REVIEW ARTICLE

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Analytical Approaches for Determination of Nebivolol

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Abstract

A highly selective ß1-adrenergic receptor antagonist, nebivolol has a unique pharmacologic profile from other medications in its class. Nebivolol causes nitric oxide-mediated vasodilation in addition to cardioselectivity mediated by ß1 receptor blockage by activating endothelial nitric oxide synthase via 3 agonism. Contrary to other vasodilatory -blockers (carvedilol, labetalol), which work by blocking -adrenergic receptors, this vasodilatory mechanism is unique. In the US and Europe, nebivolol is authorized for the treatment of hypertension and heart failure, respectively. Although -blockers are not advised as the first-line therapy for the treatment of essential hypertension according to the most recent US guidelines, with a relatively low risk of adverse effects, nebivolol has demonstrated comparable efficacy to presently indicated medications in decreasing peripheral blood pressure in persons with hypertension. Compared to other -blockers, nebivolol also has favorable effects on central blood pressure. Nebivolol may also be helpful for patients who have suffered erectile dysfunction while taking other -blockers, according to clinical studies. Here, we discuss nebivolol's pharmacological profile, the clinical evidence that supports its use as a monotherapy, add-on therapy, and combination therapy for hypertension, as well as the information pointing to its beneficial effects on heart failure and endothelial dysfunction. There are some analytical methods for the analysis of the drug in the biological fluids and pharmaceutical preparations. In this work, we have collected these methods with the aim to present the different options for the nebivolol determination.

Keywords: Nebivolol; pharmaceuticals; analytical determinations; biological samples; review.

1. Introduction

Cardiovascular diseases (CVDs) are one of the most common and dangerous diseases which lead to mortality over the world. CVDs are group of diseases related to the heart and blood vessels, such as hypertension, coronary artery disease, rheumatic heart disease and heart failure **(González et al. 2015; WHO 2016)**

CVDs are the main cause of death worldwide. According to statistics, 17.9 million deaths worldwide in 2019 were attributable to CVDs, or 32% of all fatalities. Heart attack and stroke deaths accounted for 85% of these fatalities. The majority

of CVDs mortalities occur in low- and middle-income nations **(Nabel 2003)**.

Hypertension (HTN) and congestive heart failure (CHF) are the most familiar types of CVDs. With a prevalence of about 20% in the general population, HTN is the most prevalent disease in industrialized countries **(WHO 2016)** , while CHF is a serious disorder with significant morbidity and mortality , with prevalence 1 to 2% of middle-aged and older adults, 2 to 3% of patients over the age of 65, and 5 to 10% of patients over the age of 75 **(Yamani and Massie 1993)**.

The etiology of CVDs is complex and multifactorial. There is a group of risk factors, which plays an important role contributing to increase of CVDs. Among them, we can find factors such as hyperlipidemia, obesity and diabetes mellitus **(WHO 2016)**

When people are diagnosed with CVDs, they may be treated in several different ways. Controlling risk factors that can be managed (stop smoking, make exercise, eat healthy food, weight loss, and decrease cholesterol). Combinations of two or more drugs that have different mechanism will be next step **(Arsenault and Després 2017)**.

Nebivolol (NEB) has the chemical name 1-(6-fluorochroman-2-yl)-2-[(2-(6-fluorochroman-2-yl)-2-hydroxy-ethyl] amino] ethanol (Figure 1). Its molecular formula is $C_{22}H_{25}F_2NO_4$, molecular weight 405.435 g/mole, its pKa 8.9 and 13.52, log p 3.21 (Pubchem 2022) .

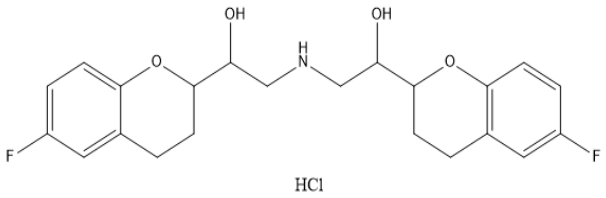

Figure 1: Chemical structure of Nebivolol HCl

Physical properties of NEB powder is a white powder which is soluble in methanol, dimethyl sulfoxide, N,N imethylformamide, sparingly soluble in ethanol, propyl glycol, polyethylene glycol and very slightly soluble in dichloromethane, hexane and toluene **(FDA 2022)**.

NEB is approved for the treatment of hypertension in the US, and for hypertension and heart failure in Europe. While β-blockers are not recommended within the current US guidelines as first-line therapy for treatment of essential hypertension, nebivolol has shown comparable efficacy to currently recommended therapies in lowering peripheral blood pressure in adults with hypertension with a very low rate of side effects. Nebivolol also has beneficial effects on central blood pressure compared with other β-blockers **(Fongemie and Felix-Getzik 2015)**, as well as for the treatment of stable mild or moderate CHF in elderly patients older than 70 years old when combined with other therapy **(Moen and Wagstaff 2006)**.

NEB is a potent antihypertensive medication that is well tolerated by hypertension patients. The medication was usually successfully reduced the composite endpoint of death and cardiovascular hospital admission in older individuals with CHF. For individuals with uncomplicated mild to severe essential hypertension and elderly CHF patients, nebivolol should be taken into consideration as an alternate first-line therapeutic option **(Olawi et al. 2019)**.

Several analytical methods have been reported for determination of NEB either alone or in combination with other medication in pharmaceutical preparation or biological fluids cited below.

1. UV/VIS spectrophotometric and spectro-fluorimetric methods:

Reported spectroscopic methods for determination of NEB in pharmaceutical preparations are summarized in **Table 1**

2. Chromatographic method

Reported chromatographic methods high performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Ultra performance liquid chromatography (UPLC) for the assay of NEB in its pharmaceuticals or biological fluids are summarized in **Table 2.**

Table 1: Reported UV/ VIS spectrophotometric and spectrofluorimetric for determination of NEB in pharmaceutical preparation.

1- UV/ VIS spectrophotometric methods			
Matrix	**Reagent**	**λ max**	**Ref.**
NEB & valsartan (VAL)	Ratio Difference	240nm and 255nm	**(Meselhy et al. 2020)**
	First Derivative ratio (DD^1)	295 nm	
	Mean Centering of ratio spectra	291 nm	
	Bivariate	270 and 285 nm	
	H-point	280 nm and 285nm	
NEB	Direct UV	282 nm	**(Kamila et al. 2007)**
	1,2-naphtha quinone-4-sulphonate in alkaline media	at 460	**(Dadi, Neeharika, and Rambabu 2013)**
	cobalt thiocyanate	630 nm	
	Second derivative	296 nm	**(Malipatil et al. 2011)**
	Third derivative	290 nm	
NEB & Hydrocholothiazide (HCZ)	principal component regression (PCR) and partial least squares (PLS)	231–310 nm	**(Gowda et al. 2009)**
	first derivative (D^1)	294.6 nm	**(Shah et al. 2019)**
NEB & beta- blockers	principal component regression and partial least squares	290–350 nm	**(Abdel Hameed, Abdel Salam, and Hadad 2015)**
2- Spectrofluorimetric methods			
NEB & amlodipine (AML)	second derivative synchronous fluorimetry	282 nm	**(Ibrahim et al. 2015)**
NEB	eosin Y	545 nm (λex. 301.5 nm	**(Derayea et al. 2016)**
NEB & VAL	first derivative spectrofluorimetric method	294 nm	**(Anumolu et al. 2014)**

Table 1: Reported Liquid chromatographic methods for the determination of NEB in pharmaceutical preparations and biological fluids

TLC				
Matrix	**Stationary Phase**	**Mobile phase**	**Densitometric Detection**	**Ref.**
NEB	Silica gel 60 F254 TLC plates	Ethyl acetate: toluene: methanol: ammonium hydroxide (1:6:2:0.1 v/v/v/v)	282 nm	**(Patel, Suhagia, and Shah 2007)**
		Toluene: methanol: triethylamine (3.8:1.2:0.2 v/v/v)	281 nm	**(Shirkhedkar, Bugdane, and Surana 2010)**
NEB & HCZ		Ethyl acetate: methanol: acetic acid 6.5:1:0.5 (v/v)	280 nm	**(YANG et al. 2016)**
		1, 4-dioxane: toluene: triethylamine (5:3:0.1 v/v).	281 nm	**(Kumbhar et al. 2011)**
HPLC				
Matrix	**Stationary Phase**	**Mobile phase**	**UV Detection**	**Ref.**
NEB	phenomenex Gemini C-18	Methanol: acetonitrile: 0.02 M potassium dihydrogen phosphate (60:30:10, v/v/v; pH 4.0	280 nm	**(Shah et al. 2008)**
	a Hypersil ODS C18	Methanol: water (80:20 v/v)	282 nm	**(Sahoo et al. 2009)**
	Lichrospher 100 C-18	50 mM KH_2PO_4 buffer (pH 3.0 acetonitrile: (45:55 v/v)	282 nm	**(Patel, Suhagia, and Shah 2007)**
	a BDS Hypersil C18	0.1 % trifluoroacetic acid: acetonitrile (60:40, v/v)	281 nm.	**(Szabó et al. 2014)**
	a Phenomenex Luna C_{18}	Methanol: water: formic acid (70:30:0.1)	LC-MS/MS	**(YANG et al. 2016)**
NEB	a Phenomenex Luna C8	Acetonitrile: pH 3.5 phosphate buffer (35: 65, v/v)	280 nm	**(Kachhadia, Doshi, and Joshi 2008)**
NEB &VAL	HIQ sil C_{18}	Methanol: water (80:20 v/v) and 0.1 % 1-hexanesulfonic	289 nm	**(Kokil and Bhatia 2009)**

		acid monohydrate sodium salt		
	Inertsil ODS C_{18}	Acetonitrile: methanol: pH4.0 0.02M Potassium hydrogen phosphate buffer (50:20:30 v/v)	210nm	**(Nekkala et al. 2014)**
NEB &HCZ	C-18 Primesil	Methanol: 0.05% ortho phosphoric acid pH 2.5 (60:40v/v)	281.0nm	**(Ambhore J 2018)**
NEB & VAL & AML	An Inertsil C18	(Buffer pH 3.0: acetonitrile: methanol) (30:20:50 v/v %)	281 nm.	**(Annadi, Shoheib, and Mohamed 2020)**
NEB & AML	Phenomenex Kinetex C18 column	Acetate buffer (pH: 4.5): acetonitrile (Gradient elution)	265 nm	**(ÖNal et al. 2021)**
UPLC				
Matrix	**Stationary Phase**	**Mobile phase**	**UV Detection**	**Ref.**
NEB & VAL& AML	An Eclipse plus C18 column	Phosphate buffer pH 3.0: acetonitrile (55:45 v/v %)	240 nm	**(Annadi, Shoheib, and Mohamed 2020)**
NEB	Ethylene Bridge Hybrid C18 (2.1 x 50mm	Potassium di hydrogen phosphate (25%) and Acetonitrile (75%)	281 nm	**(Sujana et al. 2016)**
NEB	BEH C18	10 mM ammonium acetate and acetonitrile (Gradient elution)	222 nm	**(Prasad et al. 2016)**

3. Electrochemical method:

- Voltametric techniques using high-quality graphene/nafion nanocomposite modified electrode was developed for NEB assessment (Er, Çelikkan, and Erk 2016).Another voltametric methods using cyclic and square wave voltammetry were used for the determination of amlodipine and nebivolol **(Jadon 2017).**

- Sadikovic and Mirela developed electrochemical sensor utilizing carbon nanotubes decorated with zirconium oxide nanoparticles was developed for the determination of nebivolol at the potential of +1.05 V (vs. Ag/AgCl) **(Sadikovic 2017)**.

References:

Abdel Hameed, E. A., R. A. Abdel Salam, and G. M. Hadad. 2015. 'Chemometric-assisted spectrophotometric methods and high performance liquid chromatography for simultaneous determination of seven β-blockers in their pharmaceutical products: a comparative study', *Spectrochim Acta A Mol Biomol Spectrosc*, 141: 278-86.

Ambhore J, Bangale G and Chandak M. 2018. 'Development of Stability Indicating Assay Method for Estimation of Nebivolol and Hydrochlorothiazide in Tablet Dosage Form', *Austin Journal Analytical and Pharmaceutical Chemistry*, 5: 1095-104.

Annadi, Abdelaziz, Ragaa Elsheek Shoheib, and Ashraf Mohamed. 2020. 'Assessments of Valsartan in the presence of Nebivolol or Amlodipine in solid formulations and its discriminative dissolution behavior via RP-HPLC and RP-UPLC methods. %J Egyptian Journal of Chemistry', 63: 2837-51.

Anumolu, Panikumar D, S Gurrala, R Bagirath, P Santoshi Vani, and G %J Int J Pharma Sci Rev Res Archana. 2014. 'Zerocrossing point derivative simultaneous spectrofluorimetric method for quantification of nebivolol hydrochloride and valsartan combination in tablets', 27: 164-68.

Arsenault, Benoit , and Jean-Pierre Després. 2017. 'Cardiovascular disease prevention: lifestyle attenuation of genetic risk', *Nature Reviews Cardiology*, 14: 187.

Dadi, Murali, T. Neeharika, and Cheedalla Rambabu. 2013. 'Spectrophotometric determination of nebivolol hydrochloride in bulk, pharmaceutical formulations', *Asian Journal of Chemistry*, 25: 2981-84.

Derayea, Sayed M, Mahmoud A Omar, Mohamed Aboel-Kasem Abdel-Lateef, and Ahmed I. Hassan. 2016. 'Development and validation of a new spectrofluorimetric method for the determination of some beta-blockers through fluorescence quenching of eosin Y. Application to content uniformity test %J Open Chemistry', 14: 258-66.

Er, Engin, Hüseyin Çelikkan, and Nevin Erk. 2016. 'Highly sensitive and selective electrochemical sensor based on high-quality graphene/nafion nanocomposite for voltammetric determination of nebivolol', *Sensors and Actuators B: Chemical*, 224: 170-77.

Fongemie, J., and E. Felix-Getzik. 2015. 'A Review of Nebivolol Pharmacology and Clinical Evidence', *Drugs*, 75: 1349-71.

González, Oskar, Nerea Ferreirós, María Blanco, and Rosa Alonso. 2015. 'Cardiovascular drug determination in bioanalysis: an update', *Bioanalysis*, 7: 2399-417.

Gowda, N., S. Panghal, K. Vipul, and M. Rajshree. 2009. 'Validation and application of high-performance liquid chromatographic and spectrophotometric methods for simultaneous estimation of nebivolol and hydrochlorothiazide: a novel approach to multivariate calibrations by R-Software Environment', *J AOAC Int*, 92: 1356-65.

Ibrahim, F., N. El-Enany, S. H. Shalan, and R. A. Abo Shabana. 2015. 'Second-derivative synchronous spectrofluorimetric determination of nebivolol hydrochloride and amlodipine besylate in their combined dosage form', *Luminescence*, 30: 1011-9.

Jadon, Nimsha. 2017. 'Simultaneous Determination of Amlodipine and Nebivolol at PANI-CeO₂ Modified Carbon Paste Electrode', *ECS Meeting Abstracts*, MA2017-01: 1757-57.

Kachhadia, Pankaj, Ashish Doshi, and Hitendra Joshi. 2008. 'Development and Validation of a Stability-Indicating Column High-Performance Liquid Chromatographic Assay Method for Determination of Nebivolol in Tablet Formulation', *J AOAC Int*, 91: 557-61.

Kamila, M. M., N. Mondal, L. K. Ghosh, and B. K. Gupta. 2007. 'A validated UV spectrophotometric method for estimation of nebivolol hydrochloride in bulk and pharmaceutical formulation', *Pharmazie*, 62: 486-7.

Kokil, S. U., and M. S. Bhatia. 2009. 'Simultaneous Estimation of Nebivolol Hydrochloride and Valsartan using RP HPLC', *Indian J Pharm Sci*, 71: 111-4.

Kumbhar, ST, GK Chougule, VS Tegeli, GB Gajeli, YS Thorat, US %J International Journal of Pharmaceutical Sciences Shivsharan, and Drug Research. 2011. 'A validated HPTLC method for simultaneous quantification of nebivolol and hydrochlorothiazide in bulk and tablet formulation', 3: 62-66.

Malipatil, SM, M Deepthi, SK Patil, and K %J Int. J. Pharm. Pharm. Sci Jahan. 2011. 'Second and third order derivative spectrophotometric estimation of nebivolol hydrochloride in bulk and pharmaceutical dosage forms', 3: 13-15.

Meselhy, E. M., A. A. Aboul Kheir, M. M. El Henawee, and M. S. Elmasry. 2020. 'Simultaneous determination of Nebivolol hydrochloride and Valsartan in their binary mixture using different validated spectrophotometric methods',

Spectrochim Acta A Mol Biomol Spectrosc, 230: 118083.

Moen, Marit D., and Antona J. Wagstaff. 2006. 'Nebivolol', *Drugs*, 66: 1389-409.

Nabel, Elizabeth G 2003. 'Cardiovascular disease', *New England Journal of Medicine*, 349: 60-72.

Nekkala, K, V Shanmukha, D Ramachandran, Ganji Ramanaiah, and Ganta %J American Journal of Advanced Drug Delivery Srinivas. 2014. 'Method development and validation of stability indicating RP-HPLC method for simultaneous estimation of Nebivolol HCL and valsartan in bulk and its pharmaceutical formulations', 2: 624-37.

Olawi, Nasima, Marcus Krüger, Daniela Grimm, Manfred Infanger, Markus %J Basic Wehland, Clinical Pharmacology, and Toxicology. 2019. 'Nebivolol in the treatment of arterial hypertension', 125: 189-201.

ÖNal, Cem, Kadir AluÇ, Çiğdem AluÇ, and Armağan ÖNal. 2021. 'High-performance Liquid Chromatography Analysis of Nebivolol and Amlodipine and Some Related Compounds in Synthetic Mixture', *Bezmialem Science*, 9: 264-70.

Patel, L. J., Bhanubhai Suhagia, and P. B. Shah. 2007. 'RP-HPLC and HPTLC methods for the estimation of nebivolol hydrochloride in tablet dosage form', *Indian J Pharm Sci*, 69.

Prasad, Kancherla, Pallavi Alegete, Keesari Srinivas, Bhavyasri Khagga, Sridhar Siddiraju, Mukkanti Khagga, and Parthasarathi Das. 2016. 'Stability-Indicating RP-UPLC Method Development and Validation for the Process Related Impurities of Nebivolol and Structural Characterization of Its Forced Degradation Products by LC-MS/MS', *British Journal of Pharmaceutical Research*, 14: 1-13.

Sadikovic, Mirela. 2017. 'Development of Electrochemical Platform Based on Carbon Nanotubes Decorated with Zirconium Oxide Nanoparticles for Determination of Nebivolol', *International Journal of Electrochemical Science*, 12: 9675-88.

Sahoo, MK, RK Giri, CS Barik, SK Kanungo, and BVV %J E-Journal of Chemistry Kumar. 2009. 'RP-HPLC method for the estimation of nebivolol in tablet dosage form', 6: 915-19.

Shah, D. A., K. K. Bhatt, R. S. Mehta, S. L. Baldania, and T. R. Gandhi. 2008. 'Stability Indicating RP-HPLC Estimation of Nebivolol Hydrochloride in Pharmaceutical Formulations', *Indian J Pharm Sci*, 70: 591-5.

Shah, Dimal A, Kashyap K Bhatt, Rajendra S Mehta, and Sunil L Baldania. 2019. 'Determination of Nebivolol Hydrochloride and Hydrochlorothiazide in Tablets by First-Order Derivative Spectrophotometry and Liquid Chromatography', *J AOAC Int*, 91: 1075-82.

Shirkhedkar, Atul, Prasad Bugdane, and Sanjay Surana. 2010. 'Stability-Indicating TLC-Densitometric Determination of Nebivolol Hydrochloride in Bulk and Pharmaceutical Dosage Form', *Journal of chromatographic science*, 48: 109-13.

Sujana, Kamepalli, P. Jagadish, S. Venu, and T. Priyanka. 2016. 'Stability Indicating Validated UPLC Method for Determination of Nebivolol HCl in Bulk and Pharmaceutical Dosage Form', *Research Journal of Pharmacy and Technology*, 9: 533-37.

Szabó, Zoltán István, TÍMea SzabÓ, Emoke Redai, and Emese Sipos. 2014. 'Validated HPLC method for determination of nebivolol in pharmaceutical dosage form and in vitro dissolution studies', *Studia Universitatis Babeș-Bolyai. Chemia*, 59: 195-203.

WHO. 2016. 'Health topic: cardiovascular diseases www.who.int/topic/cardiovascular_diseases/en/

Yamani, Mohamad, and Barry M Massie. 1993. "Congestive heart failure: insights from epidemiology, implications for treatment." In *Mayo Clinic Proceedings*, 1214-18. Elsevier.

YANG, Le-ting, Hui-ru XIE, Xi-qian ZHANG, Ling WANG, and Xue-hua %J Chinese Journal of Pharmaceutical Analysis JIANG. 2016. 'HPLC-MS/MS determination of nebivolol in human plasma and its application in pharmacokinetic study', 36: 2094-100.