

# Hydralazine and Isosorbide Dinitrate: An Analytical Review

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## ABSTRACT

Hydralazine is antihypertensive agent which is smooth muscle relaxant helps to treat high blood pressure. Hydralazine comes in a class of medications called vasodilators. It works by relaxing the blood vessels so that blood can flow more easily through the body. Isosorbide Dinitrate is antianginal agent which is used to prevent chest pain in patients with a certain heart condition (coronary artery disease). This medication belongs to a class of drugs known as nitrates. It works by relaxing and widening blood vessels so blood can flow more easily to the heart. This is the literature review of developed various analytical method for validation and estimation of Hydralazine, Isosorbide Dinitrate in combination. The analytical method like RP-HPLC (Reverse Phase High Performance Liquid

Chromatography), quality by design, Ultraviolet (UV) Spectrophotometry, Simultaneous Equation Method (SEM), stability indicating method were reported for Hydralazine Hydrochloride, Isosorbide Dinitrate. These analytical methods can be used for qualitative and quantitative estimation of Isosorbide Dinitrate and Hydralazine Hydrochloride in single dosage form as well as in combination with other drugs.

**Keywords:** Antianginal, Antihypertensive, HPLC (High Performance Liquid Chromatography), Hydralazine, Isosorbide nitrate

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## ABBREVIATIONS

HLZ: Hydralazine Hydrochloride; ISD: Isosorbide Dinitrate; RP-HPLC: Reverse Phase High Performance Liquid Chromatography; CGMP: Cyclic Guanosine Mono Phosphate; PKG: Protein Kinase-G; NO: Nitric oxide; V-HeFT: Vasodilator-Heart Failure Trial; CHF: Cardiac Heart Failure; A-HeFT: African-American Heart Failure; DBH: Dopamine Beta-Hydroxylase

## INTRODUCTION

In the 1950s Hydralazine was developed as a malaria treatment, along this hydralazine showed antihypertensive ability. Hydralazine chemically, 1-Hydrazinophthalazine and derived from Phthalazine. Hydralazine is orally bioavailable vasodilator. It relaxes (widens) veins and arteries, which make it easier for your heart to pump. It is sold under the brand name Apresoline to treat high blood pressure and heart failure. It also used as Anti-hypertensive agent in case of preeclampsia. It comes under the class of Smooth muscle relaxant. The mechanism of its action, Hydralazine is direct arteriole vasodilator. The mode of action is relates with intracellular calcium homeostasis. It inhibits Inositol trisphosphate (IP<sub>3</sub>)-induced release of calcium from the smooth muscle cells sarcoplasmic reticulum, it also inhibit myosin phosphorylation within the arterial smooth muscle. This results in the reduction in peripheral vascular resistance and leads to a compensatory baroreceptor-mediated release of epinephrine and norepinephrine, this cause increase in venous return and cardiac output. Hydralazine may cause stimulation of the sympathetic nervous system and that may cause tachyphylaxis and tachycardia, so it is sometimes given with a beta-blocker or diuretic for better patient tolerance (McComb MN, *et al.*, 2016).

The metabolism of Hydralazine takes place in liver *via* polymorphic acetylation. Slow acetylators needs lower doses of the drug. Both the acetylated drug and unchanged drug are excreted in the urine and feces (Kirsten R, *et al.*, 1998). In the clinical studies it was found to be HLZ shows more effective together with Isosorbide Dinitrate, for the treatment of people of African descent. HLZ shows instant effect within the time period of 15 min and last up to 6 hours. It is administered by not only orally but also injected into vein (Stuart MC, *et al.*, 2009).

Isosorbide Dinitrate chemically known as 1, 4:3, 6-dianhydro-D-glucitol-2, 5-dinitrate, which is a nitrate ester and a glucitol derivative. Isosorbide Dinitrate is antianginal agent used to prevent chest pain from less supply of blood to heart i.e. angina pectoris and also used to treat heart failure and esophageal spasms. It is sold under the brand name Isordil and Sorbitrate. The clinical study says that ISD is useful in heart disorder due to systolic dysfunction along with hydralazine (Chavey WE, *et al.*, 2008). It can be used sublingually at the time of attack as well as orally for chronic prophylaxis.

Isosorbide is a nitrate that shows its pharmacologic activity by releasing Nitric Oxide (NO) and an Endothelium-Derived Relaxing Factor (EDRF). Nitric oxide is endogenously produced in the cell in endothelium to dilate the blood vessels. Isosorbide Dinitrate undergoes bioactivation process in the cell organelle endoplasmic reticulum *via* cytochrome P450 (cytochrome P450) enzymes to release nitric oxide (Daiber A and Münzel T, 2015), this NO activates the enzyme soluble guanylyl cyclase in the vascular smooth muscles, this result in increasing the levels of intracellular cyclic Guanosine Monophosphate (cGMP) and the associated protein kinases such as cGMP-dependent protein kinases (cGK-I). The cGMP activates the Myosin Light Chain Phosphatase (MLCP). This MLCP cause dephosphorylation of the myosin light chain. CGMP-cGK-I inhibits the Inositol-1, 4, 5-trisphosphate (IP<sub>3</sub>)-dependent calcium release, so intracellular calcium level is decreased (Etter EF, *et al.*, 2001; Lincoln TM, *et al.*, 1994). Due to the decreased level of intracellular calcium inhibits the Myosin Light Chain Kinase (MLCK) (Divakaran S and Loscalzo J, 2017). The MLCK with the unphosphorylated myosin light chain, causes the myosin head to detach from the actin component of the smooth muscle, resulting in smooth muscle relaxation and causing widening of vessel (Daiber A and Münzel T, 2015).

## LITERATURE REVIEW

### History of BiDil

BiDil is the first Drug combination approved by Food and Drug Administration (FDA) marketed for a single racial-ethnic group. Hydralazine and Isosorbide Dinitrate is the fixed dose combination sold under the brand name BiDil. The BiDil is approved by

FDA by 23-Jun-2005 to treat and prevent heart failure. In 1980-1985, Dr. Jay Cohn from the University of Minnesota led a clinical trial in collaboration with the US Veterans Administration called the Vasodilator-Heart Failure Trial (V-HeFT I), in this trial it was tested whether Hydralazine and Isosorbide Dinitrate combination increases survival rate of patients with heart failure, the results was positive and next trial was held under V-HeFT II, which tested the combination of Hydralazine and Isosorbide Dinitrate against drug enalapril (Krinsky S and Sloan K, 2011). In 1989, Scientist Cohn got patent for combination treatment of Hydralazine and Isosorbide Dinitrate as US patent 4868179. Cohn licensed his patent with Medco Company. In early 1990s Medco prepared New Drug Application (NDA) for FDA's approval for BiDil on the basis of V-HeFT trials. But the trials were not designed to support NDA and FDA rejected the application in 1997 because statistical power was not enough to show whether the combination really worked (Krinsky S and Sloan K, 2011).

The scientist Cohn again analysed the data and found a signal that drug combination of Hydralazine and Isosorbide Dinitrate may work better than previous, he made V-HeFT trials on self-identified African-American patients and published a paper on his work and applied for new patent on the use of BiDil in black patients (Krinsky S and Sloan K, 2011). It had already known that African-Americans with Congestive Heart Failure (CHF) respond less effectively to conventional CHF treatments (Exner DV, *et al.*, 2001).

Cohn licensed new patent and old patent to the NitroMed Company, this company ran a clinical trial called the African-American Heart Failure Trial (A-HeFT), in 2004 the result of this trial were published in the New England Journal of Medicine (Taylor AL, *et al.*, 2004). The results were reduced mortality rate by 43%, reduced hospitalizations by 39% (Taylor AL, *et al.*, 2004; PubChem, 2022). In June-2005, FDA approved BiDil on the basis of A-HeFT. Heart Failure Society of America included the use of the fixed dose combination of Hydralazine and Isosorbide Dinitrate as the standard of care in the treatment of heart failure in blacks in 2006 (Figures 1 and 2).

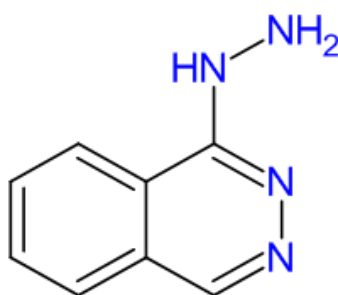


Figure 1: Structure of Hydralazine

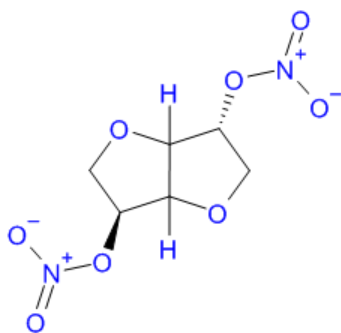


Figure 2: Structure of Isosorbide Dinitrate (ISD)

## Mode of action

BiDil is a vasodilator, shows effect on both arteries and veins, BiDil on administration, which releases NO causing activating guanylyl cyclase to relax vascular smooth muscle. It also helps to normalize NO levels, wide the veins and arteries to increase productive blood flow, and decrease workload on the heart (Table 1).

Table 1: Drug profile (Pubchem, 2022)

S. No	Properties	Hydralazine	Isosorbide Dinitrate (ISD)
1.	Color	Orange solid	White solid
2.	Molecular weight	160.18 gm/mol	236.14 gm/mol
3.	Molecular formula	C <sub>8</sub> H <sub>8</sub> N <sub>4</sub>	C <sub>6</sub> H <sub>8</sub> N <sub>2</sub> O <sub>8</sub>
4.	Category	Vasodilator	Organic nitrates
5.	Melting Point (MP)	172°C-173°C	70°C
6.	pKa value	-3.9	7.1
7.	Solubility	Acetic acid, Methanol, Ether	Acetone, Alcohol, Ether
8.	pH	3.5-4.5	9-10

## Medical use of Hydralazine and Isosorbide Dinitrate

Along with antihypertensive activity, Hydralazine may shows following activity (Drug Bank, 2007):

- Beta-Lysine 5, 6-aminomutase inhibitor.
- Cyclin-Dependent Kinase 9 (CDK9)/cyclin T1 inhibitor.
- Thromboxane B2 antagonist.
- Glucose oxidase inhibitor.
- Arylacetonitrilase inhibitor.
- Guanylate cyclase stimulant.
- Aryl-alkyl acylamidase inhibitor.
- Aspartate-phenylpyruvate transaminase inhibitor.
- Aldehyde dehydrogenase (pyrroloquinoline-quinone) inhibitor.
- N-methylhydantoinase (ATP-hydrolysing) inhibitor.

## Inhibitory action of hydralazine on catecholamine-synthesizing enzymes

The Hydralazine cause direct effect on catecholamine-synthesizing enzymes, it causes concentration-dependent inhibition of Tyrosine Hydroxylase (TH) obtained from bovine adrenal medulla and more promissable effect was shown when enzyme are incubated with the drug before the enzymatic assay (Morita K, *et al.*, 1986).

The pharmacokinetic study showed that hydralazine increases the apparent Km value of the enzyme for tyrosine and cofactor, 6, 7-dimethyl-5, 6, 7, 8-tetrahydropterin (DMPH4), without changing V<sub>max</sub>. The inhibitory effect of hydralazine was irreversible and excessive amount of FeSO<sub>4</sub> were unable to restore the enzyme activity inhibited by this drug. Hydralazine also inhibits the Dopamine Beta-Hydroxylase (DBH) in chromaffin granule membranes. This kinetic study has also shown that Hydralazine increased the apparent Km value of DBH for ascorbic acid without changing in the V<sub>max</sub> but it decreased the V<sub>max</sub> of the tyramine enzyme without any change in the apparent Km value. The observation of this study concludes hydralazine presumably causes the inhibition of catecholamine-synthesizing enzymes this result into allosteric alterations in the molecular structures of these enzymes. Thus it was confirmed that inhibitory action of hydralazine is due to its metal-chelating activity (Morita K, *et al.*, 1986).

Along with antianginal activity, Isosorbide Dinitrate may show following activity:

- Nitric oxide donor.
- Myocardial ischemia treatment.
- Mannotetraose 2- $\alpha$ -N-acetylglucosaminyltransferase inhibitor.
- Beta glucuronidase inhibitor.
- Saccharopepsin inhibitor.
- Histidine kinase inhibitor.
- Sugar-phosphatase inhibitor.
- Ribulose-phosphate 3-epimerase inhibitor.
- Aspulvinone dimethylallyltransferase inhibitor.
- ISD used in phobic disorders treatment.

**Isosorbide Dinitrate a guanylate cyclase stimulant:** The guanylyl cyclase is soluble in the heme-protein. The heme-protein soluble guanylyl cyclase is the intracellular receptor for nitric oxide. Guanylyl cyclase is heterodimeric enzyme with  $\alpha$  and  $\beta$  subunits having heme moiety which is essential for binding of NO and activation of enzyme. The physiological responses like smooth muscle relaxation, inhibition of inflammation, and thrombosis are mediated by stimulation of guanylyl cyclase (Nossaman B, *et al.*, 2012).

### Methodology

Different analytical methods like UV spectrophotometry, HPLC, High-Performance Thin-Layer Chromatography (HPTLC) and bio-analytical methods are developed for simultaneous estimation of Hydralazine and Isosorbide Dinitrate.

**UV spectroscopy:** The principle of UV-Visible spectroscopy is based on the absorption of ultraviolet light by drugs which produce different spectra. Spectroscopy deals with interaction of light and matter. When drug sample is bombarded with light then it absorb the light and get excited and after some instant come ground state, this result in the production of a spectrum.

When drug sample absorb UV radiation the electron undergoes excitation, followed by relaxation i.e. jump to ground state. It is important to know that the energy difference between ground state and excited state is amount of UV radiation absorbed by sample, this is how UV spectroscopy works.

The different researchers developed and validate UV spectroscopic method for Hydralazine and Isosorbide Dinitrate.

Mastannama S, *et al.* the objective of this study was to present a simple, accurate and precise UV spectrophotometric method for simultaneous estimation of HLZ and ISD in bulk and their combined dosage form (Mastannama SK and Sridhar TA, 2015). In this study, the drug concentration is estimated by using Simultaneous Equation Method (SEM) at 232 nm and 208.28 nm (i.e.,  $\lambda_{max}$  values of HLZ and Histidinol Dehydrogenase (HISD) respectively). The corrected absorbance was carried at 208 nm and calibration curves were linear with correlation coefficient 0.999 over the concentration range of 2-10  $\mu$ g/mL for both HLZ and ISD (Mastannama SK and Sridhar TA, 2015).

**HPLC method:** HPLC is the High Performance Liquid Chromatography, the chromatographic separation is done on the basis of distribution of the analyte i.e. sample between a mobile phase i.e. eluent and stationary phase (packing material of the column). On the basis of chemical structure of the analyte, the molecules are retarded while passing the stationary phase. The specific intermolecular interaction between the molecules of a sample and the packing material define their on column. So the different constituents of sample are eluted at different time, that's how the separation of the sample ingredients is achieved.

The separation takes place on column in two ways i.e. normal phase and reverse phase. Normal phase is called Normal phase high performance li-

quid chromatography, reverse phase is termed as reverse phase high performance liquid chromatography.

The RP-HPLC, UPLC (Ultra Performance Liquid Chromatography) and various stability determining methods are reported for simultaneous estimation of HLZ and ISD.

Neelima K, *et al.* work in this paper is focusing on development of simple, sensitive, linear, precise and accurate RP-HPLC method for simultaneous estimation of Hydralazine, Isosorbide Dinitrate in bulk and tablet formulation. The Zodiac C18 (250 mm  $\times$  4.6 mm) 5  $\mu$  column is used for chromatographic separation of drug in an isocratic mode. The mobile phase consists of 0.01 M Ammonium acetate: Acetonitrile: Methanol in the ratio of 50:30:20 v/v and pH adjusted to 3 using ortho phosphoric acid was delivered at a flow rate of 1 ml/min and effluents were monitored at 270 nm. The Retention Factor (RF) value of Hydralazine, Isosorbide Dinitrate was found to be 2.337 and 3.413 min, respectively. Calibration curves were linear with a correlation coefficient of 0.994 for Hydralazine, and 0.997 for ISD over the concentration range of 45-105  $\mu$ g/ml for Hydralazine, and 24-56  $\mu$ g/ml for ISD and precise with (% Relative Standard Deviation (%RSD)<2). This method was used for analysis of Isolazine tablet sample and the conclusion of this assay was obtained within specification limit (Neelima K and Prasad YR, 2014).

Mastanamma S, *et al.* in this article the chromatographic separation of Hydralazine and Isosorbide Dinitrate from their degradation products using Zorbax C18 (250 mm  $\times$  4.6 mm I.D; 5  $\mu$ m) column at detection wavelength 278 nm, using a mobile phase consists of Orthophosphoric acid (0.1%) pH 2.1 and Methanol (60/40) in an isocratic elution mode at a flow rate of 1 ml/min. The retention times for Hydralazine HCl and Isosorbide Dinitrate were found to be 3.7 and 4.7 min respectively. The proposed method is suitable for application in quality-control laboratories for quantitative analysis of both the drugs individually and in combination dosage forms, since it is simple and rapid with good accuracy and precision (Mastanamma S, *et al.*, 2016).

Kassey S, *et al.* found a simple, sensitive, linear, precise and accurate RP-HPLC method for simultaneous estimation of ISD and Hydralazine Hydrochloride in tablet formulation was reported. The chromatographic separation of the two drugs was achieved on INERTSIL ODS C18 (150  $\times$  4.6  $\times$  5  $\mu$ ) column in an isocratic mode. The mobile phase consisting of 0.1 M sodium dihydrogen phosphate: methanol in the ratio of 80:20 v/v and was delivered at a flow rate of 1 ml/min and effluents were monitored at 270 nm. The retention time of was found to be 1.679 and 3.430 min, respectively (Kassey S, *et al.*, 2014).

Madhuri PL, *et al.* in this article the stability indicating Reversed-Phase Liquid Chromatography (RP-LC) method for simultaneous quantitative estimation of Hydralazine Hydrochloride and Isosorbide Dinitrate in bulk drug and combined tablet dosage form was reported. Chromatographic separation was done in isocratic mode at ambient temperature on hypersil C18 column having size 150  $\times$  4.6 mm and 5  $\mu$  particle size using mobile phase comprising of potassium dihydrogen orthophosphate (0.01 M, pH 5.0 adjusted with dilute ammonia solution) and acetonitrile in the proportions of 50:50 V/V. The developed method could separate HLZ and ISB from its degradation products with good resolution (Madhuri PL and Gowri S, 2015).

Santhosh G, *et al.* found a simple, precise, rapid, specific and accurate RP-HPLC method was reported for simultaneous estimation of Isosorbide Dinitrate and Hydralazine HCl in pharmaceutical dosage form. Separation of drug was carried on Agilent Zorbax (C18) (4.6 mm  $\times$  250 mm, 5  $\mu$ m) column, with mobile phase consist of mixture of buffer (pH 6.5, adjusted with potassium dihydrogen phosphate), acetonitrile in the ratio of 70:30 v/v, at the flow rate 0.8 ml/min. The detection was carried out at 274 nm. The retention times of Hydralazine HCl and Isosorbide Dinitrate was found to be 2.7 and 3.6 min respectively. The linearity, accuracy, precision,



limit of detection and limit of quantitation, robustness and ruggedness were validated according to ICH guidelines. Linearity of Isosorbide Dinitrate was found in the range of 50-150 µg/mL and that for Hydralazine HCl was found to be 50-150 µg/mL. The correlation coefficient for Isosorbide Dinitrate and Hydralazine HCl were 1 and 0.999 respectively (Santosh G, *et al.*, 2014) (Table 2).

**Table 2: High Performance Liquid Chromatography (HPLC) methods**

S. No	Method	Parameter	Author
1.	RP-HPLC	SP: Zodiac C18 column	Neelima K and Prasad YR, 2014
		MP: Ammonium acetate:Acetonitrile:Methanol	
		(50:30:20 v/v)	
		Wavelength: 270 nm	
		Flow rate: 1 ml/min	
		Temp: Ambient	
2.	Stability determining-RP-HPLC	SP: Zorbax C18 column	Mastanamma S, <i>et al.</i> , 2016
		MP: Orthophosphoric acid:Methanol (60/40)	
		Wavelength: 278	
		Flow rate: 1 ml/min	
		Temp: Ambient	
3.	RP-HPLC	SP: Inertsil ODS C18 column	Kassey S, <i>et al.</i> , 2014
		MP: Sodium dihydrogen phosphate:Methanol (80:20 v/v)	
		Wavelength: 270 nm	
		Flow rate: 1 ml/min	
		Temp: Ambient	
4.	Stability indicating RP-LC	SP: Hypersil (BDS) C18 column	Madhuri PL and Gowri S, 2015
		MP: Potassium dihydrogen orthophosphate:Acetonitrile (50:50 v/v)	
		Wavelength: 275nm	
		Flow rate: 1 ml/min	
		Temp: Ambient	
5.	RP-HPLC	SP: Agilent Zorbax C18 column	Santosh G, <i>et al.</i> , 2014
		MP: Potassium dihydrogen phosphate:Acetonitrile (70:30 v/v)	
		Wavelength: 274 nm	
		Flow rate: 0.8 ml/min	
		Temp: Ambient	
<b>Note:</b> SP: Stationary Phase; MP: Mobile Phase; RP-HPLC: Reverse Phase High Performance Liquid Chromatography; RP-LC: Reversed-Phase Liquid Chromatography			

**Green-Analytical Method:** Chanduluru HK, *et al.* the objective of this study an eco-friendly determination of Isosorbide Dinitrate and Hydralazine Hydrochloride using a green analytical quality by design-based UPLC Method. The separation of Hydralazine Hydrochloride and Isosorbide Dinitrate, as well as their degradation product was reported with the help of Phenomenex C18 (50 × 2.1 mm, 2 µm) column containing ethanol and 0.1% trifluoroacetic acid (60%:40% v/v) at a flow rate of 0.5 mL min<sup>-1</sup>. The linearity range of 10-60 mg/mL-1 and 18.75-112.5 mg/mL with R<sup>2</sup> of 0.9998 and 0.9992 for ISD and HLZ, respectively along with accuracy, re-

producibility, and selectivity (Chanduluru HK and Sugumaran A, 2021).

## DISCUSSION

Hydralazine is an orange crystalline powder, whereas Isosorbide Dinitrate is the white colored odorless crystalline powder. HLZ has lower molecular weight than ISD. The melting point difference is also large between HLZ and ISD. As we know that lower the pKa value stronger the acid, so the pKa value of HLZ is higher than ISD, since HLZ is more acidic than ISD. Both above mentioned drugs are soluble in organic solvent. In HPLC the chromatographic separation is carried using C18 column as stationary phase and various mobile phase in different proportion at different concentration the flow rate monitored at specific time interval in specific amount.

## CONCLUSION

The above-mentioned data is concise collective information about the analysis of the Hydralazine and Isosorbide dinitrate in combination. All the methods mentioned are validated according to the ICH guidelines and are useful in the analysis of the mentioned drugs. The prominent method for the analysis of the above mentioned drugs is carried out by RP-HPLC with mobile phase phosphate buffer and acetonitrile at wavelength ranging from 270-280 nm. There is single eco-friendly method carried out by UPLC, degradation study also carried.

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