Development and Validation of Rp-Hplc Method For Estimation of Bisoprolol Fumarate In Bulk and Tablets

Dr. Bhupendra L. Deore[1], Mr. Sagar Kailas Wagh[1]
Department of Pharmaceutical Quality Assurance,
DCS's A.R.A. College of Pharmacy Nagoan Dhule, India.

Received : 12 December 2023 Accepted : 28 December 2023

Abstract

A RP-HPLC method has been developed and validated for the determination of Bisoprolol fumarate in bulk and in tablet formulation. The HPLC analysis was performed on the **Fortis RP C_{18} column** (250 mm ×4.6 mm) 5µm particle size in gradient mode, at 30^0c using **Methanol: Water (80:20) pH adjusted to 8 with triethylamine**as mobile phase**;** flow rate was set at **1.0** mL/min. The detection was carried out at 230nm. The retention time forBisoprolol fumarate was found to be found to be **6.312± 0.02 min.** Bisoprolol fumarate followed linearity in the concentration range of **4- 24 µg/mL** (r^2 **= 0.999**). The method has successively been applied for the determination of Bisoprolol fumaratein marketed formulation. There was no interference from the excipients routinely present in the tablet. The drug content for Bisoprolol fumarate was found to be **99.08 % ± 0.52**. Accuracy of the method was studied by the recovery studies at three different levels i.e. 80 %, 100 % and 120 % level. The % recovery was found to be within the limits of the acceptance criteria within range of **99.03 – 99.31 %**.

The precision of the method was studied as repeatability of sample application, intra-day and inter-day precision. The results were examined as %RSD values of concentration of drugs determined. The low value of %RSD (less than 2) indicates high precision of the method. The method proved to be adequately sensitivity as indicated by low values of **LOD** and **LOQ**.

Keywords - Bisoprolol fumarate and RP-HPLC Method.

Introduction

Bisoprolol fumarate is chemically: (*RS*)-1-[4-[[2-(1- Methylethoxy) ethoxy] methyl] phenoxy] -3-[(1 methyl ethyl) amino] propan-2-ol fumarate. It is a Beta-adreno receptor antagonist and used as an Anti - Hypertensive Drug. It is official in BP, USP Bisoprolol fumarate alone (or) in combined.

Author Correspondence
Dr. Bhupendra Deore
DCS's A.R.A. College of Pharmacy Nagoan Dhule
Email : bldeore@aracopdhule.org

In this study a simple, accurate and precise UV-spectrophotometric method was developed for the estimation of bisoprolol fumarate (BF) in bulk and tablet dosage form. The method was based on measurement of absorbance of BF aqueous solution at 271nm. Validation was conducted in accordance to ICH guidelines. The calibration curve was linear in the concentration range 5-25 µg/mL with correlation coefficient not less than 0.9986. The limit of detection and limit of quantification were 0.22 µg/ml and 0.66 µg/ml,

respectively. Intraday and intermediate precision of the developed method were reflected by the low RSD% values (1.19 and 0.854, respectively). The recovery percentage was 105.0 ± 1.3%, n=3. The proposed method was applied for the assay of BF in three different brands.

Methods and Materilas :

Reagent and Chemicals

Pure standard Bisoprolol fumarate powder was provided by Cipla Pharmaceutical Mumbai. Acetonitrile, Methanol, water and triethylamine all of HPLC grade were purchased from Merck, Mumbai (India). Bisoheart ® - 5tablets having 5mg label claim was purchased from a local market.Double distilled water was prepared by distillation assembly.

Optimization of Chromatographic conditions

The Fortis RP C18 column (250 mm x 4.6 mm i.d., 5µm) was used for separation of drug, which gives satisfactory resolution and run time. Mobile phase was optimized with a view to separate Bisoprolol fumarate. Initially, methanol and water in the various proportions were tried as mobile phase but the tailing of the peak was observed as shown in **Figure 5.a**. Adjustment of pH of aqueous phase combination of methanol and water was tried for resolution of the drug but till the problem was not solved, peak was shown in **Figure 5.b**.Finally,good resolution and symmetric peak was obtained for drug when the methanol in combination with water used and pH of water adjusted to 8 with triethyl amine. The flow rate of the mobile phase was 1.0 mL/min. Under optimum chromatographic conditions, the retention time for bisoprolol fumarate was found to be 6.312 ± 0.02 min and the detection was carried out at 230 nm.The chromatographic conditions were shown in **Table 5.1** while the typical chromatograph was showed in **Figure 5.c.**

Optimization of mobile phase pH

From the chromatographic conditions, it was observed that; pH of water set at 8was suitable for drug.

Optimization of Detection Wavelength

Detection using PDA detector at different wavelength was performed. Finally, 230 nm wavelengths were selected as detection wavelength.

Preparation of Stock Standard Solution

Stock standard solution was prepared by dissolving 10 mg of Bisoprolol fumarate in 100 mL of methanol to obtain concentration 100µg/mL.

Linearity Studies

From stock standard solution, an appropriate volume in the range of 0.4 – 2.4 mL were transferred into six separate 10mL volumetric flask and volume was made up to the mark to obtain concentration in the range of 4- 24 µg/mL. From each volumetric flask, a volume of 20µL solution was manually injected with the help of Hamilton Syringe. All measurements were repeated six times for each concentration and calibration curves were constructed by plotting the peak area *versus* the corresponding drug concentration.Calibration curves are shown in **Figure5.d** and results for linearity shown in **Table 5.2.Analysis of Bulk Material**

Accurately weighed quantity 10 mg of bisoprolol fumarate was transferred in to 100 mL volumetric flask. It was dissolved in methanol and volume was adjusted to mark. The solution was further diluted to get concentration 12 µg/mL which was subjected to proposed method and amount of bisoprolol fumaratewas determined. The procedure was repeated for six times; results are shown in **Table 5.3.**

Analysis of marketed Formulation

Ten tablets were weighed accurately and powdered. An amount of the powder equivalent to 5mg of bisoprolol fumaratewas transferred to 100 mL volumetric flaks containing 50mL of methanol, shaken manually for 20 min and volume was made up to the mark using same solvent and filtered

through Whatmann filter no. 41. From it,2.4 mL was diluted to 10mL to get 12µg/mL solutions and injected into system for analysis. Results are shown in **Table5.4**

Validation

The proposed method was validated as per ICH guidelines. The solutions of the drug were prepared as per the earlier adopted procedure given in the experiment.

Accuracy (Recovery Study)

Accuracy was determined by performing recovery studies by spiking different concentration of pure drug in pre-analyzed sample solution. To analyzed sample solution(8 µg/mL), a known amount of stock standard solution was added at different level i.e. 80%, 100% and 120%. The solutions were re-analyzed by proposed method; the results are shown in Table 5.5**.**

Precision

Precision of the method was studied as intra-day and inter-day variations and also as repeatability.

Intra – day and Inter – day Precision

Intra-day precision was determined by analyzing 8, 12 and 20 µg/mL of bisoprolol fumarate solutions for three times in the same day. Inter-day precision was determined by analyzing same concentration at three different days over a period of week ;the results are shown in **Table 5.6.**

Repeatability

Repeatability was determined by analyzing 8µg/mL concentration of bisoprolol fumarate for six times within short interval of time within a day and the results are shown in **Table 5.7.**

Sensitivity

The sensitivity of measurement of bisoprolol fumarate by the use of the proposed method was determined in terms of the LOD and LOQ. The LOD and LOQ were calculated using equation LOD = 3.3 X N/B and LOQ = 10 X N/B; Where, 'N' is standard deviation of the peak areas of the drug (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve. It was performed in range 4-8 µg/mL.

Specificity and Selectivity

The analyte should have no interference from other extraneous components and be well resolved from them. Specificity is a procedure to detect quantitatively the analytein presence of component that may be expected to be present in the sample matrix,while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix.The method is quite selective. There was no other interfering peak around the retention time of bisoprolol fumarate; also, the base line did not show any significant noise.

Ruggedness

Appropriate volume of sample solution, 8µg/mL was prepared and analyzed by two different analysts using similar operational and environmental conditions. Area was measured for same concentration solutions, six times.The results are shown in **Table 5.8.**

Robustness

Robustness of the method was studied by making deliberate variations in the chromatographic conditions and effects on the peak areas were recovered. Different chromatographic parameters such as variations in flow rate, mobile phase composition and change in pH of mobile phase were made. It was performed using 12µg/mL of bisoprolol fumarate and the effects on the peak areas were recorded. The each parameter was repeated for six times. The results are shown in **Table 5.9.**

Table 5.2: Linearity Study of Bisoprolol fumarate

Concentration of Bisoprolol fumarate [µg/mL]	Peak Area [Mean ± SD; n = 5]	% RSD
4	32508.12 ± 378.61	1.10
8	68275.61 ± 335.73	0.49
12	98398.25 ± 897.86	0.91
16	136772.75 ± 1126.80	0.82
20	170724.75 ± 613.28	0.35
24	267636.25 ± 710.10	0.34

Table 5.3: Analysis of bulk material

Component	Amount taken in[µg/mL]	Amount Found [µg/mL]± SD	% Amount found	%RSD [n=6]
Bisoprolol fumarate	12	11.93	99.41	0.31

Table 5.4: Analysis of Tablet formulation

Brand Name: Bisoheart ® - 5Mfg. By: Mankind Pharmaceutical

Component	Amount takenin [µg/mL]	Amount Found [µg/mL] ± SD [n = 6]	% Amount found ± SD	% RSD
Bisoprolol fumarate	12	11.89	99.08 ± 0.52	0.52

Table 5.5: Accuracy studies

Drug	Initial amount [µg/mL]	Excess drug added to the analyte [%]	Amount recovered ± S.D. [µg/mL]	Recovery [%]	%RSD [n = 3]
Bisoprolol fumarate	8	80	14.3± 0.12	99.30	0.45
	8	100	15.89± 0.15	99.31	0.34
	8	120	17.43± 0.15	99.03	0.65

Table 5.6: Precision studies

Drug	Conc. [µg/mL]	Intra –day Amount Found [%] [n = 3]		Inter- day Amount Found [%] [n = 3]	
		Mean	% RSD	Mean	% RSD
Bisoprolol fumarate	8	99.23	0.49	99.54	0.71
	12	99.12	0.68	99.78	0.86
	20	98.83	0.91	99.01	1.02

Table 5.7:Repeatability studies

Drug	Concentration [µg/mL]	Peak Area Mean ± SD, [n = 6]	% RSD
Bisoprolol fumarate	8	68200.75	0.56

Table5.8: Ruggedness study

Drug	Amt in µg/mL	% Amount Found [n = 6]		% RSD	
		Analyst I	Analyst II	Analyst I	Analyst II
Bisoprolol fumarate	12	99.65	99.33	0.74	0.92

Table 5.9: Robustness Studies

Sr. No.	Parameter	% RSD
	Mobile phase composition	
1.	a) Methanol : water (78: 22 *v/v*)	0.53
	b) Methanol : water (82: 18 *v/v*)	0.38
	pH of Mobile Phase	
2.	a) 8.1	0.97
	b) 7.9	0.47
	Change in Flow Rate	
3.	a) 0.9	0.65
	b) 1.0	0.82

Table 5.10: System Suitability Test

System suitability Parameters	Bisoprolol Fumarate
Retention time (min)	6.3
Theoretical Plates(N)	8386.15
Tailing Factor	1.01
Capacity factor	1.5

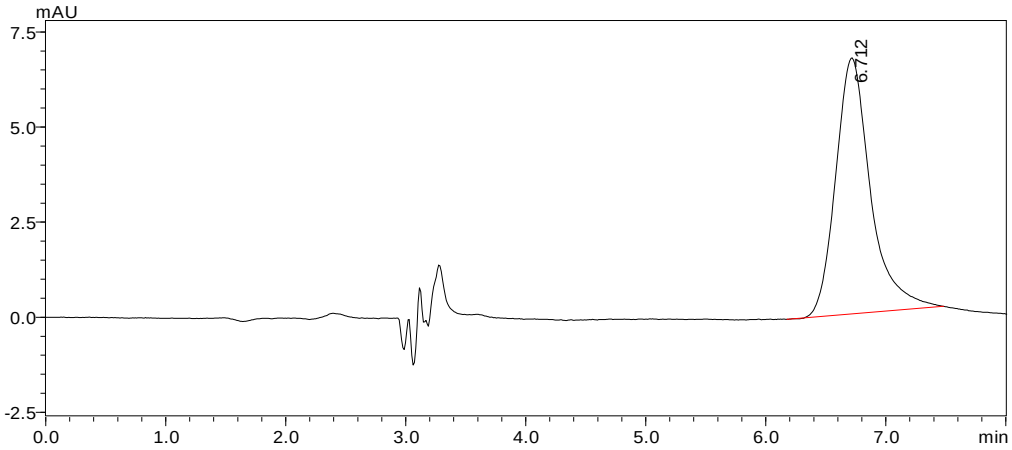

Figure 5.a Chromatogram of Bisoprolol fumarate in methanol : water (80:20 % *v/v*) as mobile phase.

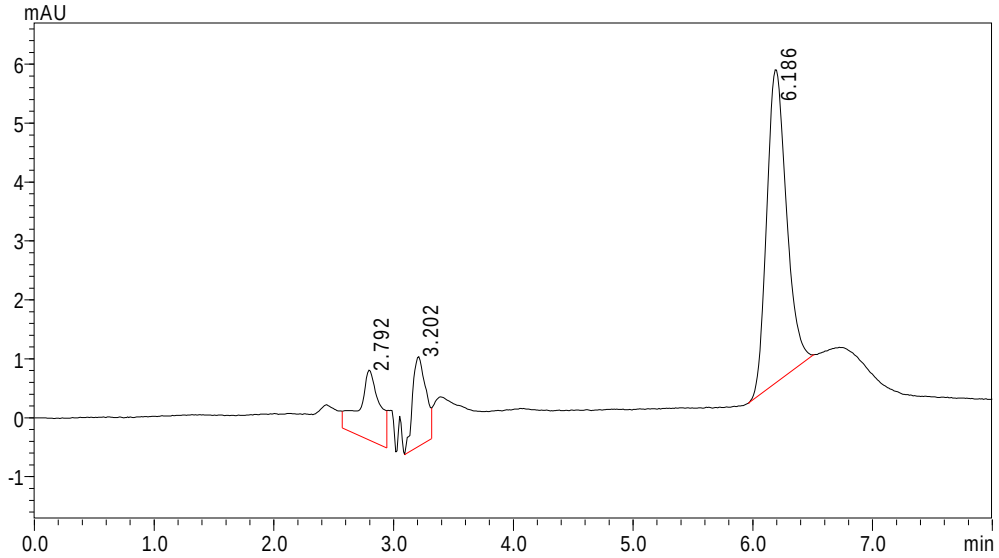

Figure 5.b Chromatogram of Bisoprolol fumarate in methanol : water at pH8(70:30 % *v/v*) as mobile phase.

Summary and Conclusion :

Bisoprolol is a beta-1 adrenergic blocking agent used to prevent myocardial infarction and heart failure and to treat mild to moderate hypertension. Bisoprolol is a cardioselective β1-adrenergic blocking agent used to treat high blood pressure. It is considered a potent drug with a long-half life that can be used once daily to reduce the need for multiple doses of antihypertensive drugs. Bisoprolol is generally well tolerated, likely due to its β1-adrenergic receptor selectivity and is a useful alternative to non-selective β-blocker drugs in the treatment of hypertension such as Carvedilol and Labetalol. It may be used alone or in combination with other drugs to manage hypertension and can be useful in patients with chronic obstructive pulmonary disease (COPD) due to its receptor selectivity.

It is available in Indian market in the form of tablet dosage form.As very few analytical methods are available in literature for analysis of Bisoprolol fumarate in bulk and in tablet formulation; therefore, established RP-HPLC method and summarized as follows

Method :

Development and Validation of RP-HPLC method for estimation of beta-blocker Bisoprolol fumarate in Bulk and Tablets

A RP-HPLC method has been developed and validated for the determination of Bisoprolol in bulk and in tablet formulation. The HPLC analysis was performed on the **Fortis RP C_{18} column** (250 mm ×4.6 mm) 5µm particle size in gradient mode, at 30^0c using **Methanol: Water (80:20) pH adjusted to 8 with triethylamine** as mobile phase**;** flow rate was set at **1.0** mL/min. The detection was carried out at 230nm. The retention time forBisoprolol fumarate was found to be found to be **6.312± 0.02 min.** Bisoprolol fumarate followed linearity in the concentration range of **4-24 µg/mL** (**r^2 = 0.999**). The method has successively been applied for the determination of Bisoprolol fumarate in marketed formulation. There was no interference from the excipients routinely present in the tablet. The drug content for Bisoprolol fumarate was found to be **99.08 % ± 0.52**. Accuracy of the method was studied by the recovery studies at three different levels i.e. 80 %, 100 % and 120 % level. The % recovery was found to be within the limits of the acceptance criteria within range of **99.03 – 99.31 %**.

The precision of the method was studied as repeatability of sample application, intra-day and inter-day precision. The results were examined as %RSD values of concentration of drugs determined. The low value of %RSD (less than 2) indicates high precision of the method. The method proved to be adequately sensitivity as indicated by low values of **LOD** and **LOQ** .Summary of developed method is shown in **Table 6.1**

Table 6.1: Summary of RP-HPLC method

Parameter	**Bisoprolol fumarate**
Linearity range [µg/mL]	4-24
Correlation coefficient	0.999
LOD [µg]	0.39
LOQ [µg]	1.17
% Recovery [n = 3,% RSD]	0.45-0.65
Ruggedness	
Analyst I [n = 6]	0.74
Analyst II [n =6]	0.92

Precision [% RSD]	
Repeatability of Injection [n = 6]	0.56
Intra-day [n = 3]	0.49-0.91
Inter-day [n = 3]	0.71-1.02
Robustness	Robust
Specificity	Specific
Retention time [t_R]	6.312
Theoretical plates [N]	8386.15
Capacity factor [k']	1.5
Tailing Factor [T]	1.01

References :

1. Katz, E., 2009. Quantitative analysis using chromatographic techniques. John Wiley & Sons.
2. D. A. Skoog, F. J. Holler and T.A. Nieman, Principles of Instrumental Analysis, 5th edn Thomson Brook/cole, 2005, pp. 674-696.
3. K. A. Connors, Liquid Chromatography- A Textbook of Pharmaceutical Analysis, 3rd edn., Willey Interscience, New York, 1999, pp. 373-438.
4. H. Beckett, J. B. Stenlake, Practical Pharmaceutical Chemistry, 4thedn., Part II, CBS Publications and Distributors, New Delhi, 1997, pp. 1, 275-300.
5. E. Heftman, Chromatography- Fundamentals & applications of Chromatography and Related differential migration methods, 6thedn, Elsevier, Amsterdam, Vol. 69A, 2004, pp. 253-291.
6. P. D. Sethi, Introduction – High Performance Liquid Chromatography, 1stedn, CBS Publishers, New Delhi, , 2001, pp.1-28.
7. J. Swadesh, HPLC –Practical and Industrial Applications–CRC Press, Boca Raton, 1997, pp.20-25.
8. P. W. Scott, Liquid Chromatography Column Theory, John Willey and Sons, Chi Chester,2001, pp. 1-13.
9. C. D. Gary, Analytical chemistry, 5th edn. John Wiely& sons, Inc., 2001, pp. 1- 3.
10. L. R. Synder, J. J. Kirkland, L. J. Glajch, Practical HPLC Method Development, 2ndedn., JohnWiley & sons, Inc, 1997, pp. 21-57, 653-660.
11. Reviewer Guidance, Validation of Chromatographic Methods (CDER), Nov.1994
12. Vogel's Text book of quantitative chemical analysis, 5th edn., Longman Group UK Ltd., 1989, pp. 1-5.
13. K. Sharma, Instrumental Method of chemical Analysis, 21stedn., Goel publishing house,2002, pp. 9-16.
14. P. W Scott, Raymond, Encyclopedia of Chromatography, 10th edn., Marcel Dekker, Inc. USA,2001, pp. 252-254.
15. H. H. Willard, L. L. Merritt, J. A. Dean, and F. A. Settle, Instrumental Method for Analysis, 7th edn, CBS publishers and distributors, New Delhi, 1986, pp. 513-530.
16. V. Kasture, S. G. Wadodkar, K. R. Mahadik, and H. N. More, Textbook of Pharmaceutical Analysis – II, 11thedn., Published By Nirali Prakashan, 1996, pp. 156-165.
17. E. Heftman, Chromatography- Fundamentals & applications of Chromatography and Related differential migration methods,6thedn., Elsevier, Amsterdam, Vol. 69A, 2004, pp. 72-87.
18. J. Cazes, P. W. Scott, Raymond, Chromatography Theory, Marcel Decker, Inc, NY, 2002, pp. 443-454.

19. P. D. Sethi, HPTLC: Quantitative Analysis of Pharmaceutical formulation, 1st edn., CBS Publications, New Delhi, 1996, pp.162-165.
20. E. Stahl, Thin Layer Chromatography: A Laboratory Handbook, 2nd edn, Springer InternationalEdition, pp.1-30
21. K. Sharma, Instrumental Method of Chemical Analysis, 21th ed., Goel PublishingHousing, 2002,pp. 3.
22. K. Conners, Textbook of Pharmaceutical Analysis, 3rd ed.; A Wiley- IntersciencesPublication,1999, pp. 616.
23. H. Beckett, J. B. Stenlake, Practical Pharmaceutical Chemistry, 4th ed.; Part II, CBS Publications and Distributors: New Delhi, 1997, pp. 275-277.
24. H. H. Willard, L. L. Metritt, J. A. Dean, F. A. Settal, Instrumental methods of Analysis, 7thed., CBS Publishers and Distributers, 1986, pp. 118.
25. Yadav, L.D.S., 2013. Organic spectroscopy. Springer Science & Business Media.
26. US pharmacopoeia 24, Validation of compendia methods, section 1225, united states pharmacopeialconvension, Rockville, MD, 1999, pp. 2149
27. International conference on harmonization (ICH), Q2b: Validation of analytical
procedure:Methodology, USFDA federal register, Vol. 62, May 1997, pp. 27463
28. US pharmacopoeia 23, Chromatography section <621>, united statesPharmacopeialConvension, Rockville, MD, 1994, pp. 1776
29. International Conference on Harmonization (ICH), Q2A: Text on Validation of Analytical Procedure USFDA federal register, vol 60, May 1995 pp. 11260
30. G. A. Shabir, Journal of Chromatography A, 987, 57-66 (2003)
31. Chandran, S. and Singh, R.S.P., 2007. Comparison of various international guidelines for analytical method validation. Die Pharmazie-An International Journal of Pharmaceutical Sciences, 62(1), pp.4-14.
32. Swartz, M.E. and Krull, I.S. eds., 2018. Analytical method development and validation. CRC Press.
33. O'Neil, M.J. (ed.). The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Cambridge, UK: Royal Society of Chemistry, 2013., p. 236
34. USP 31 NF 26, United States Pharmacopoeia U.S. Pharmacopeial Convention,
Inc., Rockville, MD, 2008.
35. Indian Pharmacopoeia 2018. Government of India, Ministryof Health and Family Welfare, Indian Pharmacopoeia Commission,Gaziabad, India.
36. Mohammed, S., Adam, M. and Shantier, S., 2017. Development and validation of UV spectrophotometric method for determination of bisoprolol fumarate in bulk and pharmaceutical dosage forms. *Mediterranean Journal of Chemistry*, *6*(5), pp.196-199.
37. Ramzy, S., Abdelazim, A.H. and Shahin, M., 2022. Quantitative analysis of two pharmaceutical combinations containing amlodipine with either bisoprolol or candesartan using different UV spectrophotometric methods. *Journal of AOAC International*, *105*(4), pp.1200-1204.
38. Pawar, S., Tamboli, A. and Patil, S., 2020. UV spectrophotometric area under curve method for the simultaneous determination of bisoprolol fumarate and cilnidipine in pharmaceutical dosage form. *World Journal of Pharmaceutical Sciences*, *9*(5), pp.1691-9.
39. Patil, S., Tamboli, A., Jokar, S. and More, S., 2020. Development and Validation Of Uv Spectrophotometric Method For Bisoprolol Fumarate In Bulk And Tablet Dosage Form. *World J. Pharm. Res.*, *9*.
40. Gudruman, A.D., Murarasu, A. and Dorneanu, V., 2012. Spectrophotometric determination of bisoprolol using methyl orange as reagent. *Farmacia*, *60*(5), pp.634-
41. adhav, R.S. and Jagdish, V.B., 2018. Analytical Method Development and Validation for

estimation of Bisoprolol Fumarate in bulk and tablet dosage form by UV spectroscopic method. *International Journal of Universal Science and Technology-4 (1)*, pp.008-017.

42. Patel Hetal, R., Damahe, D.P., Luhar, S.V. and Sachin, B., 2018. Development and validation of uv spectrophotometric method for the simultaneous estimation of cilnidipine and bisoprolol fumarate in tablet dosage form.
43. Joshi, S.J., Karbhari, P.A., Bhoir, S.I., Bindu, K.S. and Das, C., 2010. RP-HPLC method for simultaneous estimation of bisoprolol fumarate and hydrochlorothiazide in tablet formulation. *Journal of pharmaceutical and biomedical analysis*, *52*(3), pp.362-371.
44. Vora, D.N. and Kadav, A.A., 2008. Development and validation of a simultaneous HPLC method for estimation of bisoprolol fumarate and amlodipine besylate from tablets. *Indian journal of pharmaceutical sciences*, *70*(4), p.542.
45. Rolim, C.M.B., Brum Jr, L., Fronza, M., Malesuik, M.D., Bajerski, L. and Dalmora, S.L., 2005. Development and validation of an RP-HPLC method for the dissolution studies of Bisoprolol in pharmaceutical dosage forms. *Journal of liquid chromatography & related technologies*, *28*(3), pp.477-486.
46. Turner, R.M., Fontana, V., Bayliss, M., Whalley, S., Castelazo, A.S. and Pirmohamed, M., 2018. Development, validation and application of a novel HPLC-MS/MS method for the quantification of atorvastatin, bisoprolol and clopidogrel in a large cardiovascular patient cohort. *Journal of Pharmaceutical and Biomedical Analysis*, *159*, pp.272-281.
47. Tutunji, M.F., Ibrahim, H.M., Khabbas, M.H. and Tutunji, L.F., 2009. Simultaneous determination of bisoprolol and hydrochlorothiazide in human plasma by HPLC coupled with tandem mass spectrometry. *Journal of Chromatography B*, *877*(16-17), pp.1689-1697.
48. Hassan, S.A., Nashat, N.W., Elghobashy, M.R., Abbas, S.S. and Moustafa, A.A., 2020. Stability-indicating RP-HPLC and CE methods for simultaneous determination of bisoprolol and perindopril in pharmaceutical formulation: a comparative study. *Journal of Chromatographic Science*, *58*(8), pp.747-758.