How to Cite:
Mujawar, T., Ahmad, S., Gadilohar, H., Deshmukh, N., Gaikwad, A., Sable, V., & Bhosale, A. (2022). Analytical method development and validation for the simultaneous estimation of Metoprolol and Benidipine by RP-HPLC in bulk and tablet dosage forms. *International Journal of Health Sciences*, *6*(S6), 8188–8202.
https://doi.org/10.53730/ijhs.v6nS6.12288

Analytical method development and validation for the simultaneous estimation of Metoprolol and Benidipine by RP-HPLC in bulk and tablet dosage forms

Dr. Tabrej Mujawar
Department of Pharmacology, Gangamai College of Pharmacy, Nagaon, Dist. Dhule, Affiliated to Kavayitri Bahinabai Chaudhari North Maharashtra University, Jalgaon, Maharashtra, India
Email: tabrej_27@yahoo.com

Dr. Sufiyan Ahmad
Department of Quality Assurance, Gangamai College of Pharmacy, Nagaon, Dist. Dhule, Affiliated to Kavayitri Bahinabai Chaudhari North Maharashtra University, Jalgaon, Maharashtra, India
*Corresponding author email: sufipharm1712@gmail.com

Hitesh Gadilohar
Department of Quality Assurance, Gangamai College of Pharmacy, Nagaon, Dist. Dhule, Affiliated to Kavayitri Bahinabai Chaudhari North Maharashtra University, Jalgaon, Maharashtra, India
Email: sufimpharm@rediffmail.com

Dr. Nitin Deshmukh
Research Scholar in Pharmaceutical Sciences, Department of Science and Technology, Savitribai Phule Pune University, Pune, Maharashtra, India
Email: ni3deshmukh@gmail.com

Dr. Asmita Gaikwad
Department of Pharmaceutical Chemistry, Sharadchandra Pawar College of Pharmacy, Otur, Tal. Junnar, Affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India
Email: asmitavgaikwad@gmail.com

Dr. Vijay Sable
Department of Pharmaceutics, Gourishankar Education Society's Satara College of Pharmacy, Satara, Affiliated to Dr. Babasaheb Ambedkar Technological University, Lonere, Dist. Raigad, Maharashtra, India
Email: vijaysabale1038@gmail.com

Manuscript submitted: 9 April 2022, Manuscript revised: 18 June 2022, Accepted for publication: 27 July 2022

Dr. Avinash Bhosale
Department of Pharmaceutical Chemistry, Gourishankar Education Society's Satara College of Pharmacy, Satara, Affiliated to Dr. Babasaheb Ambedkar Technological University, Lonere, Dist. Raigad, Maharashtra, India
Email: bhosalea1@gmail.com

Abstract*---Objective:*** The day by day new combinations drugs are being introduced in market. Then the multiple therapeutic agents which acts at different sites are used in the management of various diseases and disorders are done. Thus it is necessary to develop methods for analysis with the help of number of analytical techniques which are available for the estimation of the drugs in combination. An accurate, precise and reproducible RP-HPLC method was developed for the simultaneous quantitative determination of METO and BENI in tablet dosage forms. *Methods:* Younglin (S. K.) gradient system UV detector and C_{18} column with 250 mm x 4.6 mm i. d. and 5µm particle size Acetonitrile: OPA water (45: 55v/v) pH 2.5 was used as the mobile phase for the method. The detection wavelength was 230 nm and flow rate was 1ml/min. *Results:* In the developed method, the retention time of Metoprolol and Benidipine were found to be 2.9833 min and 7.3833 min. The developed method was validated according to the ICH guidelines. *Conclusion:* The developed method was validated according to the ICH guidelines. In this methods linearity, precision, range, robustness was within the limits as specified by the ICH guidelines. The method was found to be simple, accurate, precise, economic and reproducible. So, it is worthwhile that, the proposed methods can be successfully utilized for the routine quality control analysis Metoprolol and Benidipine in bulk drug as well as in formulations.

***Keywords*---**metoprolol, benidipine, method- development, validation, HPLC.

Introduction

Pharmaceutical Analysis plays a vital role in quality assurance and quality control of bulk drugs and their formulations. Pharmaceutical analysis is a particular branch of analytical chemistry, which includes isolating, identifying and determining the relative amounts of compounds in a sample matter. It is concerned with chemical characterization of matter both quantitative and qualitative. In recent years many analytical techniques have been developed. Analytical method is a particular utilization of a procedure to solve a problem. Analytical instrumentation assumes an imperative part in the production and evaluation of new products and protection of Consumers and the environment. This instrumentation provides the lower detection limits required to assure safe foods, medications, water and air.

Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications. There are two important reasons for validating assays in the pharmaceutical industry. The first, and by for the most important, is that assay validation is an integral part of the quality control system. The second is that current good manufacturing practice regulation requires assay validation. Metoprolol (MET) is beta blocker, which is official in IP,[1] chemically it is 1-[4-(2-methoxyethyl) phenoxy]-3-[(1-methylethyl) amino]-2-propanol Fig. 1.[2, 3] Literature reveals UV spectrosctroscopy ,[4] HPLC,[5-8] chemometric-assisted spectrophotometric and HPLC method,[9-10] GC-MS,[11] liquid chromatography tandem mass spectrometry methods have been reported for the estimation of metoprolol.[12]

Benidipine (BEN) 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 Fig. 2.[13, 14] 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.[15, 16] Analytical method development and validation play important roles in the discovery, development and manufacture of pharmaceuticals.[17] As per the literature review, there are no analytical methods are reported for the simultaneous estimation of Metoprolol and Benidipine hydrochloride in combined pharmaceutical dosage form. Various publications are available regarding the UV simultaneous estimation, HPTLC and RP-HPLC method development of Metoprolol succinate,[18-22] either alone or in combination with other drugs in pharmaceutical dosage form. But there are a few methods developed for the estimation of Benidipine hydrochloride in its single dosage form and in combination with other drugs.[23-26] The present study aimed to develop a simple, sensitive, short retention time and accurate RP-HPLC method for the simultaneous determination of both Metoprolol and Benidipine together in pure and tablet dosage forms with high sensitivity, selectivity that can be used for the routine analysis of production samples.

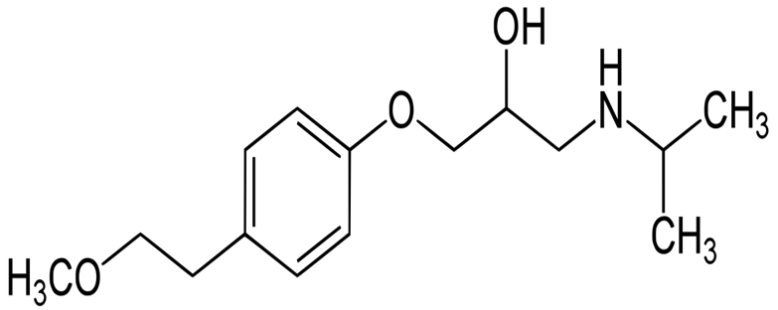

Fig 1. Structure of Metoprolol

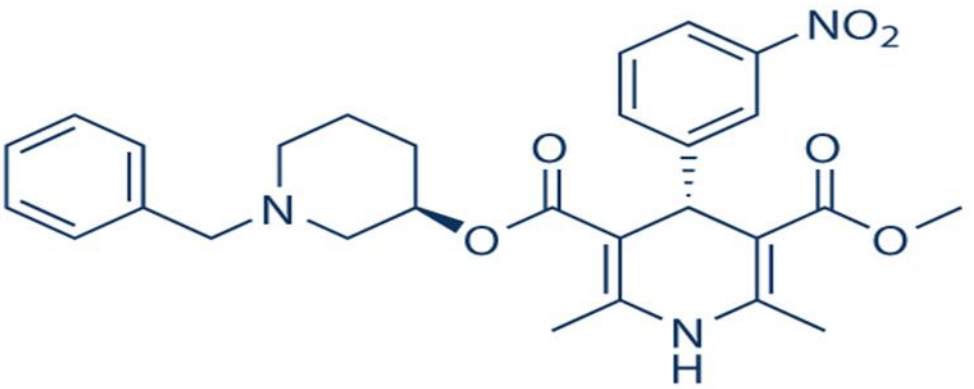

Fig 2. Structure of Benidipine

Materials and Methods

Materials and Reagents

The analysis of the drug was carried out on Youngline (S. K.) gradient system UV Detector. Equipped with reverse phase (Grace) C_{18} column (4.6mm x 250mm; 5µm), a SP930D pump, a 20µl injection loop and UV730D Absorbance detector and running autochro-3000 software. Metoprolol and Benidipine were procured from R.S.I.T.C Jalgaon. Orthophopsphoric acid (OPA) (Avantor Performance material India Ltd. Thane, Maharashtra) and methanol, acetonitrile, (HPLC grade Merck Specialties Pvt. Ltd. Shiv Sager Estate 'A' Worli, Mumbai.), water, 0.45 µm filter (Millipore, Bangalore). Metoprolol and Benidipine are obtained as gift samples from R.S.I.T.C Jalgaon. Marketed formulation Benidip M (Precia Pharma Pvt. ltd.) was procured from the local market.

Chromatographic Conditions

Column C_{18} (250 mm× 4.6 mm); particle size packing 5µm ; detection wavelength of 230 nm; flow rate 1 ml/min; temperature ambient; sample size 20 µl; mobile phase Acetonitrile: water (OPA 0.1% PH 3) (45:55); run time of 15 min.

Preparation of standard stock solution

50mg of Metoprolol and 40 mg of Benidipine were weighed accurately and transferred to a 10 ml volumetric flask dissolved in methanol and diluted to 10 ml with the mobile phase Acetonitrile + 0.1% OPA water (45 + 55% v/v) to give a stock solution of 5000 µg/ml Metoprolol and 1000 µg/ml Benidipine Table 1 and Fig. 3.

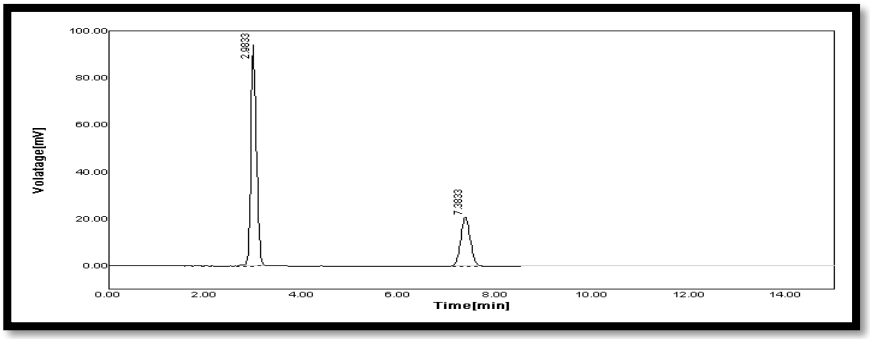

Fig 3. Chromatogram of standard Combination of Metoprolol and Benidipine

Table 1
Details of chromatogram of standard combination containing MET and BEN

No.	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	2.9833	747.6716	72.57	2776.2	1.2143	0.000
2	7.3833	282.5911	27.43	6439.3	1.000	12.5714
Sum		1030.2627				

Method development and validation

Serial dilutions were done to prepared various concentration stock (Standard solution and diluted to get required concentration for calibration plot and which was injected) [26-34].

Assay preparation for commercial formulation

For analysis of the tablet dosage form, weigh 20 Metoprolol (50mg) and Benidipine (4mg) combination tablets and calculated the average weight, accurately weigh and transfer the sample equivalent to 12.2 mg MET and BEN into 10 ml volumetric flask. Add about 10ml methanol of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45 µm nylon membrane filter. Then volume was made up to the mark with Acetonitrile + 0.1% OPA water (45 + 55% v/v). The simple chromatogram of test MET and BEN shown in Fig. 4. The amounts of MET and BEN per tablet were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated five times with tablet formulation. Tablet Assay for % Label claim for % RSD Calculated, Result was shown in Table 2.

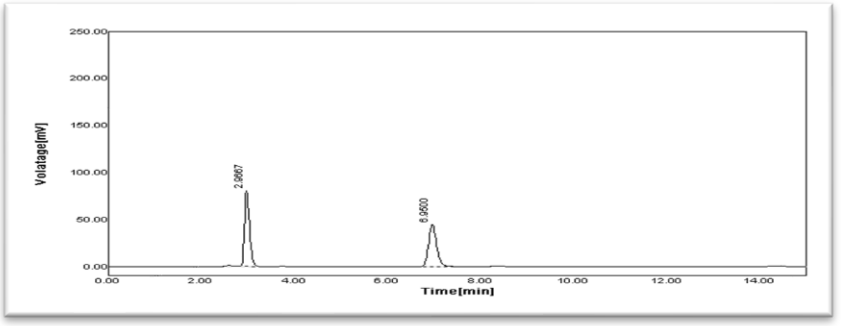

Fig 4. Chromatogram for marketed formulation

Table 2
Analysis of marketed formulation

Assay	Drug	Label Claimed	Amt. Found	% Label Claim	S.D.	%RSD
RP-HPLC Method	MET	200	200.71	100.36	3.16	0.51
	BEN	16	16.51	103.19	3.64	0.11
	MET	200	202.15	101.08	1.02	4.10
	BEN	16	16.30	102.25	0.65	0.51

Results

Linearity and Range

The data obtained in the calibration experiments when subjected to linear regression analysis showed a linear relationship between peak areas and concentrations in the range 50-250 µg/ml for MET and 4-20 µg/mL for BEN Table 3 and 4 depict the calibration data of MET and BEN. The respective linear equation for Metoprolol was y = 3.1148x - 3.991 and Benidipine equation y = 32.639x + 19.904where x is the concentration and y is area of peak. The correlation coefficient was 0.9987. The calibration curve of MET and BEN shown in Fig. 5 and 6.

Table 3
Linearity data for Metoprolol

Method	Conc µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
RP-HPLC Method	50	144.25	146.26	145.26	1.42	0.98
	100	309.471	303.03	306.25	4.55	1.49
	150	478.61	475.63	477.12	2.11	0.44
	200	625.34	616.33	620.84	6.37	1.03
	250	772.04	761.28	766.66	7.61	0.99
	Equation		y = 3.1148x - 3.991			
	R^2		0.9987			

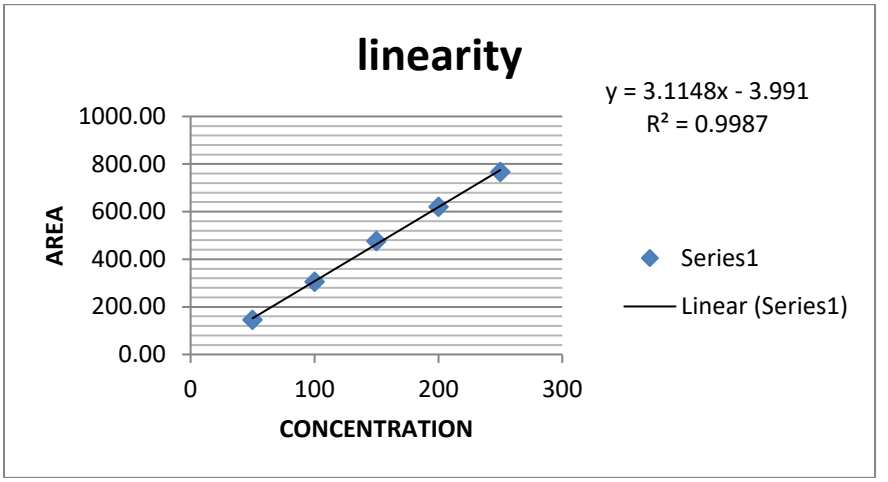

Fig 5. Calibration curve of Metoprolol

Table 4
Linearity data for Benidipine

Method	Conc. µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
RP-HPLC Method	4	141.59	144.83	143.21	2.29	1.60
	8	285.61	279.66	282.64	4.21	1.49
	12	415.71	422.16	418.94	4.56	1.09
	16	555.91	547.05	551.48	6.26	1.14
	20	658.02	665.1	661.56	5.01	0.76
	Equation		y = 32.639x + 19.904			
	R^2		0.9981			

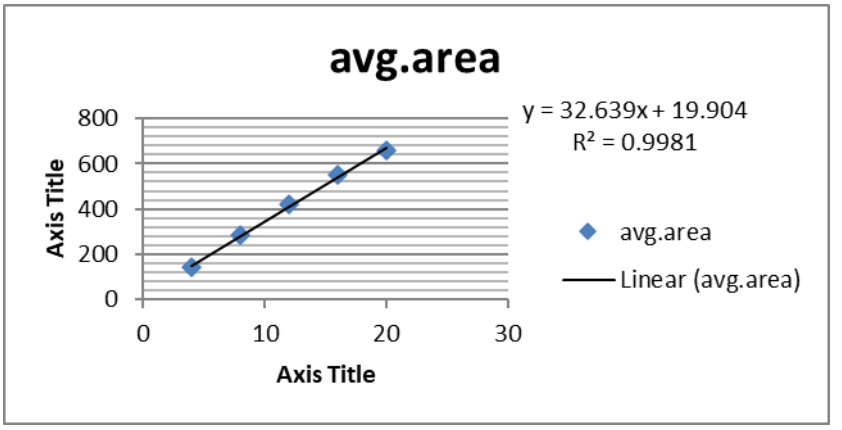

Fig 6. Calibration curve of Benidipine

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To a pre-analysed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed**.** The % recovery was found to be within 98-101%. Statistical validation of recovery studies are shown in Table 5, 6 and Fig. 7, 8 and 9.

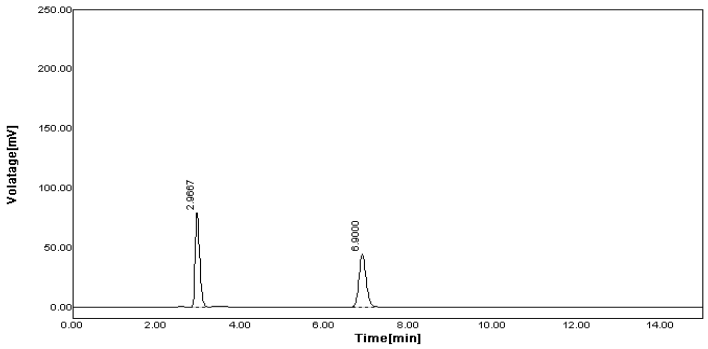

Fig 7. Chromatogram of Accuracy 80%

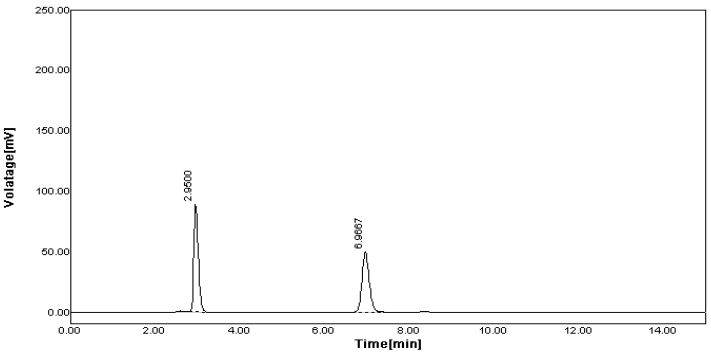

Fig 8. Chromatogram of Accuracy 100%

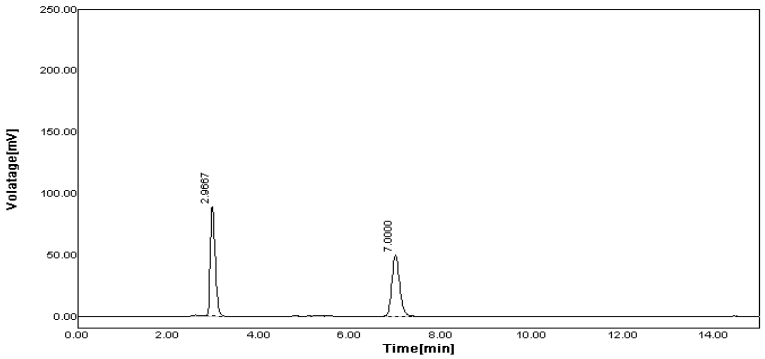

Fig 9. Chromatogram of Accuracy 120%

Table 5
Result of recovery data for Metoprolol and Benidipine

Method	Drug	Level (%)	Amt. taken (µg/ml)	Amt. Added (µg/ml)	Absorbance Mean* ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
RP-HPLC Method	MET	80%	50	40	89.47±0.37	39.47±0.37	98.19±0.21
		100%	50	50	99.53±0.23	49.56±0.23	99.07±0.46
		120%	50	60	109.88±0.42	59.88±0.42	99.81±0.71
	BEN	80%	4	3.2	7.18±0.06	3.18±0.06	99.37±1.84
		100%	4	4	7.96±0.04	3.96±0.04	99.15±1.01
		120%	4	4.8	8.78±0.08	4.78±0.08	99.63±1.61

*mean of each 3 reading for RP-HPLC method

Table 6
Statistical validation of recovery studies Metoprolol and Benidipine

Method	Level of Recovery (%)	Drug	Mean % Recovery	SD*	% RSD
RP-HPLC Method	80%	MET	98.19	0.21	0.22
		BEN	99.37		1.84
	100%	MET	99.07	0.46	0.46
		BEN	99.15		1.01
	120%	MET	99.81	0.71	0.71
		BEN	99.63		1.61

*Denotes average of three determinations for RP-HPLC method

System suitability parameters

To ascertain the resolution and reproducibility of the proposed chromatographic system for estimation of MET and BEN system suitability parameters were studied. The result shown Fig. 10 and Table 7.

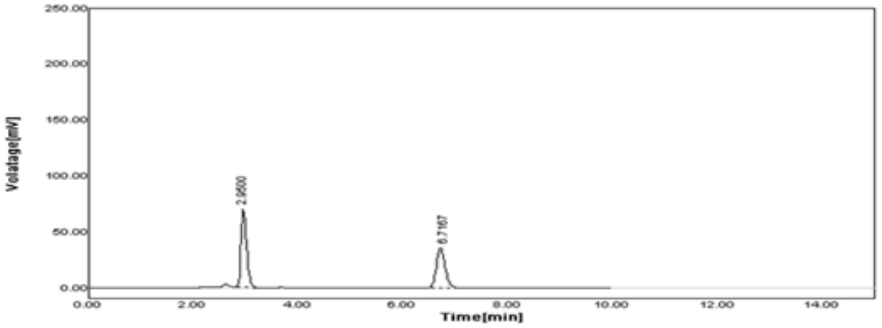

Fig 10. Chromatogram of system suitability

Table 7
Repeatability studies on RP-HPLC for Metoprolol and Benidipine

Method	Conc. of MET and BEN (mg/ml)	Peak area	Amount found (mg)	% Amount found
RP-HPLC Method for MET	150	470	149.64	99.76
	150	470.23	149.72	99.81
	150	479.34	152.64	101.76
	150	473.82	150.87	100.58
	150	469.95	149.63	99.75
		Mean	150.50	100.33
		SD	1.31	0.87
		%RSD	0.87	0.87
RP-HPLC Method for BENI	12	411.41	11.99	99.98
	12	411.57	12.00	100.02
	12	410.91	11.98	99.83
	12	412.23	12.02	100.17
	12	417.34	12.20	101.67
		Mean	12.04	100.33
		SD	0.09	0.75
		%RSD	0.76	0.75

Precision

The method was established by analyzing various standards of MET and BEN. All the solution were analyzed thrice in order to record any intra-day & inter-day variation in the result. The result obtained for interday and intraday variation are shown in the Table 8.

Table 8
Result of Intra day and Inter day Precision studies on RP-HPLC method for MET and BEN

Method	Drug	Conc. (µg/ml)	Intraday Precision		Interday Precision	
			Mean± SD	%Amt Found	Mean± SD	%Amt Found
Rp-HPLC	MET	100	306.31± 4.99	99.64	0.1499±0.0014	0.94
		150	475.35±6.34	100.9	0.5635±0.001	0.025
		200	623.22±3.07	100.7	0.9881±0.008	0.08
	BEN	8	286.62± 2.98	8.174	0.0565±0.0007	100.00
		12	414.96±3.54	12.1	0.1815±0.004	99.54
		16	546.79±2.97	16.14	0.3092±0.0010	99.23

*Mean of each 3 reading for RP-HPLC method

Robustness

To evaluate the robustness of the proposed method, small but deliberate variations in the optimized method parameters were done. The effect of changes in mobile phase composition and flow rate on retention time and tailing factor of drug peak was studied. The results indicate that less variability in retention time and tailing factor were observed Table 9 and 10.

Table 9
Result of robustness study of Metoprolol

Parameters	Conc. (µg/ml)	Amt. of detected (mean ±SD)	%RSD
Chromatogram of flow change 0.9ml	150	465.7±6.34	1.36
Chromatogram of flow change 1.1 ml	150	525.59±5.26	0.99
Chromatogram of comp change 46 ACN+54 water	150	578.89±6.69	1.16
Chromatogram of comp change 44ACN+ 56 water	150	581.02±0.82	0.14
Chromatogram of comp change wavelength change 229 nm	150	545.48±2.37	0.43
Chromatogram of comp change wavelength change 231nm	150	598.80±2.02	0.34

Table 10
Result of robustness study of Benidipine

Parameters	Conc. (µg/ml)	Amt. of detected (mean ±SD)	%RSD
Chromatogram of flow change 0.9ml	12	435.64±0.83	0.19
Chromatogram of flow change 1.1 ml	12	465.82±2.84	0.61
Chromatogram of comp change 46 ACN+54 water	12	468.36±7.01	1.50
Chromatogram of comp change 44ACN+ 56 water	12	479.37±8.55	1.78
Chromatogram of comp change wavelength change 229nm	12	486.20±8.39	1.72
Chromatogram of comp change wavelength change 231nm	12	475.96±7.69	1.61

Discussion

The proposed methods for simultaneous estimation of MET and BEN in tablet dosage forms were found to be simple, accurate, economical and rapid. The method was validated as per the ICH Q2 (R1) guidelines. Standard calibration yielded correlation coefficient (r^2) 0.999 for both MET and BEN at all the selected wavelengths. The values of % RSD are within the prescribed limit of 2 %, showing high precision of methods and recovery was close to 100% for

both drugs. Results of the analysis of pharmaceutical formulations reveal that the proposed method is suitable for their simultaneous determination with virtually no interference of any additive present in pharmaceutical formulations. Hence, the above methods can be applied successfully for simultaneous estimation of MET and BEN in formulations.

Conclusion

The developed HPLC methods in that linearity, precision, range, robustness were found to be more accurate, precise and reproducible. The methods were found to be simple & time saving. All proposed methods could be applied for routine analysis in quality control laboratories.

Conflicts of interest

All authors have none to declare.

Acknowledgements

The authors are thankful to the Principal, Gangamai College of Pharmacy, Nagaon, Dist. Dhule for providing necessary facilities for research work. They are also grateful to R.S.I.T.C Jalgaon for providing gift samples of pure drugs. Authors are also thankful to two anonymous reviewers.

Abbreviation used

HPLC: High performance liquid chromatography; UV: Ultraviolet; ICH: International Conference on Harmonization; LOQ: Limit of quantitation; LOD: Limit of detection; RSD: Relative standard deviation; RT: Retention time; OPA: Orthophosphoric acid; MET: Metoprolol; BEN: Benidipine; FDA: Food and Drug Administration; SD: Standard deviation.

References

1. Ahmad, S., Ashwin, B., Rahman, B. A., Gorad, R. U., & Tare, H. (2022). Quality by design (qbd) approach to develop stability indicating rp-hplc method development for naproxen and pantoprazole. International journal of health sciences, 6(s4). Https://doi.org/10.53730/ijhs.v6ns4.11131
2. Ahmad, S., Khairnar, M., Bakhshi, A. R., Tare, M., Baheti, D., & Tare, H. (2022). QBD APPROACH TO DEVELOP STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT FOR LEVOSULPIRIDE AND ILAPRAZOLE. INTERNATIONAL JOURNAL OF HEALTH SCIENCES, 6(S5), 7413–7429. Https://doi.org/10.53730/ijhs.v6ns5.11625
3. AHMED O ALNAJJAR, ABUBAKR M IDRIS, MAHESH M ATTIMARAD. Capillary electrophoresis assay method for metoprolol and hydrochlorothiazide in their combined dosage form with multivariate optimization. Journal of chromatographic science. 2013; 51(3): 92-97.
4. CHAWLA S, GHOSH S, SIHORKAR V, NELLORE R, KUMAR TR, SRINIVAS NR. High-performance liquid chromatography method development and validation for simultaneous determination of five model compounds,

antipyrine, metoprolol, ketoprofen, furosemide and phenol red, as a tool for the standardization of rat in situ intestinal permeability studies using timed wavelength detection. Biomed chromatogr., 2005; 20: 349-357.
5. CHIU FC, DAMANI LA, LI RC, TOMLINSON B. Efficient high-performance liquid chromatographic assay for the simultaneous determination of metoprolol and two main metabolites in human urine by solid-phase extraction and fluorescence detection. J chromatogr b biomed sci appl. 1997; 696: 69-74.
6. ESEN BELLURATICI, BEKIR KARLIGA. Identification, synthesis and characterization of process related impurities of benidipine hydrochloride, stress-testing/stability studies and hplc/uplc method validations. Journal of pharmaceutical analysis. 2015; 2(3): 1-15.
7. FDA. Draft guidance for industry: stability testing of drug substances and drug products, fda. Rockville, md. 1998.
8. Food and drug administration draft guidance for industry on analytical procedures and methods validation. Chemistry, manufacturing, and controls documentation; availability, fed. Regist, 2000; 65 (169), 52776–52777.
9. FURBERG CD, PSATY BM, MEYER JV, NIFEDIPINE: dose-related increase in mortality in patients with coronary heart disease. Circulation. 1995; 92:1326-1331.
10. GOHIL K, TRIVEDI P, MOLVI KI, spectrophotometric analysis of amlodipine besylate in bulk and in tablet dosage forms. Indian j pharma sci., 2005; 67: 376-378.
11. GOWDA KV, MANDAL U, SELVAN PS, SOLOMON WD, GHOSH A, SARKAR AK, ET AL . Liquid chromatography tandem mass spectrometry method for simultaneous determination of metoprolol tartrate and ramipril in human plasma. J chromatogr., 2007; 858:13-21.
12. Https://en.m.wikipedia.org/wiki/benidipine [july 24, 2018].
13. ICH HARMONIZED TRIPARTITE GUIDELINE Q1F 2003.stability data package for registration applications in climatic zones iii and iv, london.
14. ICHIYUSHOU, LI NING WANG, YUTAKA TAKAHASHI. Effects of benidipine hydrochloride on antioxidant enzyme activity in stroke-prone spontaneous hypertensive rats (shr-sp). Journal of clinical laboratory analysis. 1997; 11(2): 158-162.
15. INDIAN PHARMACOPOEIA, VOL. 1. NEW DELHi: government of india, the controller of publication. 1996; p. 387-389.
16. International conference on harmonisation guideline on validation of analytical procedures 1995. Definitions and terminology; availability, fed. Regist, 60 (40), p. 11260–11262.
17. International conference on harmonisation guidance for industry, q1a(r2) 2000. Stability testing of new drug substances and products, ifpma. Geneva.
18. International conference on harmonisation guideline on the validation of analytical procedures. Methodology; availability, fed. Regist, 1997; 62 (96): 27464-27467.
19. JADHAV A S, TARKASE K N, DESHPANDE A P. Quantitative determination of metoprolol succinate in bulk and tablet dosage form through comparative study of uv and derivative spectroscopy. Scholars research library. 2012; 4(3): 763-767.
20. Japanese pharmacopoeia. Official monographs. 17th edition. 2016; 476-478.

21. KALYAN L, KUNTURKAR, HEMANT KUMAR JAIN. Development and validation of uv spectrophotometric method for determination of s (-) metoprolol succinate and clopidogrel bisulphate in bulk and tablet dosage form. International journal of pharmacy and pharmaceutical sciences. 2013; 5(3): 593-598.
22. MANISHA B JADHAV, SACHIN S SURYAWANSHI, SACHIN R JAJANE. Development and validation of uv spectrophotometric method for determination of metoprolol succinate and telmisartan in tablet dosage form. International journal of pharmacy and pharmaceutical sciences. 2012; 4(3): 387-389.
23. MARIA PQ, ANNA MB, LUCIANO M, GUISEPPE F, FABIO T. Simultaneous determination of propranolol or metoprolol in the presence of butyrophenones in human plasma by gas chromatography with mass spectrometry. J pharm sci., 1993; 82:187-190.
24. MASOOM R S, ZEID A A, NAFISUR R. Analytical techniqes in pharmaceutical analysis: a review. Arabian journal of chemistry., 2017: 10(2): s1409-s1421.
25. MUNTWYLER J, FOLLATH F. Calcium channel blockers in treatment of hypertension. Prog cardiovasc dis. 2001; 44: 207-216.
26. PAI PN, SHENOY KR, PANDEY J. Simultaneous reverse phase liquid chromatographic determination of metoprolol tartarate and hydrochlorothiazide in tablets., indian j pharma sci. 2005; 67: 608-610.
27. PSATY BM, heckbert sr, koepsell td, siscovick ds, raghunathan te, weiss ns, et al. The risk of myocardial infarction associated with antihypertensive drug therapies. Jama. 1995; 274: 620-5.
28. QIN LI, RUI W. Simultaneous analysis of tramadol, metoprolol and thier metabolites in human plasma and urine by hplc. Chinese med j. 2006; 119: 2013-7.
29. RONTOGIANNI MA, markopoulou ck, koundourellis je. Hplc and chemometrically-assisted spectrophotometric estimation of two binary mixtures for combined hypertension therapy. J liquid chromatogr related technol., 2006; 29: 2701-19.
30. SALEM H, ABDALLAH OM. Determination of metoprolol and felodipine in binary mixture using chemometric-assisted spectrophotometric and high-performance liquid chromatographic-uv methods. Am j appl sci., 2007; 4: 709.
31. SEEMA M DHOLE, DINESH R CHAPLE, MADHULIKA T HARDE. Validated uv spectrophotometric method for simultaneous estimation of metoprolol succinate and amlodipine besylate in their combined tablet dosage form. International journal of analytical and bioanalytical chemistry., 2013; 3(3): 82-85.
32. SRAVANI M. Estimation of benidipine by using hplc. International journal of pharma and chemical research. 2017; 3(4): 775-806.
33. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Health and treatment of diabetes mellitus. International journal of health sciences, 5(1), i-v. Https://doi.org/10.53730/ijhs.v5n1.2864
34. SWEETMAN SC. Martindale, the complete drug reference. 32nd ed. Pharmaceutical press; p. 822, 907.
35. VEERASKARAN V, KATAKDHOND SJ, KADAM SS, JADHVI RR. Simultaneus spectrophatometric estimation of hydrochlorthiazide and metoprolol tartarate from combined dosage form. Indian drug. 2001; 38:187.

36. Widjaja, G. (2021). Impact of human resource management on health workers during pandemics covid-19: systematic review. International journal of health & medical sciences, 4(1), 61-68. Https://doi.org/10.31295/ijhms.v4n1.850