIES FOR TELMISARTAN

S. no.	Concentration (µg/ml)	Area
1	10	955
2	20	1887
3	40	3784
4	60	5407
5	80	6921

TABLE 2: LINEARITY STUDIES FOR METOPROLOL SUCCINATE

S. no.	Concentration (µg/ml)	Area
1	6.25	176
2	15.5	315
3	25	683
4	37.5	924
5	50	1313

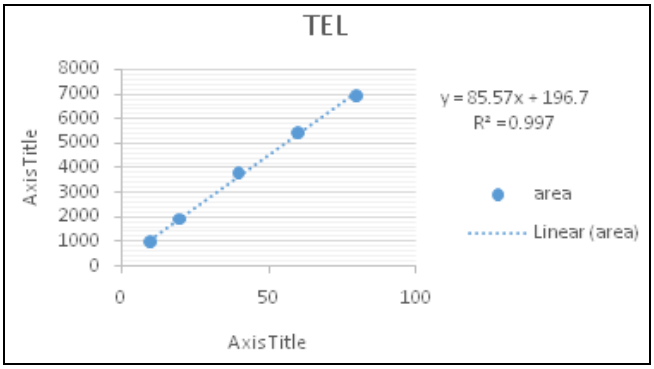

FIG. 7: CALIBRATION CURVE OF TELMISARTAN

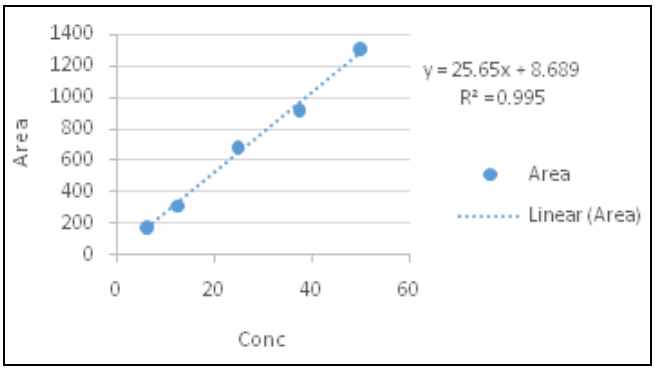

FIG. 8: CALIBRATION CURVE OF METOPROLOL SUCCINATE

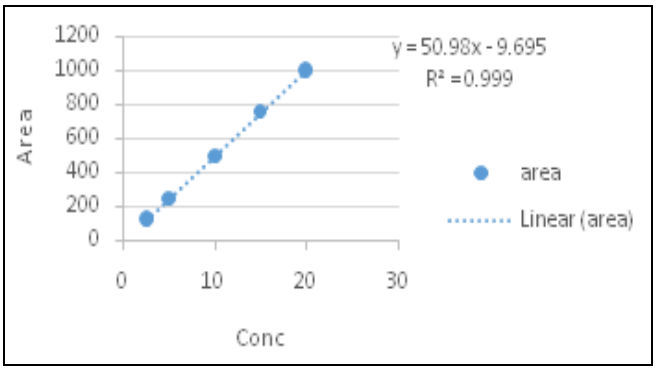

FIG. 9: CALIBRATION CURVE OF CLINIDIPINE

TABLE 3: LINEARITY STUDIES FOR CLINIDIPINE

S. no.	Concentration (µg/ml)	Area
1	2.5	122
2	5	242
3	10	494
4	15	762
5	20	1008

Limit of Detection (LOD) and Limit of Quantitation (LOQ): The sensitivity of proposed method has estimated by limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were calculated using the following formula;

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where 'σ' is the standard deviation of the response and 'S' is the slope of the corresponding calibration curve of the analyte.

The linearity equation was found to be y = 85.574x + 196.7 for TEL, y = 25.658x + 8.689 for TEL and y = 50.98x – 9.6951 for CLN. The results are given in **Table 4**.

TABLE 4: LOD AND LOQ DATA FOR TEL, MET AND CLN

Parameters	Telmisartan (µg/ml)	Metoprolol Succinate (µg/ml)	Clinidipine (µg/ml)
LOD	4.35	1.83	0.57
LOQ	13.18	5.56	1.74

TABLE 5: ACCURACY STUDIES

Sample	Amount of Sample Taken (µg/ml)	Amount of Standard Spiked to Sample (%)	Amount of Standard Spiked to Sample (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)
Telmisartan	20	80	16	16.08	100.5
	20	100	20	20.2	101
	20	120	24	24.1	100.4
Metoprolol succinate	12.5	280	10	9.96	99.6
	12.5	100	12.5	12.44	99.5
	12.5	120	15	14.91	99.4
Clinidipine	5	80	4	4.07	101.7
	5	100	5	5.04	100.8
	5	120	6	6.1	101.6

Accuracy: The accuracy of the method has evaluated in triplicates by recovery studies at three different concentration levels 80%, 100%, and 120% known amount of standard drug concentration were added to the sample. Then, the amount of drug recovered is calculated in terms of % recovery. The accuracy data and results were shown in **Table 5.**

Precision: The precision of the method has determined by analyzing the corresponding responses 6 times on the same day for the same concentration of telmisartan (40 µg/ml), metoprolol succinate (25 µg/ml), and clinidipine (10 µg/ml). The standard deviation (SD) and % RSD have been calculated. The results are given in **Table 6.**

TABLE 6: PRECISION STUDIES

Telmisartan		Metoprolol Succinate		Clinidipine	
Concentration (µg/ml)	**Area**	**Concentration (µg/ml)**	**Area**	**Concentration (µg/ml)**	**Area**
40	4396	25	730	10	595
40	4234	25	718	10	570
40	4413	25	711	10	580
40	4233	25	704	10	593
40	4329	25	714	10	595
40	4366	25	712	10	598
Average	4328	Average	714	Average	588
SD	78.93	STD	8.72	STD	11.04
% RSD	1.82	% RSD	1.22	% RSD	1.87

Robustness: Small but deliberate changes in a method like flow rate, wavelength, and temperature are made, but there were no recognized changes in the result and are within range as per ICH guidelines. The results of robustness are given in **Table 7**.

TABLE 7: ROBUSTNESS STUDIES

Parameters		Telmisartan	Metoprolol Succinate	Clinidipine
Wavelength (nm)	227	5688	838	568
	231	5659	565	600
Flow rate (ml/min)	0.9	6395	806	660
	1.1	5244	668	556

CONCLUSION: A validated simple, rapid, sensitive, and accurate RP-HPLC method was developed for the determination of metoprolol succinate, telmisartan and clinidipine in bulk and pharmaceutical formulation. No interference of the excipients with the absorbance of the interested analyte; hence the proposed method is applicable for the routine estimation of MET, TEL, and CLN in the pharmaceutical combined dosage form.

The drugs have been resolved properly with retention time 2.0 min, 2.8 min, and 6.8 min for telmisartan, metoprolol succinate, and clinidipine, respectively.

The method is linear in the range 10-80, 6.25-50 and 2.5-20 µg/ml with regression coefficients 0.997, 0.995, and 0.999 for telmisartan, metoprolol succinate, and clinidipine, respectively. The method is accurate, having % recovery values within limits for all three drugs. The % recovery studies indicated that there is no interferon's from the excipients present in the formulation. The %RSD values are within limits, *i.e*., less than 2.

ACKNOWLEDGEMENT: The authors are thankful to the Principal and Management of D. S. T. S. Mandal's College of Pharmacy, Solapur, Maharashtra, India, for providing the required facilities to carry out this research work.

CONFLICTS OF INTEREST: Authors do not have conflicts of interest.

REFERENCES:

1. Drug profile of Metoprolol succinate available: https://www.drugbank.ca/salts/DB SALT000863 last access in 17 September 2019.
2. Brunton LL: Goodman & Gilman's The Pharmacological Basis of Therapeutics. The McGraw-Hill Companies. Twelfth Edition 2011; 767-77.
3. Beale JM and Block JH: Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry. Wolter's Kluwer Health, Lippincott Williams & Wilkins. Twelfth Edition 2011; 612-13.

4. Rang HP, Ritter JM, Flower RJ and Hendorson G: Pharmacology. Edinburgh Churchill Livingstone Eighth Edition 2016.
5. Drug profile of Telmisartan available: https://www.drugbank.ca/drugs/DB00966 last access in 17 September 2019.
6. Lemke TL and Williams DA: Foye's Principles of Medicinal Chemistry. Wolter's Kluwer Health, Lippincott Williams & Wilkins. Seventh Edition 762-63.
7. Drug profile of Clinidipine available:https://www.drugbank.ca/drugs/DB09232 last access in 17 September 2019.
8. Hinge MA, Desai DK, Chavda RN, Patel DR, Shing RD and Patel ES: Analytical method development and validation for simultaneous estimation of cilnidipine and metoprolol succinate by RP-HPLC. Der Pharmacia Lettre 2015; 7(5): 333-40.
9. Approved new drugs–CDSCO available: https://cdsco.gov.in/opencms/opencms/en/Approval_new/Approved-New-Drugs/ last access in 17 September 2019.
10. Guguloth R, Madhukar A, Umadevi GT: Lalitha and Ravinder A: Analytical Method Development and Validation for the Determination of Metoprolol Succinate in tablet dosage form by RP-HPLC techniques. Journal of Scientific Research in Pharmacy 2016; 5(6): 74-77.
11. Ashok P, Narenderan S, Meyyanathan S, Babu B and Vadivelan R: Development and Validation of a RP-HPLC Method for Estimation of Telmisartan in Human Plasma. International Journal of Applied Pharmaceutics 2019; 11(1): 237-40.
12. Ruhina T and Mamatha T: A Novel RP HPLC Method for Developmentand Validation of Clinidipine in Bulk and Pharmaceutical Dosage Form. Asian Journal of Pharmaceutical Technology & Innovation 2017; 5(14): 72 -81.
13. Suthakaran R, Bikshapathi D, Shankar C, Prem Kumar B and Ghouse S: A Validated RP-HPLC Method for Estimation of Telmisartan and Metoprolol in its Bulk Form. International Journal of Biomedical Investigation 2018; 1(2): 1-13.
14. Prasad KC, Krishna MR, Babu DJ, Kumar NA and Babu GR: Stability indicating Simultaneous Estimation of Metoprolol and Clinidipine by using RP-HPLC. World Journal of Pharmacy and Pharmaceutical Sciences 2017; 6(1): 1395-03.
15. Nagaraju K and Chowdary YA: Simultaneous Estimation of Telmisartan and Clinidipine in Combined Tablet Dosage Form by RP-HPLC. International Journal of Engineering Science Invention 2018; 18: 39-42.

How to cite this article:
Kalshetti MS and Kankure SS: Development and validation of RP-HPLC method for simultaneous es-timation of metoprolol, telmisartan and clinidipine in tablet. Int J Pharm Sci & Res 2021; 12(3): 1651-57. doi: 10.13040/IJPSR.0975-8232.12(3).1651-57.