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Analytical Methodologies for the Determination of Hydralazine: A Review.

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Review Article

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ABSTRACT

Hydralazine belongs to the hydrazinophthalazine class of drugs and it is a potent vasodilator, which is used in combination with a suitable β-blocking drug to treat hypertension. Hydralazine is not only a peripheral vasodilator but also a potent and irreversible inhibitor of Semicarbazide sensitive amine oxidase (SSAO). It was sold under the brand name Apresoline® by Novartis and approved by the U.S. Food and Drug Administration (FDA) in 1953. A widespread review of the literature published in various pharmaceutical journals has been conducted and the instrumental analytical methods which are developed and used for determination of Hydralazine have been reviewed. This review includes Potentiometric titration, Colorimetric method, HPLC-UV, HPLC with Electrochemical detection, HPLC-MS/MS, GC-ECD, GC-FID and GC-NSD. The applications of these methods for the determination of Hydralazine in pharmaceutical formulations and biological sample have also been discussed in this article

INTRODUCTION

Hydralazine (1-hydrazinylphthalazine, HDZ) (Fig 1) is a direct acting smooth muscle relaxant used to treat hypertension by acting as a vasodilator primarily in arteries and arterioles. Vasodilators act to decrease peripheral resistance, thereby lowering blood pressure and decreasing afterload. It has also clinical application in after heart valve replacement and in the treatment of chronic – resistant heart failure [1, 2]. It is widely used in combination with β-blocking drug (to balance the reflex tachycardia) and a diuretic (to decrease sodium retention) for the treatment of essential hypertension. HDZ increases cyclic guanosine monophosphate (cGMP) levels, increasing the activity of protein kinase G (PKG). Active PKG adds an inhibitory phosphate to myosin light-chain kinase (MLCK) - a protein involved in the activation of cross-bridge cycling (i.e. contraction) in smooth muscle. This results in blood vessel relaxation [3, 4]. Excessive or habitual uptake of hydralazine can cause toxic symptoms, such as headache, joint or muscle pain, swollen ankles, nausea, sweating, tachycardia, arrhythmia and precipitation of angina [5]. The substance is first sold under the brand name of Apresoline® by Novartis and approved by the U.S. Food and Drug Administration (FDA) in 1953. The available marketed formulations of LEF are enlisted below in table no. 1 [6, 7].

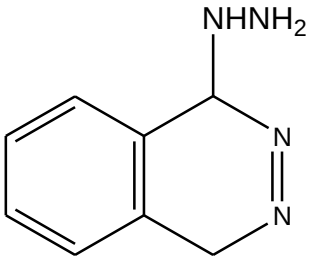

Figure 1: Chemical structures of Hydralazine

Development and validation of analytical methods are of basic importance to optimize the analysis of drugs in the pharmaceutical industry and to guarantee quality of the commercialized product. [8] Method development is required to develop quantitative methods to determine concentration of drug and if necessary metabolites in biological matrix. These methods are used to support several activities in drug development including formulation research, GLP, toxicology, clinical pharmacology and clinical research studies. [9] Method validation is performed to demonstrate that a particular method used for quantitative measurement of drug and/or

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metabolite is reliable and reproducible for intended use. The validated method is applied to the study samples with known samples with predefined acceptance criteria. The obtained values are used to calculate the pharmacokinetics parameters for the anticipated end results [10].

Table 1: Marketed formulations of LEF

Generic Name	Dosage Form	Strength	Company
Hydralazine Hydrochloride	Injection	20 mg/ml	AKORN, FRESENIUS KABI USA, LUITPOLD, NAVINTA LLC
Hydralazine Hydrochloride	Tablet	Multiple strengths	ACTAVIS GRP PTC, ALKEM LABS LTD, GLENMARK PHARMS LTD, MYLAN
Hydralazine Hydrochloride	Tablet	25 and 50 mg	WATSON LABS
Hydralazine Hydrochloride	Tablet	10, 25, 50 and 100 mg	PAR PHARM, PLIVA
Hydralazine Hydrochloride	Tablet	Multiple strengths	HETERO LABS LTD, HERITAGE PHARMS INC, INVAGEN PHARMS, STRIDES ACROLABLTD

A wide variety of analytical methods have been reported for the determination of HDZ in pharmaceutical preparations and in biological fluids. It includes Potentiometric Titration, Colorimetric Method, Gas Chromatography - Nitrogen Selective Detector (GC-NSD), Gas chromatography - Flame ionization detector (GC-FID), High Performance Liquid Chromatography - Ultraviolet Visible Spectroscopy (HPLC-UV), High Performance Liquid Chromatography - Electrochemical Detection and Liquid Chromatography - Electron Spray Ionization - Tandem Mass Spectrometry (LC-ESI-MS/MS). Among HPLC and GC methods different internal standards, reversed phase columns with different size and different mobile phase compositions have been used for the quantification purpose. The aim of the present review is to summarize these validated techniques for the determination of HDZ in pharmaceuticals and biological matrix.

Various Analytical Methods Developed for Hydralazine

HDZ is a highly reactive phthalazine that rapidly forms hydrazones with endogenous α-keto acids, such as pyruvic acid. To determine unchanged hydralazine the analyte must be derivatized to stable form immediately upon sample collection.

Official Methods

Indian Pharmacopoeia 2010 and British Pharmacopoeia 2009 include Potentiometric Titration for HDZ quantification. Here, 0.1 g drug was dissolve in a mixture of water and hydrochloric acid. After that titrate it with 0.05 *M* Potassium Iodate and determining the end point potentiometrically using a calomel reference electrode and a platinum indicator electrode [11, 12].

United State Pharmacopoeia 30 National Formulary 25 includes HPLC - UV method for quantification of HDZ. This separation was carried out on 4 mm × 25 cm column that contains 10 micron packing L10 by using 1.44 g of sodium dodecyl sulphate, 0.75 g of tetra butyl ammonium bromide in 770 ml water and 230 ml of acetonitrile (pH-3) at a flow rate of 1 ml/min and detected by UV detection at 230 nm [13].

Spectrophotometric Method

Stewart *et al.* 1983 reported a colorimetric assay for determination of HDZ in pharmaceutical dosage forms based on the interaction with 9-methoxyacridine and developed yellow color solution was measured by 455 nm. Here, color development was affected by the quantity of acridine reagent used and time of heating or standing at ambient temperature. For maximum absorbance, the solutions were heated at 50±1^{0}C for a minimum of 5 min or allowed to stand at ambient temperature for at least 15 min. In result, HDZ was determined in the 0.1-12 µg/ml range with correlation coefficient of 0.9993. Here also good precision and accuracy was observed, which 0.15 to 3.29 %RSD is and 98.8 % to 101.2 %RSD, respectively [14].

Miralles *et al.* 1993 described a colorimetric method for determination of dihydralazine by using interaction with ethanolic solution of 2-hydroxy-1-naphthaldehyde to developed a water-insoluble yellow product, 1,4-bis[(2-hydroxy-1-naphthyl)methylene hydrazinelphthazine was measured at 420 nm. The method was successfully applied for the determination of dihydralazine in mixtures containing other drugs (reserpine,

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hydrochlorothiazide, oxprenolol, xanthinol, rutoside, chlorthalidone and bietaserpine). The process is stable at 50^{0}C. The most precise results were obtained for heating at 25^{0}C for 1 h. The result parameters are tabulated in table no. 2 [15].

Table 2: Validation Parameters reported by Miralles*et al.* 2012

Parameters	Result
Linearity range	0.4 - 8 µg/ml
Correlation coefficient	0.998
Limit of Detection (LOD)	0.08 µg/ml
Precision (% RSD)	2.02%

Chromatographic Methods

Gas Chromatography (GC)

Jack *et al.* 1975 performed a gas chromatographic method for the determination of HDZ in plasma. For derivatization nitrous acid was used and a formed stable compound was extracted with organic solvent and determined quantitatively using 1-hydrazino-4-methylphthalazine as an internal standard (IS). The separation was achieved on Chromosorb W-HP (80-100 mesh) packed with 3% of OV-223 at 220^0 of column oven temperature with using nitrogen as a carrier gas at a flow rate of 30 ml/min and detected by electron capture detector. Here, the linear relationship was observed between the ranges of 10 - 200 ng/ml [16].

Smith *et al.* 1977 described a gas chromatographic method for the quantification of HDZ in tablet formulations based on the interaction with 2,4-pentanedione to yield 1-(3,5-dimethylpyrazole)phthalazine and determined by flame ionization detector using 4-methyl hydralazine as an IS. The separation was carried out on U-shaped glass tube packed with 10 % SE-30 (6ft × 4mm I.D.) at 200^{0}C of column temperature by using oxygen free nitrogen as a carrier gas at a flow rate of 55 ml/min. Here, recovery of spiked sample was observed 98.1 and 98.5% for HDZ and IS, respectively [17].

Degen *et al.* 1979 established a gas chromatographic method for the specific determination of unchanged HDZ in plasma. Derivatization was done with 2,4-pentadione with 4-methyl HDZ as an IS and determined by nitrogen selective detector. The chromatographic conditions include Chromosorb W-HP glass column (2 m x 2 mm I.D.) packed with 3 % OV-17 as a stationary phase at a column temperature of 230^{0}C with helium as a carrier gas and at a flow rate of 35ml/min. Here, the linear relationship was obtained between the range of 9.5 - 240 ng/ml and the retention time were observed at 3.4 and 4.6 for HDZ derivative and IS, respectively [18].

Angelo et al. 1980 proposed gas chromatographic method for simultaneous quantification of HDZ and its acetylated metabolite 3-methyl-s-triazolo[3,4-α]phthalazine (MTP) with 4-methyl HDZ as an IS. HDZ and IS converted into their formylated derivatives by derivatized with formic acid and extracted with toluene and detected with nitrogen selective detector. Here chromatography was done on Chromosorb W glass column packed with 1% SP 1000 with helium as a carrier gas at a flow rate of 30 ml/min. The lower limits of detection for HDZ and MTP were 0.13 and 0.27 µmol/l, respectively with the linear range of 1-15µmol/l. Here, also found that, after the addition of ascorbic acid, serum samples were stable at -20°C for at least 7 months [19].

High Performance Liquid Chromatography (HPLC)

Molles et al. 1985 reported HPLC method which utilizes the derivatization product of HDZ with p-hydroxybenzaldehyde or p-anisaldehyde as an IS. The chromatographic separation was performed on an µBondapak Phenyl column (30 cm x 3.9 mm I.D., 10 µm) at a column temperature of 35^{0}C, in isocratic mode using methanol: 2% acetic acid solution (60:40, v/v), at a flow rate of 1ml/min and detected by UV detector at 295 nm. The method validation parameters are tabulated in table no. 3. Here also identified that if HDZ was extracted from the dosage form in methanol and diluted with water within 2 h, no significant degradation occurred. This method is applicable where multiple sample assays were needed [20].

Table 3: Validation Parameters reported by Molles *et al.* 1985

Parameters	Result
Retention Time	7.4 min
Linearity Range	9.7 – 29.1 µg/ml
Correlation coefficient	0.999
Recovery	100.9%
Precision (%RSD)	0.8%

Wong et al. 1987 established reversed phase HPLC method with electrochemical detection for extraction and analysis of HDZ in plasma. HDZ and 4-methyl HDZ (IS) were derivatized at room temperature with salicylaldehyde and extracted with a mixture of heptane, methylene chloride and isopentyl alcohol. The separation was achieved on a Supercoil LC-18-DB (5 um) column kept at 28°C with using 66% methanol in 0.055 A4 citric acid/0.02 M dibasic sodium phosphate (pH 2.5) as mobile phase at a flow rate of 1.5 ml/min. both derivatives were detected by the electrochemical detector with the following screen oxidation mode: conditioning cell potential at +0.20 V, detector 1 (coulometric electrode) at +0.25 V and detector 2 (amperometric electrode) at +0.60 V. The method validation parameters are tabulated in table no. 4. This method was used for routine patient monitoring or pharmacokinetic studies of free (unmetabolized) HDZ [21].

Table 4: Validation Parameters reported by Wong *et al.* 1987

Parameters	Result
Concentration range	25 - 500 ng/ml
Correlation coefficient	0.994
Limit of detection (LOD)	200pg/ml
Recovery (%CV)	94%

Manes *et al.* 1990 described HPLC method for determination of HDZ and its metabolite in human plasma using Methyl Red as IS. This method involves pre-column derivatization with 2-hydroxy-1-naphthaldehyde at pH 1.2 and both were extracted into dichloromethane and detected by UV detector at 406 nm. The chromatography was done on an ODS-2 column packed with spherisorb (250 × 4, 3 µm) by using acetonitrile: aqueous triethylamine phosphate buffer (80:20, v/v - pH 3) as eluent at a flow rate of 0.7 ml/min. The method result parameters are tabulated in table no. 5 [22].

Table 5: Validation Parameters reported by Manes *et al.* 1987

Parameters	Result
Concentration range	10 - 500 ng/ml
Correlation coefficient	0.997
Limit of detection (LOD)	1 ng/ml
Recovery (%CV)	50 - 55%

Liu *et al.* 2011 introduced a HPLC method using MS/MS detection for HDZ quantification in BALB/C mouse plasma and brain. Derivatization was done with 2,4-pentadione at 50^{0}C for 1 h, and a step of solid phase extraction to purify and concentrate HDZ derivative. The chromatographic separation was performed on an Agilent ZORBAX SB-C18 column with column chamber temperature at 30^{0}C, in isocratic mode using 0.01 mol/l methanol: ammonium acetate (60:40, v/v) at a flow rate of 0.2 ml/min and for detection multiple reaction monitoring transition used at m/z 225.2→129.5 in the electrospray positive ionization mode. The method result parameters are tabulated in table no. 6. [23]

Table 6: Validation Parameters reported by Liu *et al.* 1987

Parameters	Result
Concentration range	10 - 200 ng/ml
Correlation coefficient	Plasma:0.999 Brain: 0.998
Limit of detection (LOD)	Plasma: 0.49 ng/ml Brain: 1.05 ng/ml
Limit of Quantification (LOQ)	Plasma: 1.5 ng/ml Brain: 3.18 ng/ml
Recovery (%CV)	96.2 - 113%
Intra-day precision	10.9%
Inter-day precision	18.9%

CONCLUSION

The review presents specific, sensitive and accurate spectrophotometric and chromatographic analytical methods applied for determination of Hydralazine in pharmaceutical preparations and biological fluid. However, still there is more focus require to develop other methods using spectrofluorimeter and HPTLC as well as degradation kinetic study can also be develop by suitable stability indicating method.

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