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Review Article

**AMLODIPINE AND NEBIVOLOL - A REVIEW ON HPLC  
METHOD****Rajveer Bhaskar<sup>1</sup>, Monika Ola<sup>2</sup>, Harshjeet Sisode\*<sup>1</sup>, Rakeshsing Rajput<sup>1</sup>,  
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**Article Received:** December 2018**Accepted:** February 2019**Published:** March 2019**Abstract:**

*The review on focuses on recent analytical method development on HPLC high performance liquid chromatography Method for estimation of Amlodipine and nebivolol along with combination on both/other drug on hplc method development of drug dosage form. HPLC method can able to separate, detect, and quantify the various drug it can degradants that can from storage or manufacturing detect and quantify any drug and drug related impurities introduce during synthesis. It was separation technique based on solid stationary phase and liquid mobile phase in these system can various Advantages of HPLC system is pharmaceutical, clinical, ecological scientific etc. Validation can process of establishing characterization and limitation of method. Parameter of validation is Accuracy, Precision, Reputability, Intermediate precession, Linearity, Detection limit, Quantification limit, Specificity, Range, Robustness, System suitability determination, Force degradation study, Stability study. Amlodipine drug can use on high blood pressure coronary artery virus and category is calcium channel blocker, Angina pectoris. Side effect on dizziness, fatigue, headache, palpitations and nausea. It has been molecular weight is 506.06 g/mole. White powder, class 1st drug etc. nebivolol can vital hypertension disorder associate with endothelial dysfunctions and angina pectoris. It has been side effect on headache, dizziness, paresthesia, constipation, nausea, and diarrhea. It has been molecular weight is 405.435 g/mole white talc class 2<sup>nd</sup> drug.*

**Keywords:** HPLC technique, Development and validation, stability study, Amlodipine, Nebivolol.**Corresponding author:****Harshjeet Sisode,**

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**INTRODUCTION:**

HPLC is play vital part on pharmaceutical analysis. It was separation techquine based on solid stationary phase and liquid mobile phase. Chromatography was mass transfer process involve in absorption.[1] The active component like column is adsorbent granular material of solid particle like silica & polymer. The code of separation is normal phase mode & reverse phase mode is adsorption in which substance transportable on according to their comparative affinity.[2] The solvent typically flows through column with the support of gravity however in HPLC technique the Solvent will be required under high forces up to 400 airs so that sample can be separated into changed constituents with the support of difference in comparative affinities

**Instrumentation:**

- ❖ Solvent reservoir.
- ❖ Pump.
- ❖ Sample injector.
- ❖ Column.
- ❖ Detector.
- ❖ DATA collector.

**Application:**

The HPLC has some uses in the playing field of druggists, criminal, atmosphere and scientific. It also helps in the parting and sanitization of numerous

- Pharmaceutical Applications: The pharmaceutical applications include regulatory of medication steadiness, dissolution studies and QC
- Ecological Applications: Monitoring of toxins and identifying components of drinking water.

- Criminal Applications: Analysis of fabric dyes, quantification of medications and steroids in organic samples.
- Food and Aromas Applications: Sugar examination in fruit liquids, identifying polycyclic complexes in Vegetables, examination of preservatives.
- Scientific Applications: Identifying endogenous neuropeptides, study of biological samples like plasma and urine. [3]

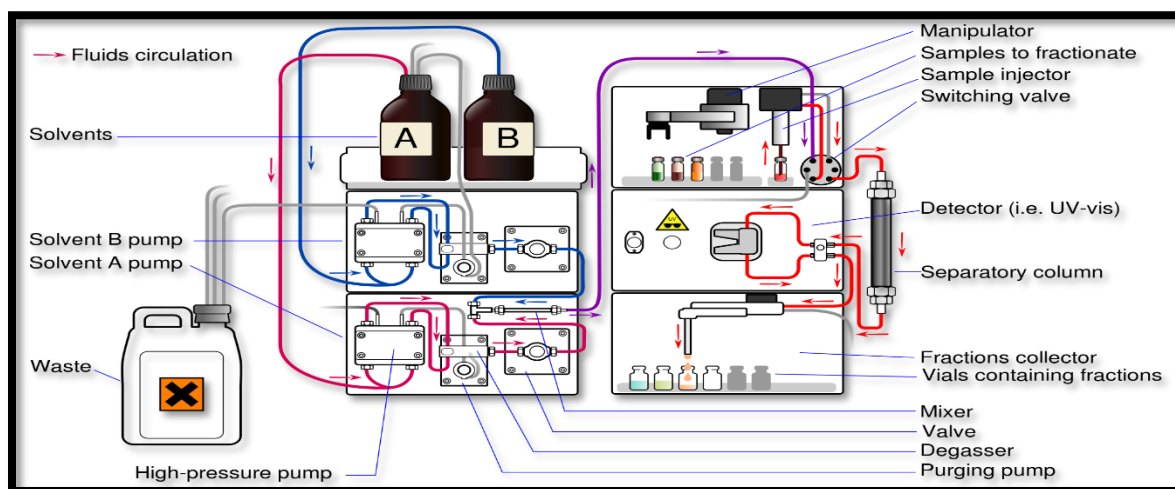
**Method validation:**

It can analytical process which establish by laboratory studies that can characterization of the procedure meet the requirement for intended use. These process can collection on data to analytical procedure or analyzing clinical sample it well need to be validated as per ICH guideline. [4]

**Validation parameter.**

The following are typical performance characteristics which may be tested during method validation.

1. Accuracy.
2. Precision.
3. Reputability.
4. Intermediate precession.
5. Linearity.
6. Detection limit.
7. Quantification limit.
8. Specificity.
9. Range.
10. Robustness.
11. System suitability determination.
12. Force degradation study.
13. Stability study.

**(DIAGRAM\_ OF HPLC INSTRUMENT)**

**INTRODUCTION TO DRUG PROFILE:****AMLODIPINE:****Introduction**

Amlodipine, sold below the trade title Norvasc amongst others, is a drug used to delight high blood pressure and coronary artery virus.[5] It is a long-acting calcium channel blocker of the dihydropyridine (DHP) type. Mutual side effects include puffiness, feeling tired, abdominal pain, and nausea. Serious side belongings include low blood pressure or a stroke[6]

**Scientific indications, pharmacodynamics and pharmacokinetics**

Amlodipine is showed for the action of high blood pressure (BP) and angina. In calculation, a sum of randomized courts-martial have find out its value in angina pectoris. Amlodipine is a long acting, lipophilic, third group dihydropyridine (DHP) that exerts its action done reserve of calcium influx into vascular flat influence cell and myocardial cells,

which marks in reduced peripheral vascular resistance (PVR).[7] Starting reflex mechanisms, such as improved PVR and raised up heart rate, can cause injurious effects on lipid and carbohydrate digestion.[8] These famous adverse effects are usually seen with other managers including the first group  $\beta$ -blockers (BBs; such as atenolol and metoprolol) and earlier group of DHPs.

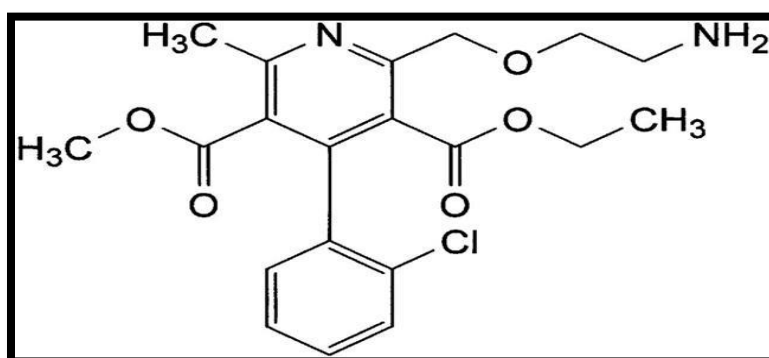
**Adverse effect**

The most usually stated adversative result delaying agreement with amlodipine is peripheral oedema. Include faintness, fatigue, headache, shivers and nausea, while these are usually not troublesome enough to cause termination of the medication.[9]

**Use**

Calcium channel blocker

Angina pectoris[10]

**Chemical Structure****AMLODIPINE DRUG PROFILE.**

<b>Molecular Formula</b>	C <sub>20</sub> H <sub>25</sub> CIN <sub>2</sub> O <sub>5</sub>
<b>Molecular Weight</b>	567.05
<b>Chemical Name</b>	RS)-3-ethyl 5-methyl 2-[(2-amino ethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate.
<b>Description</b>	White powder.
<b>Melting Point</b>	199-201°C
<b>Solubility</b>	Chloroform, Ethanol, Methanol, A little Solvable in H <sub>2</sub> O
<b>Adverse Effects</b>	dizziness, fatigue, headache, palpitations and nausea
<b>Bioavailability</b>	64-90%
<b>Category</b>	Calcium channel blocker
<b>Pka</b>	8.6
<b>BSC Class</b>	1 medication
<b>Half life</b>	30 to 50hours. [11]

**NEBIVOLOL:****Introduction**

Nebivolol is a third group, very careful  $\beta$  adrenoceptor adversary specified 1 for action of vital hypertension. Vital hypertension is a disorder associated with endothelial dysfunction which is instigated by manufacture of oxygen free die-hard that abolish nitric oxide and spoil its useful and caring effects on vessel wall. In adding to its beta blocking effects, nebivolol has an endothelium reliant on vasodilator property which is mediated via L-arginine/NO pathway.[12]

**Pharmacodynamics**

Nebivolol predicaments to the  $\beta$  receptor on lockup film leading to 1 beginning of adenylyl cycles subsequent in buildup secondary runner cAMP.[13] This cAMP dependent protein kinase reliant on production. This mechanism leads to effective control of blood pressure by vasodilatation of blood vessels.[14] Decreases resting heart rate and cuts exercise induced tachycardia Reduces total lipid and low density lipoprotein heights[15]. Reduces plasma renin and aldosterone planes

**Pharmacokinetic**

Absorption:

Oral bioavailability is 12% in extensive metabolizers and 96% in poor metabolizers, with plasma half-life of 10.3hrs and 31.9hrs respectively[16]

**Distribution:**

The plasma protein binding is 98%, with limited distribution in adipose tissue due to its lipophilicity and hence no need for dosage adjustment in obese patient

**Metabolism and Excretion:**

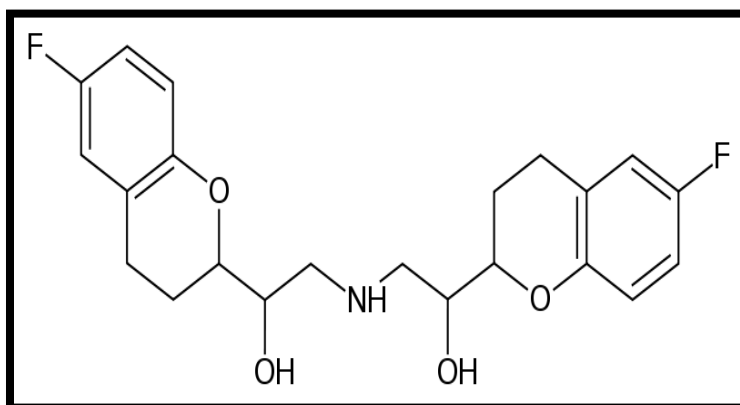
It undergoes extensive first pass metabolism and produces active  $\beta$ - blocking hydroxylase metabolites The metabolism of nebivolol shows genetic polymorphism in gene encoding the CYP2D6 isoenzyme where individuals may be phenotypically divided as "poor" or "extensive "metabolizers it is about 24hrs.. 38% of dose is excreted in urine.

**Adverse effects:**

The adverse effects with a frequency of 1-10% incidence included headache, dizziness, paresthesias, dyspnea, constipation, nausea, diarrhea, tiredness and edema. The less frequently reported are impaired vision, bradycardia, heart failure, hypotension, bronchospasm, pruritus and impotence[17]

**Uses**

1. Essential hypertension.
2. Angina pectoris.

**Chemical structure.**

## NEBIVOLOL DRUG PROFILE

<b>Molecular Formula</b>	C <sub>22</sub> H <sub>25</sub> F <sub>2</sub> NO <sub>4</sub>
<b>Molecular Weight</b>	405.435g/mol.
<b>Chemical Name</b>	1-(6-fluorochroman-2-yl) {[2-(6-fluorochroman 2-yl)-2-hydroxy-ethyl] amino} ethanol.
<b>Description</b>	White talc
<b>Melting Point</b>	130-133°C
<b>Solubility</b>	Solvable in methanol, acetone, acetonitrile, DMSO. Unsolvable In Water
<b>Adverse Effects</b>	headache, dizziness, paresthesia's, dyspnea, constipation, nausea, diarrhea, tiredness and edema
<b>Bioavailability</b>	12%
<b>Category</b>	B-adrenergic blocking cause.
<b>Pka</b>	8.13
<b>BSC Class</b>	class II
<b>Half life</b>	10 hr.

## NEBIVOLOL

No	Method	Short-term introduction	Ref
1	<b>RP_HPLC Development &amp; Validation.</b>  <b>Drug name.</b> NBH	<b>Mobile phase.</b> MeOH-H <sub>2</sub> O (70:30, v/v)	[18]
		<b>Column.</b> Ace C18 column (5 μm, 4.6×250 mm.) with a guard column (4 mm × 3 mm. Phenomenex)	
		<b>Detector.</b> UV/Vis	
		<b>Flow rate.</b> 1.0 ml/min	
		<b>Wavelength.</b> 282nm.	
		<b>Retention Time</b> 3.3 min	
2	<b>RP_HPLC Development &amp; Validation.</b>  <b>Drug name.</b> NBH	<b>Mobile phase.</b> H <sub>2</sub> O-MeOH (40:60 v/v.)	[19]
		<b>Column.</b> C-18	
		<b>Detector.</b> UV-vis	
		<b>Flow rate.</b> 1.0 ml/min.	

		<b>Wavelength.</b> 282nm	
		<b>Retention Time</b> 3.150 and 4.125 min	
3	<b>Stability study RP_HPLC development &amp; Validation.</b>	<b>Mobile phase.</b> Buffer-ACN (80:20 v/v)	[20]
	<b>Drug name.</b> NBH	<b>Column.</b> Hypersil BDS Phenyl (250mm x 4.6mm) 5µm	
		<b>Detector.</b> UV170 (DAD) detector operated	
		<b>Flow rate.</b> 1.2mL/min.	
		<b>Wavelength.</b> 220 nm	
		<b>Retention Time</b> 70 min	
4	<b>RP_HPLC development &amp; Validation.</b>	<b>Mobile phase.</b> ACN: Tetra butyl ammonium hydrogen sulphate buffer (350:650)	[21]
	<b>Drug name.</b> NBH	<b>Column.</b> UV detector and Class-VP software with pre-packed Phenyl column 5µ (250 x 4.6) mm	
		<b>Detector.</b> Uv detector	
		<b>Flow rate.</b> 1.0ml/min	
		<b>Wavelength.</b> 210nm	
		<b>Retention Time</b> 10 min	
5	<b>Stability study RP_HPLC development &amp; Validation.</b>	<b>Mobile phase.</b> ACN-pH 3.5 phosphate buffer (35 + 65, v/v)	[22]
	<b>Drug name.</b> NBH	<b>Column.</b> Phenomenex Luna C8(250 mm)	
		<b>Detector.</b> Photodiode array.	
		<b>Flow rate.</b> 1.0 mL/min.	
		<b>Wavelength.</b> 280 nm.	
		<b>Retention Time.</b> -	
6	<b>RP_HPLC development &amp; Validation.</b>	<b>Mobile phase.</b> MeOH: H2O (80:20 v/v)	[23]
	<b>Drug name.</b> NBH	<b>Column.</b> Hypersil ODS C18 column.	
		<b>Detector.</b> UV-Visible	
		<b>Flow rate.</b> 1.0 ml/min.	
		<b>Wavelength.</b> 282 nm	
		<b>Retention Time</b> 3.175 min and 4.158 min	

7	<b>Simultaneous study RP_HPLC method development</b>  <b>Drug name.</b> NBH VLT	<b>Mobile phase.</b> ACN : Buffer ( PH 3.5 with dilute Ortho Phosphoric acid) 60:40 v/v. <b>Column.</b> C18 Inertsil ODS column (250×4.6mm, 5µm particle size). <b>Detector.</b> UV-Visible <b>Flow rate.</b> 1ml/min. <b>Wavelength.</b> 278nm <b>Retention Time</b> 3.233min & 5.056 min	[24]
8	<b>Validated Chiral LC Method for Enantiomeric Separation in drug doses from.</b>  <b>Drug name.</b> NBH	<b>Mobile phase.</b> n-hexane–ethanol–isopropanol– diethanolamine in the ratio 42:45:13:0.1 (v/v/v/v). <b>Column.</b> Chiralpak AD-3 (250 3 4.6 mm, 3 mm) <b>Detector.</b> Photodiode array (PDA) <b>Flow rate.</b> - <b>Wavelength.</b> 280 nm. <b>Retention Time</b> -	[25]
9	<b>Liquid chromatographic impurity profiling of from bulk drug.</b>  <b>Drug name.</b> NBH HCl	<b>Mobile phase.</b> MeOH:H <sub>2</sub> O(80:20v/v) <b>Detector.</b> UV-Visible <b>Column.</b> C18 column (250 mm length ×4.6 mm) <b>Flow rate.</b> 1.0 ml/min. <b>Wavelength.</b> 222 nm. <b>Retention Time</b> 0.69 min and 0.64 min	[26]
10	<b>Stability study RP_HPLC method development</b>  <b>Drug name.</b> NBH HCL	<b>Mobile phase.</b> Buffer: methanol in the ratio of 50: 50. (PH was adjusted to 5.5 ±0.1 by using Ortho phosphoric acid) <b>Detector.</b> UV-Vis <b>Column.</b> Hypersil BDS C18, 150x4.6, 5µ. <b>Flow rate.</b> 1.0 ml/minute. <b>Wavelength.</b> 254 nm. <b>Retention Time</b> 2.625 and 6.060 min.	[27]
11	<b>Simultaneous study by RP_HPLC method development &amp; validation</b>	<b>Mobile phase.</b> 50 mm ammonium acetate buffer pH 3.5 & ACN (30:70 v/v) <b>Detector.</b> UV-visible.	[28]

	<b>Drug name.</b> NBH HCL VLT	<b>Column.</b> C18 column (25 cm x 4.6 mm 5 µm particle size)	
		<b>Flow rate.</b> 0.8 ml/min	
		<b>Wavelength.</b> 275 nm	
		<b>Retention Time</b> 3.139, 4.920 and 10.101 min	
12	<b>Simultaneous study by RP_HPLC method development &amp; validation</b>	<b>Mobile phase.</b> ACN : MeOH PH4.0 0.02M Potassium hydrogen phosphate buffer (50:20:30 v/v)	[29]
	<b>Drug name.</b> NBH HCL VLT	<b>Detector.</b> DAD	
		<b>Column.</b> C-18	
		<b>Flow rate.</b> 1.0mL/min.	
		<b>Wavelength.</b> 210 nm.	
		<b>Retention Time</b> 2.5 min and 4.3 min	
13	<b>Stability Indicating RP-HPLC Method Development.</b>	<b>Mobile phase.</b> MeOH: 20 mm Ammonium acetate (85:15, v/v) pH 4.0 adjusted with Formic acid.	[30]
	<b>Drug name.</b> NBH HCL CLP	<b>Detector.</b> PDA.	
		<b>Column.</b> Spheri-5-RP-18 (250×4.6 mm)	
		<b>Flow rate.</b> 1.0 ml/min.	
		<b>Wavelength.</b> 274nm	
		<b>Retention Time</b> 3.4 min and 8.4 min	
14	<b>Development and Validation RP_UPLC method.</b>	<b>Mobile phase.</b> 10 mm ammonium dihydrogen phosphate pH adjusted to 3.00 ± 0.02 with dilute orthophosphoric acid as buffer:ACN 60:40 (v/v)	[31]
	<b>Drug name.</b> NBH HCL VLT	<b>Detector.</b> PDA.	
		<b>Column.</b> Thermo C18 (4.6 mm×50 mm, 1.9 µm)	
		<b>Flow rate.</b> 0.4 ml/min.	
		<b>Wavelength.</b> 220nm.	
		<b>Retention Time</b> -	
15	<b>RP_HPLC Method development &amp; validation.</b>	<b>Mobile phase.</b> ACN: Buffer KH <sub>2</sub> PO 4.5PH (45:55 v/v)	[32]
	<b>Drug name.</b> NBH HCL VLT	<b>Detector.</b> UV	
		<b>Column.</b> (Agilent) C18 column (4.6mm x150mm;5µm)	
		<b>Flow rate.</b> 0.7 ml/min.	
		<b>Wavelength.</b>	



		273nm.	
		<b>Retention Time</b> 3.383min and 6.100min.	

## AMLODIPINE

1	<b>Development and optimization of RP-HPLC method</b>  <b>Drug name.</b> S (-) AMB	<b>Mobile phase.</b> Phosphate buffer: ACN (65: 35% v/v)	[33]
		<b>Detector.</b> UV	
		<b>Column.</b> Phenomenex C8 ODS column (150 x 4.6 mm),	
		<b>Flow rate.</b> 1.2ml/min	
		<b>Wavelength.</b> 239 nm.	
		<b>Retention Time</b> <b>4.20 ± 0.02 min.</b>	
2	<b>Development and Validation of a Stability indicating RP-HPLC Method for Simultaneous study.</b>  <b>Drug name</b> ALHF AMB Besylate HDCT	<b>Mobile phase.</b> 0.1 M Ammonium acetate buffer (pH adjusted to 5 using formic acid) and Acetonitrile in the ratio of 65:35 v/v	[34]
		<b>Detector.</b> PDA	
		<b>Column.</b> Inertsil-ODS, C18, 100X 4.6 mm, 5µm column	
		<b>Flow rate.</b> 1.0 ml/min.	
		<b>Wavelength.</b> 232 nm.	
		<b>Retention Time</b> 3.90 5.22 & 1.9min.	
3	<b>Validated HPLC Method for Simultaneous study.</b>  <b>Drug name.</b> AMB Besylate, ATL ASP	<b>Mobile phase.</b> Methanol: phosphate buffer PH 7.0 adjust.	[35]
		<b>Detector.</b> Uv.	
		<b>Column.</b> Hypersil BDS-C18 (250 mm × 4.6mm5.0u)	
		<b>Flow rate.</b> 1ml/min.	
		<b>Wavelength.</b> 235nm.	
		<b>Retention Time</b> 2.58min 3.40min 4.23min.	
4	<b>HPLC method for the simultaneous study</b>  <b>Drug name.</b> AMB TLM	<b>Mobile phase.</b> ACN 0.05M sodium dihydrogen phosphate buffer (60:40) PH. 6	[36]
		<b>Detector.</b> Uv	
		<b>Column.</b> C-18 ODS3	
		<b>Flow rate.</b> 0.8ml/min.	
		<b>Wavelength.</b> 254nm.	

		<b>Retention Time</b> 4.0 min & 8.2 min.	
5	<b>Simultaneous study RP_HPLC method.</b>  <b>Drug name.</b> AMB RVT	<b>Mobile phase.</b> (ACN 40, 55, 70, 40, 40) : (phosphate buffer 60, 45, 30, 60, 60)	[37]
		<b>Detector.</b> UV.	
		<b>Column.</b> kromasil C18 (100 mm, 4.6 mm, 5 $\mu$ m)	
		<b>Flow rate.</b> 1ml/min.	
		<b>Wavelength.</b> 239 nm.	
		<b>Retention Time</b> 2.40 & 4.28 min.	
6	<b>New HPLC Method development</b>  <b>Drug name.</b> AMB VLT	<b>Mobile phase.</b> ACN : phosphate buffer of pH 3.5 and methanol (45:45:10, v/v/v)	[38]
		<b>Detector.</b> UV	
		<b>Column.</b> Li chrospher (RP-18)	
		<b>Flow rate.</b> 1.0 mL·min <sup>-1</sup>	
		<b>Wavelength.</b> 255nm.	
		<b>Retention Time</b> -	
7	<b>Stability Indicating RP-HPLC Method</b>  <b>Drug name.</b> ATN AMB	<b>Mobile phase.</b> ACN & 50mM potassium dihydrogen phosphate buffer (60:40, v/v), apparent pH adjusted to 3.0 with 10% phosphoric acid solution	[39]
		<b>Detector.</b> Uv.	
		<b>Column.</b> C18.	
		<b>Flow rate</b> 1.0ml/min	
		<b>Wavelength.</b> 254nm.	
		<b>Retention Time</b> -	
8	<b>Simultaneous study RP_HPLC method.</b>  <b>Drug name.</b> AMB Besylate IDPM	<b>Mobile phase.</b> 0.02 M potassium dihydrogen phosphate– methanol (30+70, v/v) total pH-adjusted to 3 using o-phosphoric acid	[40]
		<b>Detector.</b> UV.	
		<b>Column.</b> Brownlee C-18, 5 $\mu$ m	
		<b>Flow rate.</b> 1.0 ml/ min.	
		<b>Wavelength.</b> 242 nm	
		<b>Retention Time</b> 5.9 min and 3.6 min.	

9	<b>RP-HPLC method development.</b>  <b>Drug name.</b> AMB HDCT	<b>Mobile phase.</b> Triethylamine:ACN: Methanol in the ratio of 50:25:25(pH adjusted to 3.0 with Orthophosphoric acid)	[41]
		<b>Detector.</b> UV	
		<b>Column.</b> Reverse phase C18 column (Phenomenex C18, 5 $\mu$ , 250mm x 4.6mm).	
		<b>Flow rate.</b> 2.0ml/mi.	
		<b>Wavelength.</b> 232 nm.	
		<b>Retention Time</b> 6.631 & 2.183 min	
10	<b>RP-HPLC method development</b>  <b>Drug name.</b> HDCT AMB OLMN	<b>Mobile phase.</b> TEA Buffer (40%) whose pH was adjusted to 3.5 by using Ortho Phosphoric Acid & ACN (60%)	[42]
		<b>Detector.</b> UV.	
		<b>Column.</b> Symmetry C18 (4.6 X 150mm, 5 $\mu$ m, Make: X Terra) or equivalent in an	
		<b>Flow rate.</b> 0.8ml/min	
		<b>Wavelength.</b> 230nm	
		<b>Retention Time</b> 3.034 min., 4.062 min. & 5.165 min	

## SIMULTANEOUS STUDY OF (AMLODIPINE &amp; NEBIVOLOL)

1	<b>Simultaneous study RP_HPLC method.</b>  <b>Drug name.</b> AMB NBH	<b>Mobile phase.</b> (ACN) & Phosphate buffer (pH 3.0), mixed in a ratio of (40 : 60)	[43]
		<b>Detector.</b> Uv.	
		<b>Column.</b> Lichrospher ODS RP-18 column (250 $\times$ 4 mm), particle size 5 $\mu$ m.	
		<b>Flow rate.</b> 0.8 ml/minute	
		<b>Wavelength.</b> 268 nm.	
		<b>Retention Time</b> AMB 7.47 and 10.25 of NBH	
2	<b>Development and validation of RP-HPLC method</b>  <b>Drug name.</b> AMB NBH	<b>Mobile phase.</b> 67: 33(% v/v) ACN: Phosphate buffer	[44]
		<b>Detector.</b> Uv.	
		<b>Column.</b> Thermo hypersil – keystone C18 (250 x 4.6mm)	
		<b>Flow rate.</b> 1ml/min.	
		<b>Wavelength.</b> 280 nm.	
		<b>Retention Time</b> NBH 2.1min & AMB 5.3 min	

3	<b>RP-HPLC method &amp; development.</b>  <b>Drug name.</b> AMB besylate NBH HCl	<b>Mobile phase.</b> 0.5M Ammonium acetate solution, ACN & triethylamine in the ratio 60:40:0.1 (v/v) & PH 3.0	[45]
		<b>Detector.</b> Uv.	
		<b>Column.</b> Luna C-18, 5 $\mu$	
		<b>Flow rate.</b> 1.5 ml/min.	
		<b>Wavelength.</b> 269nm	
		<b>Retention Time</b> AMB 3.911 & NBH 5.818 min.	
4	<b>RP-HPLC method &amp; development.</b>  <b>Drug name.</b> AMB besylate NBH HCl	<b>Mobile phase.</b> Water & ACN( PH 3.5 Orthophosphoric acid)	[46]
		<b>Detector.</b> Uv.	
		<b>Column.</b> A C18 (250 $\times$ 4.6 mm, 5 $\mu$ )	
		<b>Flow rate.</b> 1.0 ml min.	
		<b>Wavelength.</b> 268 nm.	
		<b>Retention Time</b> AMB (2.769) and NBH (5.236)	
5	<b>RP_HPLC Method development.</b>  <b>Drug name.</b> S-AMB besylate NBH HCl	<b>Mobile phase.</b> ACN : water (60:40v/v)	[47]
		<b>Detector.</b> Uv.	
		<b>Column.</b> Sunfire column C18 (4.6 $\times$ 250mm) with 5 $\mu$ m	
		<b>Flow rate.</b> 1 ml/min.	
		<b>Wavelength.</b> 265 nm.	
		<b>Retention Time</b> AMB 3.553 & NBH 2.970 min	
6	<b>RP_HPLC Method development.</b>  <b>Drug name.</b> AMB besylate NBH	<b>Mobile phase.</b> ACN & Phosphate buffer (pH 2.5 $\pm$ 0.1) of 60:40	[48]
		<b>Detector.</b> Uv.	
		<b>Column.</b> Thermo Hypersil C18 (250 x 4.6 mm, 5 $\mu$ m)	
		<b>Flow rate.</b> 1.0 ml/min	
		<b>Wavelength.</b> 220nm	
		<b>Retention Time</b> AMB 6.39 and NBH 7.54 min.	
7	<b>Stability Indicating HPLC Method.</b>	<b>Mobile phase.</b> 0.05M Potassium dihydrogen phosphate: ACN (pH 3.0) (60:40v/v)	[49]
		<b>Detector.</b> Uv.	

	<b>Drug name.</b> S-AMB besylate NBH	<b>Column.</b> Zorbax C8 G (250mm x 4.6mm, 5µm) <b>Flow rate.</b> 1.0ml/min <b>Wavelength.</b> 269nm. <b>Retention Time</b> AMB 5.2 and NBH 6.8 min	
8	<b>Stability Indicating HPLC Method.</b>  <b>Drug name.</b> AMB besylate NBH	<b>Mobile phase.</b> ACN & 0.01 M ammonium acetate (pH adjusted to 4.5 using glacial acetic acid) (50: 50. v/v) <b>Detector.</b> PDA. <b>Column.</b> Eclipse XDB plus C18 column (4.6 X 150 mm; 5 µm) <b>Flow rate.</b> 1.0 mL/min. <b>Wavelength.</b> 265nm. <b>Retention Time</b> AMB 2.967 and NBH 3.510 min	[50]
9	<b>Novel RP-HPLC method for simultaneous study.</b>  <b>Drug name.</b> AMB besylate NBH	<b>Mobile phase.</b> acetate Buffer pH 5& ACN (60:40v/v) <b>Detector.</b> Uv. <b>Column.</b> Kromasil ODS column (250 x 4.6mm x 5µ particle size) <b>Flow rate.</b> 1ml/min. <b>Wavelength.</b> 268nm <b>Retention Time</b> AMB 5.26min and NBH 6.84min.	[51]
10	<b>A Validated RP-HPLC Method for Simultaneous study.</b>  <b>Drug name.</b> S-ABH besylate NBH	<b>Mobile phase.</b> Ammonium acetate buffer (pH 4.5): ACN (50:50 v/v). <b>Detector.</b> PDA. <b>Column.</b> Zorbax SB CN column (250 × 4.6 mm, 5µ particle size) <b>Flow rate.</b> 1ml / min. <b>Wavelength.</b> 274 nm <b>Retention Time.</b> AMB 9.590 ± 0.04 and NBH 13.56 ± 0.05 min.	[52]

**ABBREVIATIONS:**

- ❖ Nebivolol-NBH, Amlodipine-AMB, Valsartan-VLT, Cilnidipine-CLP, Telmisartan-TLM, Rosuvastatin-RVT, Atorvastatin-ATN, Indapamide-IDPM, Hydrochlorothiazide-HDCT, Olmesartan-OLMN, ATL-Atranol, ASP-Aspirin, Aliskiren Hemifumarate-ALHF

- ❖ MeOH-methanol, H<sub>2</sub>O-water, ACN-acetonitrile, nm-nanometer,
- ❖ PDA-Photodiodearray detector, UV-Ultraviolet detector, DAD- Diode array detector.

**CONCLUSION:**

In conclusion, a broad range of techniques are available for the analysis of nebivolol and amlodipine in pharmaceutical formulations. The analysis of the published data revealed that the HPLC methods were extensively used for the determination of nebivolol and amlodipine in drug dosage form. For determination of nebivolol and amlodipine in drug samples, we commend the HPLC method, since this HPLC method separation ability to sensitivity and selectivity, allowing the unambiguous identification of NBH and AMB its metabolites. For analysis of both drug in pharmaceuticals, HPLC detection is applicable because this method provides accurate results and low cost compared to more advanced detection techniques. This review carried out an overview of the current state-of-art HPLC methods for the determination of nebivolol and amlodipine. The review would help analytical chemists in knowing the key solvents and their combinations for their available set of instruments in the analytical laboratory. The effective combination of parameters should minimize the cost of the analysis and reduce the time required for producing are liable analytical method. The methods are also useful for determining parameters for in-process evaluation during the manufacturing of API.

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