

# Development and validation of RP-HPLC determination of Ambrisentan

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**Abstract:** The aim of the present review work was to develop and validate a simple, and economical method for the analysis of Ambrisentan in bulk drug pharmaceutical dosage forms by reverse phase high pressure liquid chromatography. After analyzing various mobile phase buffers, WELCHROM C-18 reverse phase column (4.6x250 mm) with mobile phase phosphate buffer ACN (50:50 v/v) was found effective with RT 29min. and eluents were monitored at 226 nm.

**Keywords:** Ambrisentan, RP-HPLC, Whatmann Filter Paper, Anti-hypertensive

## INTRODUCTION:

Ambrisentan is anti-hypertensive agents. Orally active selective type A antagonist indicated for the pulmonary arterial hypertension A endothelin receptor Treatment of non-peptide is a highly selective endothelin. type A receptor antagonist. (1) Chemically (AMB) is (2S) - 2[(4,6 dimethyl-pyrimidin-2, yl) oxy]-3-methoxy-3-3diphenyl - propanoic acid with molecular formula C<sub>22</sub>H<sub>22</sub>N<sub>2</sub>O<sub>4</sub> molecular weight 378.421 g/mol.

**literature survey:** reveal that there are very few HPLC, UV methods were available, [2].

Hence, RP-HPLC method for estimation of Ambrisentan was developed.

Ambrisentan taken orally with or without food. it is available as 5 and 10 mg film Coated, convex, unscored tablet (3-4) Ambrisentan can reduce blood pressure in the lung and improve activity level and well-being in PAH patient (5)

## Background:

Ambrisentan is an orally active selective type A endothelin receptor antagonist indicated for the treatment of pulmonary arterial hypertension. It is approved in Europe, Canada and the United States for use as a single agent to improve exercise ability and delay clinical worsening. In addition, it is approved in the United States for use in combination with tadalafil to reduce the risks of disease progression, hospitalization and to improve exercise ability. Studies establishing the efficacy of Ambrisentan included patients with both idiopathic or heritable pulmonary arterial hypertension and those with pulmonary arterial hypertension associated with connective tissue diseases. Patients studied displayed symptoms and etiologies predominantly of WHO Functional Class II-III. As an endothelin receptor antagonist, Ambrisentan prevents endogenous endothelin peptide from constricting the muscles in blood vessels, allowing them to relax and permit a reduction in blood pressure.

## Brand Names:

Letairis

## Generic Name:

Ambrisentan

## Drug Bank Accession Number:

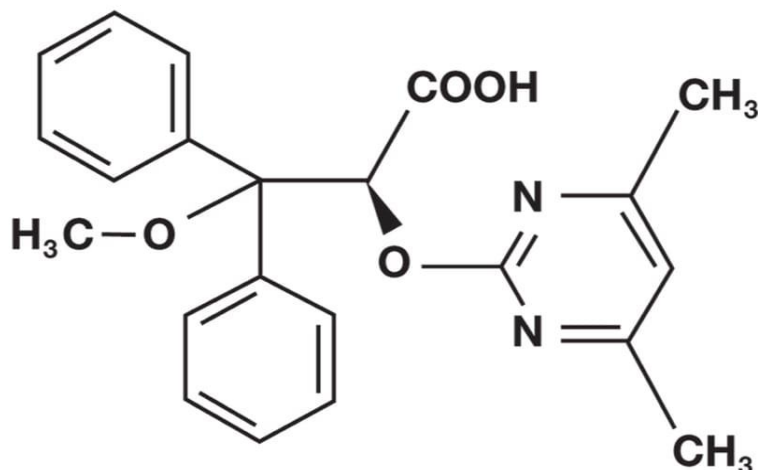
DB06403

## Type:

Small Molecule

## Groups:

Approved, Investigational

**Structure:****Molecular Weight:**

Average: 378.428

Monoisotopic: 378.157957196

**Chemical Formula:**C<sub>22</sub>H<sub>22</sub>N<sub>2</sub>O<sub>4</sub>**Synonyms:**

Ambrisentan

**Pharmacokinetics:****1) Absorption:**

Ambrisentan is rapidly absorbed with peak plasma concentration occurring around 2 hrs after oral administration .

C max and AUC increase proportionally with dose across the therapeutic dosing range.

Absolute oral bioavailability of Ambrisentan unknown.

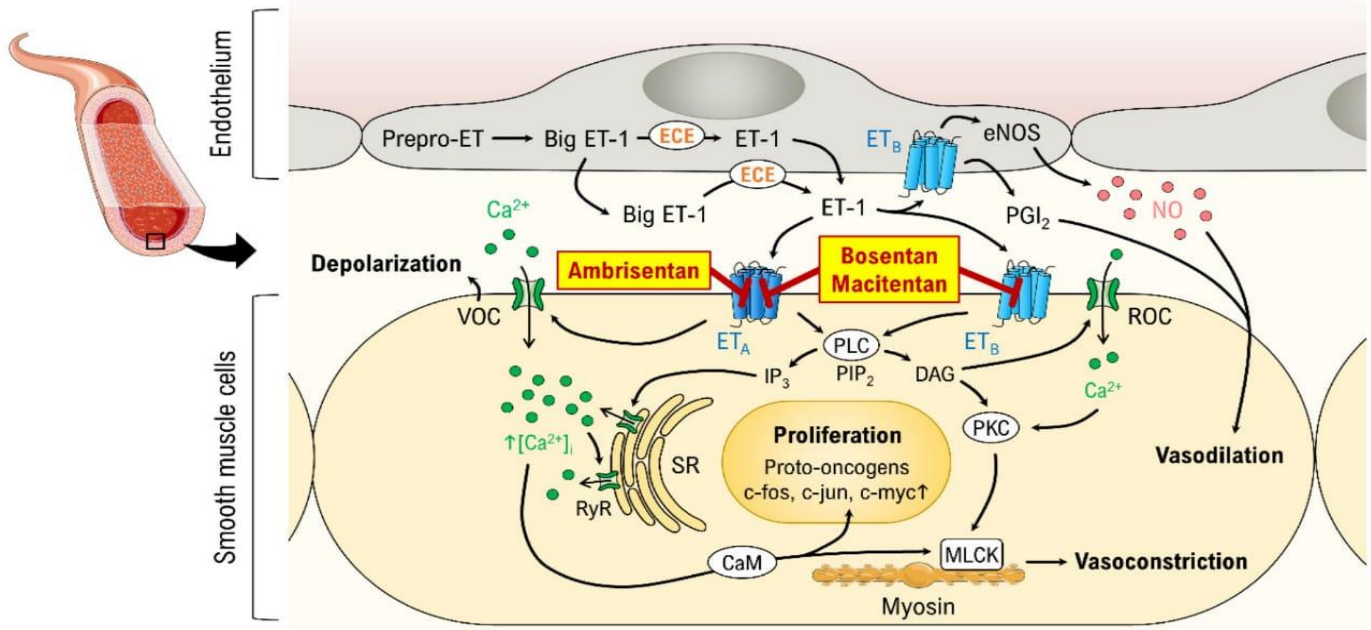
**Volume of distribution:**

Ambrisentan has a low distribution into red blood cells, with a mean blood: plasma ratio of 0.57 and 0.61 in males and females, respectively.

**Protein binding:**

Ambrisentan is 99% plasma protein bound, primarily to albumin (96.5%) and to a lesser degree alpha1-acid glycoprotein.

**Pharmacodynamics:**

**Mechanism of action of Ambrisentan:**

Endothelin -1 (ET-1) is an endogenous peptide that acts on the endothelin type A (ETA) and Endothelin type B (ETB) receptor in vascular smooth muscle and endothelium

ETA – mediated action include vasoconstriction and all proliferation where as ETB predominantly mediates vasodilation, anti-proliferation and ET-L clearance

In patient with pulmonary arterial hypertension, arterial hypertension ,ET-1 level are and co-melate with increased right arterial pressure and severity of disease .

Ambrisentan is one of several newly developed vasodilator drug that selectively target the endothelin type-A (ETA) receptor ,inhibiting its action and preventing vasoconstriction

Selective inhibition of the ETA receptor prevent phospholipase C-mediated vasoconstriction and protein kinase C-mediated all proliferation Endotheline type B (ETB) receptor function is not significantly inhibited, and nitric oxide and prostacyclin, cyclic GMP- and cyclic AMP- mediated vasodilation, & endothelin-1 (EC-1) clearance is persend.

**Metabolism:**

Ambrisentan is a metabolized primarily by uridine 5'-diphosphate glucuronosyltransferases (UGTs) 1A9S, 2B7S,1A3S to form ambrisentan glucuronide. Ambrisentan is also metabolized to a lesser extent by CYP3A4, CYP3A5 and CYP2C19 to form 4-hydroxymethyl ambrisentan which is further glucuronidated to 4-hydroxymethyl ambrisentan glucuronide.

Ambrisentan

Ambrisentan glucuronide

4-hydroxymethyl ambrisentan

4-hydroxymethyl ambrisentan glucuronide

**Route of elimination:**

Ambrisentan is primarily cleared by non-renal pathways. Along with its metabolites, ambrisentan is primarily found in the feces following hepatic and/or extra-hepatic metabolism. Approximately 22% of the administered dose is recovered in the urine following oral administration with 3.3% being unchanged ambrisentan.

**Half-life:**

Ambrisentan has a terminal half-life of 15 hours. It is thought that steady state is achieved after around 4 days of repeat-dosing.

**Clearance:**

The mean oral clearance of ambrisentan was found to be 38 mL/min in healthy subjects and 19 mL/min in patients with pulmonary artery hypertension.

**Toxicity:**

Ambrisentan is teratogenic and has a high risk of embryo-fetal toxicity. LD50 was found to be greater than or equal to 3160 mg/kg when studied in rats. There was no evidence of carcinogenic potential in 2-year oral daily dosing studies in rats and mice.

**Preparation of Standard Solution of Ambrisentan:**

About 100 mg of accurately weighed, pure Ambrisentan was and dissolved in 100 ml of mobile phase to get 1mg. ml stock me solution. working standard solution of ambrisentan was prepared with mobile phase. The final was made with the mobile phase. The standard Solution was filtered through 0.45 um nylon membrane filled and degassed by sonication [1]

**Preparation of Sample Solution of Ambrisentan:**

The content of tablets of ambrisentan were 20 weighed and transferred into a mortar accurately and ground to fine powder from this, tablet powder which is equivalent to 100 mg Of Ambrisentan was taken into 100ml Volumetric Flask and the drug was made up to the mark with mobile phase. The resulting solution was filtered using Whatmann grade no. 1.

Filter paper and degassed by sonication This Solution we further suitably diluted for chromatography. [1]

**Preparation of share standard Solution**

prepare a Solution in containing 0.1 mg/ml of ambisentan in diluent. weigh accurately 25.0 mg of Ambrisentan working Standard into a 50ml clean, dry volumetric flask. Add 30 ml of diluent and Sonicate to dissolve. Make up the volume with diluent and mix. Diluent 5 ml of to 25 ml. with diluent & mix Labeling this standard Solution as STD-1.

Prepare the standard solution in duplicate and label as STD-2.

**Sample solution:**

Prepare Solution containing al mg/ml of Ambrisentan. in diluent weigh and transfer 10 tab. into 100 ml clean, dry volumetric flask. Add about 70 ml of diluent and Sonicate for 15 min with intermittent shaking. Make up to Volume with diluent and mix. Centrifuge the sample for 5 mins at 2500 rpm. through 0.45 um syringe filter by discarding first 5ml of filtrate Dilute 5ml to 25 ml with diluent and mix.[2]

**Observation:**

The blank Solution Placebo solution Standard Solution. Impurity Solutions. Sample Solutions and Impurity spiked sample Solutions are analysed by HPLC System and checked for interference. There is no interference peak was blank, observed due to blant plaube and brown impunity at the retention time of Amhnsentan

**Conclusion:**

ABN Ambnsentan is orally active antihypertensive drug wed in the treatment of pulmonary arthal hypertension and no official method is reported. In any of the pharmacopacial for it routine Lestimation the developed HPLC methad wal Validatid For Specificity accuracy and prasion

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