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Research

Analytical Method Development and Validation for Simultaneous Estimation of Nebivolol Hydrochloride and Amlodipine Besylate in Combined Pharmaceutical Dosage Form by Rp-Hplc

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Abstract

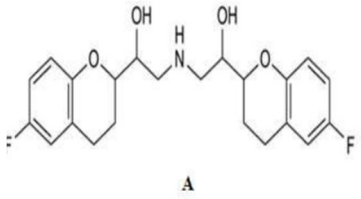
Amlodipine Besylate and Nebivolol Hydrochloride, a fixed - combination drug is used to treat for hypertension and heart failure. These both (Amlodipine Besylate and Nebivolol Hydrochloride) drugs are soluble in ethanol, pH 1.2 and poorly soluble in water. Various parameters are collected including LOD, LOQ, Correlation, Coefficient and linearity, absorbance maxima, and retention time. A simple, rapid, and efficient Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Nebivolol and Amlodipine in combined pharmaceutical dosage forms. The chromatographic separation was achieved using an X-bridge C18 column (4.6 × 150 mm, 5 µm) at a column temperature of 35°C. The mobile phase consisted of Acetone and Methanol in a 65:35 (v/v) ratio, with a flow rate of 1 mL/min and detection at 220 nm. The method was validated for various parameters, including accuracy, precision, specificity, linearity, and robustness. The method demonstrated excellent resolution, sensitivity, and reproducibility, making it suitable for routine quality control analysis of Nebivolol and Amlodipine in combined dosage forms. The method is advantageous due to its simplicity, rapidity, and reliability, ensuring precise measurement of both drugs in pharmaceutical formulations.

Keywords: Amlodipine Besylate and Nebivolol Hydrochloride, RP-HPLC Method, RP-HPLC, Analytical Method Validation, Pharmaceutical Dosage Form, Column Chromatography, Method Development.

INTRODUCTION

Amlodipine Besylate it is a dihydropyridine analogue, a long-acting Calcium Channel Blocker and Inhibits the influx of extracellular calcium across the myocardial and vascular smooth muscle cell membranes. Amlodipine is a peripheral arterial vasodilator that acts directly on the vascular smooth muscle to cause a reduction in peripheral vascular resistance and in blood pressure. Nebivolol Hydrochloride it is a β1 -Blocker, reduces peripheral vascular resistance, and significantly increases stroke volume, with preservation of cardiac output[1].

Amlodipine Besylate is an oral dihydropyridine calcium channel blocker widely used for its therapeutic effects. Combination dosage forms of NBM and AMB are available under various brand names, such as, Amlopres-NB, Nebi long-AM, and Nebi star-SA tablets.in these case of NBM and AMB in combination of HPLC has been used as analytical methods. NBH and AMB in human plasma with protein precipitation extraction. This approach is cost -effective, less time consuming, and simpler than other extraction methods[2].



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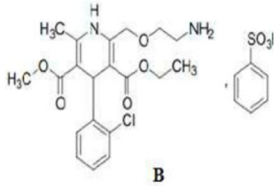

B

(A) NEBIVOLOL HYDROCHLORIDE (B) AMLODIPINE BESYLATE

Amlodipine is widely used in the treatment of high blood pressure, Amlodipine comes under the class of calcium channel blockers, it acts on blood vessels relaxation, by means heart does not have to pump hard and lower the blood pressure. Amlodipine increases the flow of blood to the heart. The chemical name of Amlodipine is 3-Ethyl 5 -Methyl (4RS) -2- (2 aminoethoxy) Methyl) 4-(2-chlorophenyl)-6- methyl -14-dihydro pyridine-3, 5-dicarbonylate benzene sulphate. Nebivolol relaxes the blood vessels and improves in lowering the blood pressure and blood flow. There are no many HPLC such as HPLC and UV spectrophotometers have been reported for this drugs.in this paper we present a specific, simple and rapid analytical method of determination for Amlodipine and Nebivolol in tablet formulations[3].

Nebivolol hydrochloride is a highly selective $\beta 1$ receptor antagonist without partial agonist activity. It is official in Martindale, the extra pharmacopeia. Amlodipine besylate is chemically 3-Ethyl 5-methyl 2-(-aminoethoxy methyl) 4-(20 chlorophenyl) -1, -4- dihydro-6-methylpyridine-3, 5 dicarboxylate mono benzene sulphonate used in the treatment of hypertension and congestive heart failure. A literature search revealed that very few methods are published for the determination of combination of Amlodipine and Nebivolol. The objective of the present work was to design a validation both drugs in tablet dosage form within a short run time and with good resolution. The present RP-HPLC methods were validated by following ICH guidelines[4].

Amlodipine besylate, it is designated as 3- ethyl 5- methyl 2-(2- aminoethoxy) methyl -4- (2-chlorophenyl) -6- methyl-1,4- dihydropyridine-3,5- dicarboxylate. It is used for treatment of anti hypertensive under the category of calcium channel blocker, clinically used for the treatment of hypertensionand chronic heart failure and chemically known as 1-(6- flourochroman-2-yl)-(26- fluorochrome -2-yl) 2- hydroxy- ethyl) amino) ethanol. A recent literature survey revealed that few individual methods were available for the determination of Amlodipine Besylate and Nebivolol Hydrochloride in tablets. For Amlodipine Besylate liquid chromatographic tandem mass spectrometry, liquid chromatography and capillary electrospray ionization mass spectrometry, HPLC method with amperometrydetection, RP- HPLC, HPLC with fluorescence detection and Nebivolol hydrochloride reported reported in HPLC with diode- array detection liquid chromatography coupled with electrospray ionization tandem mass spectrometry[5].

LITERATURE REVIEW

Author name	Title	Chromatographic conditions	Results	Journal details
S. Vidyadhara, k. Tejaswi et al[6]	Validation of a novel RP-HPLC method for simultaneous estimation of Amlodipine besylate and Nebivolol Hydrochloride in bulk and pharmaceutical dosage forms.	λmax – 268nm.	Amlodipine besylate of the LOD-0.36µg/ml LOQ- 1.10µg/ml Nebivolol hydrochloride of LOD-1.43µg.ml LOQ-4.41µg/ml.	International journal of pharmaceutical Sciences and Research, 2014.
Kajol Verma, shruti Rastogi, Meenakshi Dahiya et al[7]	Development and validation of RP- HPLC method using UV detection of Amlodipine besylate and Nebivolol Hydrochloride in fixed -dose combination tablets.	λmax -245nm.	NH-lod and loq-29.8-69.4µg/ml and amlodipine-30.4-70.8µg/ml.	Research journal of pharmacy and technology, 2024.
M.Jagadeeswaran , T. Sivakumar et al[4]	Simultaneous estimation and validation of AMB and NH in Tablet formulation by RP-HPLC method.	λmax -264nm	AMB of the LOD--0.3190 µg/ml LOQ 0.8923µg/ml and NH of the LOD-0.6845µg/ml and LOQ3.451µg/ml.	Asian journal of the research in chemistry.2010.
Deepak Sharma , Anu Rekha Jain and Alankar Shrivastava et al[8]	Simultaneous estimation of AMB and NH in tablets dosage forms by reverse phase- high performance liquid chromatographic using ultraviolet detection.	λmax -268nm.	Amb of theLOD-0.062 µg/ml And LOQ- 0.188 µg/ml. And NH of the LOD-0.10 µg/ml and LOQ-0.31 µg/ml.	Access through your organization, 2011.
AL Bratty, Mohammed, Manoharanet al[9]	Development and validation of RP-HPLC method for the simultaneous estimation of AMB and NH in raw and tablets formulations.	λmax -280nm.	AMB of the LOD-430 µg/ml LOQ- 1310µg/ml and NH of LOD -530 µg/ml and LOQ- 1610 µg/ml.	International journal of pharmaceutical research and Allied Sciences, 2016.
Kaveri , vaditake et al [(10)]	Simultaneous estimation of nebivolol hydrochloride and amlodipine besylate in human plasma employing an innovative HPLC, chromatographic method.	Λmax 215 nm	NH-4.50-180.12 µg/ml and AMB-3.50-140.06 µg/ml.	Future journal pharmaceutical scinces 10,142 (2024)
Kundan et al[(11)]	Stability indicating RP-HPLC method development for the simultaneous estimation of Nebivolol and Chlorthalidone in pharmaceutical dosage form .	Λmax226nm	NH- 12.5 to 75 µg/ml.and AMB-31.25µg/ml.	Afr.J..Bio.Sc.(2024).
S.Pratap , Ratna et al[(12)]	Development and validation of a sensitive and Rapid Bioanalytical RP-HPLC method for the Quantification of Nebivolol Hydrochloride in Rat plasma,	Λmax272nm	NH-0.80-72.00.05-4.50µg/ml. And Amlodipine-4.00144.00, 0.125-4.50 µg/ml.	Current trends in biotechnology and pharmacy,(2022) .

Bhushan M. Firake, et al.[13]	Simultaneous,spectrophotometric estimationof Amlodipine and Nebivolol in tablets dosage form.	Λmax242. 0 nm and 286.8 nm	NH:-lod-0.3µg/ml,loq-1.2µg/ml. Amlodipine:-lod-0.2µg/ml and loq-0.7µg/ml.	Analytical chemistry an indian journal(2016).
Bhushan M. Firake, et al.[13]	Simultaneous,spectrophotometric estimationof Amlodipine and Nebivolol in tablets dosage form.	Λmax242. 0 nm and 286.8 nm	NH:-lod-0.3µg/ml,loq-1.2µg/ml. Amlodipine:-lod-0.2µg/ml and loq-0.7µg/ml.	Analytical chemistry an indian journal(2016).
Cemonal,kadir Aluc et al.[14]	High performance liquid chromatography Analysis of Nebivolol and Amlodipine and some related compounds in synthetic mixture	Λmax265nm	NH0.2µg/ml10.0µg/ml .And Amlodipine:-0.25µg/ml.-10.0µg/ml.	Bezmialem science. Organisation.(2021).

CONCLUSION

According to this review RP- HPLC is a versatile, reproducible chromatographic technique for the Analysis of Amlodipine Besylate and Nebivolol Hydrochloride. These both (Amlodipine Besylate and Nebivolol Hydrochloride) drugs are soluble in ethanol, pH 1.2 and poorly soluble in water. RP-HPLC provides the highest level of precision, repeatability, reliability, and also it is a simple, rapid and robust quantitative analytical method.

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REFERENCES

1. https://pmc.ncbi.nlm.nih.gov/articles/PMC3658026/
2. Kaveri, Atul, & Shirkhedkar . (2024). Simultaneous Estimation of Nebivolol Hydrochloride and Amlodipine Besylate In Human Plasma Employing An Innovative HPLC Chromatographic Method. Vaditake and Shirkhedkar Future Journal of Pharmaceutical Sciences. 10(142), 2213-2665.
3. Dr. Mohammed Al Bratty, & Gunasekar Manoharan. (2016). Development and Validation of RP-HPLC method for the Simultaneous estimation of Amlodipine and Nebivolol in raw and tablet formulation. International journal of pharmaceutical research and Allied Sciences. 5(4), 37-44.(2016 (Issu no: - 2277-3657).
4. M. Jagadeeswaran, N. Gopal T. Sivakumar. (2010). Simultaneous Estimation and Validation of Amlodipine Besylate and Nebivolol Hydrochloride in Tablet Formulation by RP-HPLC Method. Asian Journal of Research in chemistry. 3(3), 640-642. ISSN -0974-4169.
5. Madhuri Ajay, hinge, & Jigar. (2016). Spectrophotometric and High Performance Liquid Chromatographic Determination of Amlodipine Besylate and Nebivolol Hydrochloride in Tablets Dosage Form. Pharmaceutical Methods. 7 (1), 1-7.
6. Vidyadhara, Sasidhar, k. Tejaswi, & K.Bhagyasri. (2014). Validation of a Novel RP-HPLC method for Simultaneous Determination of Amlodipine Besylate and Nebivolol Hydrochloride in bulk and pharmaceutical dosage forms. International Journal of Pharmaceutical Sciences and Research. 5(8), 3273-3278.
7. Kajol Verma, Shruti Rastogi, Meenakshi Dahiya, Gaurav Pratap Singh, Pooja Tomar,& Shobhit Kumar.(2024). Development and Validation of RP-HPLC Method Using UV Detection for Simultaneous Quantification of Amlodipine Besylate and Nebivolol Hydrochloride in Fixed-Dose Combination Tablets. Research Journal of Pharmacy and Technology. 17(2), 523-528.

8. Sharma D, Jain A, & Shrivastava A. (2011). Simultaneous Estimation of Amlodipine Besylate and Nebivolol Hydrochloride in Tablet Dosage Forms by Reverse Phase-High-Performance Liquid Chromatographic Using Ultraviolet Detection. Pharm Methods. 2(1), 9-14.
9. Mohammed AL Bratty, Gunasekaran Manoharan, & Hassan A Alhazmi. (2016). Development and Validation of RP-HPLC method for the Simultaneous estimation of Amlodipine and Nebivolol in raw and tablet formulation. International Journal of Pharmaceutical Research and Allied Sciences. 5(4), 37-44.
10. Vaditake, K.T., Shirkhedkar, A.A. Simultaneous estimation of nebivolol hydrochloride and amlodipine besylate in human plasma employing an innovative HPLC chromatographic method. Futur J Pharm Sci 10, 142 (2024).
11. Kundan Deore, Ujash Shah. Stability Indicating Rp-Hplc Method Development for The Simultaneous Estimation Of Nebivolol And Chlorthalidone In Pharmaceutical Dosage Form. Afr.J.Bio.Sc. 6(Si2) (2024).
12. Pratap, S.& Ratna, J.V. , Development and Validation of a Sensitive and Rapid Bioanalytical RP-HPLC Method for the Quantification of Nebivolol Hydrochloride in Rat Plasma. Current Trends in Biotechnology and Pharmacy, (2022) 16(3s), 87–95.
13. Bhushan,M. Firake, sanjay, Simultaneous spectrophotometric estimation of Amlodipine and Nebivolol in tablets dosage form, Analytical chemistry an indian journal, 16,issue- 5(2016)(220-225).
14. Cemonal,kadir Aluc,cigdem Aluc,Armagan Onal, High performance liquid chromatography Analysis of Nebivolol and Amlodipine and some related compounds in synthetic mixture, Bezmialem science. Organisation.(2021).9(3):264-70.
15. A. Raja Reddy, new RP -HPLC method for estimation of vericiguat in bulk drug and pharmaceutical dosage form. Journal of advanced scientific reaserch (2023), 14(09): 37-43.
16. A. Raja Reddy, T. Rama Rao, S. Sriya, Analytical Method Development And Validation Of stability Indicating Rp-Hplc Method For Estimation**,** IJAR Volume - 13 | Issue - 11 | November - 2023 | . PRINT ISSN No 2249 - 555X | DOI : 10.36106.
17. A. Raja Reddy, M. Mounika, T. Rama Rao, Analytical method development and validation for simultaneous estimation of clonidine hcl and chlorthalidone in bulk and tablet dosage form by RP-HPLC method, A. Raja Reddy, M. Mounika. T. Rama Rao, / Int. J. of Pharmacy and Analytical Research Vol-12(3) 2023 [490-499].
18. A. Raja Reddy, N. Divya, T. Rama Rao, Simultaneous estimation of enalapril maleate and losartan potassium in tablet dosage form by using RP-HPLC, Journal of Pharmacreations Vol-10(3) 2023 [144-153].
19. A. Raja Reddy, V. Kalyan, Method Development and validation of tivozanib by RP-HPLC in bulk and pharmaceutical dosage forms, Int. Journal Adv. Res. 11(08), 566-573, DOI URL: http://dx.doi.org/10.21474/IJAR01/17423.
20. A. Raja Reddy, R. Devicharan, Analytical RP-HPLC method development and validation for simultaneous estimation of Emtricitabine, Tenofovir and Bictegravir**,** Research journal Pharm. and Tech. 16(8): August 2023. https://www.doi.org/:%2010.52711/0974-360X.2023.00614.
21. A. Raja Reddy, R. Kiran, QbD Based Rp-Hplc Method Development And Validation for The Estimation Of Safinamide In Bulk And It's Dosage form, IJPCR |Volume 7 | Issue 2 | Apr - Jun - 2023.
22. A. Raja Reddy, V. Sowmya, Simultaneous estimation of Sacubitril and Valsartan in bulk and tablet Dosage form by RP-HPLC, Int. Journal of Pharmacy and Analytical Research Vol-11(4) January 2022 [419-424].
23. A. Raja Reddy, J. Lavanya, T. Rama Rao, Analytical method development and validation for simultaneous estimation of Metformin and Empagliflozin in bulk and pharmaceutical dosage form by Rp-Hplc, Ymer, Volume 21 : issue 11 (nov) – 2022.
24. A. Raja Reddy, H.kavya, Analytical Rp-Hplc method development and validation for simultaneous estimation of Bilastine and Montelukast in bulk and pharmaceutical dosage form, Ymer, Volume 21 : Issue 11 (nov) – 2022.