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Development and characterisation of process related impurity in Hydralazine Hydrochloride by some analytical technique

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ABSTRACT

The unknown impurity associated with the synthesis of Hydralazine hydrochloride bulk drug were detected by high performance liquid chromatography and were subjected to high resolution accurate liquid chromatography mass spectroscopy for identification. The proposed impurities were isolated from Hydralazine hydrochloride active pharmaceutical ingredient by preparative chromatographic method and were injected on HPLC for comparison of retention time with that of the unknown process related impurity in Hydralazine hydrochloride. The molecular ion peak of preparatively isolated impurity and that of unknown process related impurity in Hydralazine hydrochloride were compared for confirmation. The structure were determined and confirmed with the help of some analytical techniques. This impurity of Hydralazine hydrochloride is not been previously reported. A rapid Acquity H-class gradient method with runtime of 12.0 min was developed for Quantitation on Unisphere Cyno column and validated for parameters such as accuracy, precision, linearity and range, robustness. The LOD and LOQ of method were 0.081% and 0.0246% respectively.

Keywords: Acquity UPLC H-class, Hydralazine hydrochloride, HR/AM-LCMS, NMR.

INTRODUCTION

Hydralazine hydrochloride drug is used for the treatment of vasodilator which is used for to reduces blood pressure and peripheral resistance[1]. Mostly it is used in the cure hypertension, usually the once times dose should be not more than below 100 mg daily through oral, hence higher doses causes with an increased incidence of lupus erythematosus. It has also been given intravenously in the treatment of hypersensitive crises [2]. Hydralazine is usually used for above treatment with another drug in combination. In last five years, it has been found that it should be especially useful when used with beta-adrenergic blocking agents and diuretics [3].

The analytical methods on impurity detection and identification are reported in some literatures and these literatures were screened for the presence of impurities in Hydralazine hydrochloride drug. Hydralazine hydrochloride is a Pharmacopoeia product [4-7], the synthetically prepared drug and was injected on

HPLC method from pharmacopeia, a late eluting impurity peak at retention time 39 min and 72 min in chromatogram were observed during analysis respectively [4-5]. We have screened the literatures for impurities, which were produced form different synthetic process and found that this unknown impurity was not reported in any of the synthetic process related to Hydralazine hydrochloride active pharmaceutical ingredient[8-10]

To identify the impurities were very critical for its safety assessment and its isolation process. It is mandatory to identify and characterize the impurities in pharmaceutical product, if present above the accepted limit of 0.10% [11]. In this present article complete characterization of this unknown impurity were done using HR/AM-LCMS/MS, NMR, IR and rapid Acquity UPLC H-class instrument method is developed for Quantitation of this unknown impurity. However, so far there is no published report, describing the complete characterization and Quantitation of this unknown process related impurity in Hydralazine hydrochloride API.

During process development studies, impurities were detected in both crude and pure samples of Hydralazine hydrochloride using a newly developed gradient reversed phase Acquity UPLC H-class method developed for rapid analysis for Quantitation of process related unknown impurity. A comprehensive study was undertaken for the identification of this impurity using HR/AM-LCMS/MS followed by isolation and further characterization by NMR and FTIR technique. This article also describes the analytical method validation by using Acquity H-class for quantitative determination of this unknown impurity.

MATERIALS AND METHODS

Materials and reagents: Samples of Hydralazine hydrochloride API were obtained from Ipca Laboratories Ltd., HPLC grade acetonitrile, Sodium lauryl Sulphate from Sigma Aldrich life science and tetra butyl ammonium bromide from Loba Chemie. HPLC grade taken from Millipore. Dimethyl sulphoxide—*d*6 (for NMR) from Aldrich.

Liquid chromatography: Samples were analyzed on Acquity H-class (Waters, Milford, MA, USA) LC system equipped with Photo diode array (PDA) detector.

Liquid chromatography-high resolution accurate mass spectroscopy (LC-HR/AM-MS): The HR/AM-LCMS/MS and MS/MS studies were performed on Q-Exactive Orbit trap mass spectrometer (Thermo Fisher Scientific Inc. Waltham, Massachusetts United States). HESI (Heated electron spray ionization) source was used for ionization. The spray voltage was maintained at 4.0 kV, Auxiliary gas flow rate was kept at 10 and capillary temperature at 320°C.

NMR spectroscopy: ^{1}H, ^{13}C NMR and DEPT measurement of the isolated impurities were performed on AVANCE 400 (Bruker, Fallanden, Switzerland) instrument. The ^{1}H and ^{13}C chemical shift values were reported on the δ scale (ppm) relative to DMSO.

FTIR spectroscopy: The FTIR spectrum of isolated impurity was recorded in the solid state as KBr powder dispersion using (Perkin-Elmer, Beaconsfield, UK) spectrum one FT-IR spectrometer.

Preparative liquid chromatography: Impurity was isolated from the crude sample using Waters Auto purification system consisting of 2525 binary gradient pump, a 2487UV detector and 2767sample manager (Waters, Milford MA, USA). A Kromasil C18 column (150mm × 21mm i.d., particle size 5µm) was used for the separation.

Preparation of solutions for validation of Acquity UPLC H-class method: A test preparation of 1000ppm of Hydralazine hydrochloride bulk drug sample was prepared using diluents (mobile phase A 80: mobile phase B 20).

Brief synthetic preparation of Hydralazine Hydrochloride*:* Phthalazin-1(2H)-one when treated with $POCl_3$ at 75°C-85°C in the presence of 2-ethoxy ethanol and toluene gives rise to 1-chlorophtalazine, this reaction takes place by intramolecular chlorination by Vilsmeir Hack reaction [13]. The solvent is distilled under vacuum and the product containing 1-chlorophtalazine is reacted with hydrazine hydrate and 2-ethoxy ethanol at 65°C to 70°C for 2hrs, 15% HCl is added to give wet crude Hydralazine hydrochloride. This crude Hydralazine hydrochloride is purified with charcoal, EDTA and methanol to give Hydralazine hydrochloride pure.

RESULTS AND DISCUSSION

Detection of impurity by HPLC: During the analysis of Hydralazine hydrochloride API using Pharmacopeia chromatographic purity method / related substances method a late eluting impurity was found, when chromatographic run time was increased to, approximately five fold to principal peak retention time. This method was not suitable to quantify this late eluting impurity since the peak area, sensitivity of 1000ppm solution of Hydralazine hydrochloride containing unknown impurity was too low to quantitative and also the runtime was high.

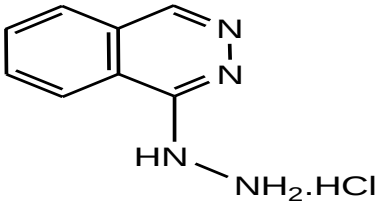

Fig.1. Structure of Hydralazine Hydrochloride

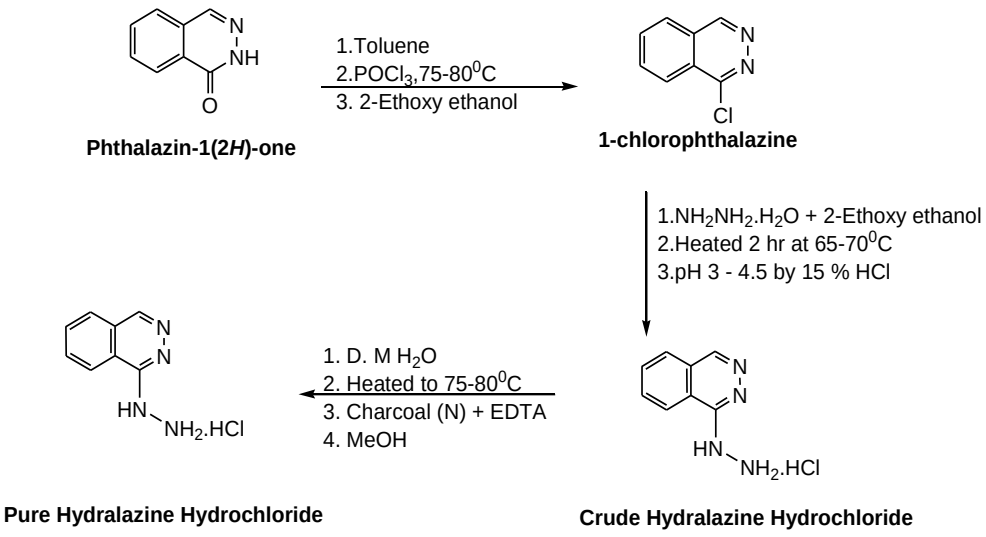

Fig.2 Synthetic Scheme for Hydralazine Hydrochloride

Further detection and confirmation was done to trace the impurity by HPLC, by injecting the crude Hydralazine Hydrochloride to check if this impurity was coming from crude Hydralazine hydrochloride. It

was observed that this unknown impurity is process related impurity and was present in crude sample and even after purification it remains in pure Hydralazine Hydrochloride.

Identification of impurities by HR/AM-LCMS/MS: HR/AM-LCMS and MS/MS were performed as per the method described to generate the mass data for the impurity. The impurity of interest was eluted at retention time 6.0min, which exhibits a protonated molecular ion peak $[M+H]^+$ 289.

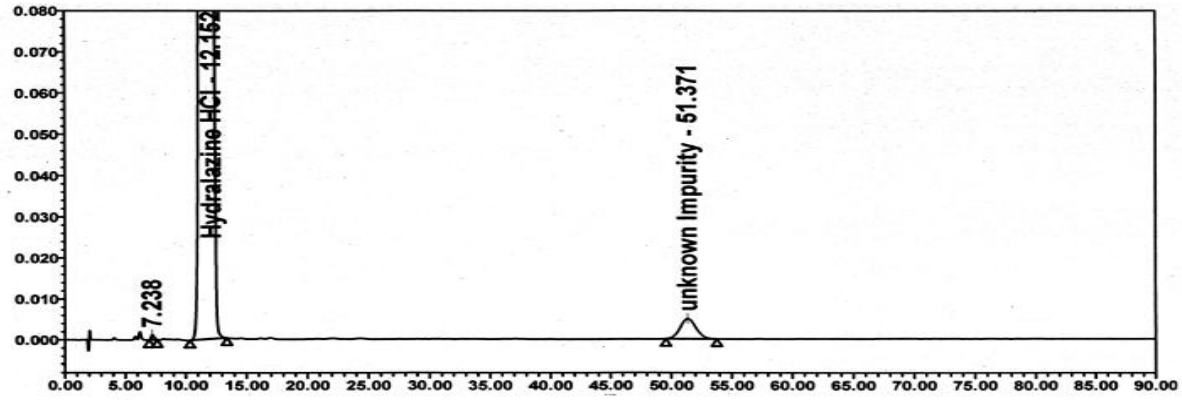

Fig. 3 Typical chromatogram of Hydralazine Hydrochloride EP Method showing unknown at 51.37min

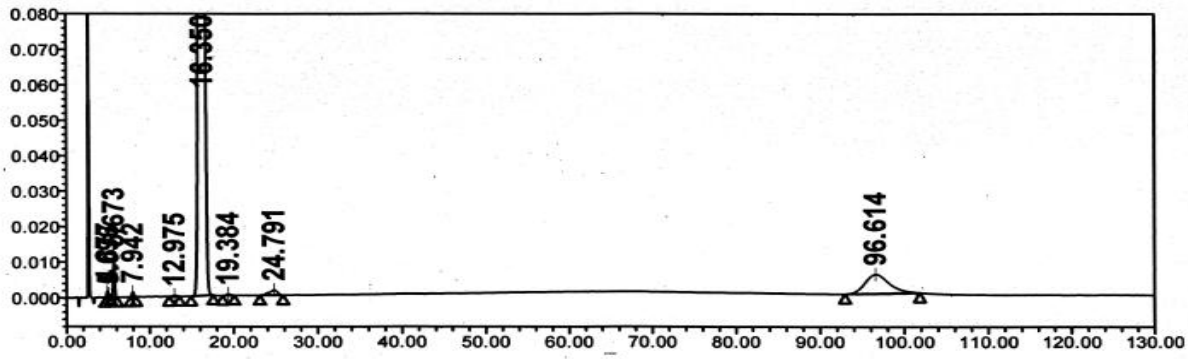

Fig.4 A typical chromatogram of Hydralazine Hydrochloride USP Method showing unknown at 96.61min

The impurity was isolated as described and this impurity was analyzed by High resolution accurate mass spectrometry giving $[M+H]^+$ 289.11963 and MS/MS of 272.09298 . The theoretical atomic formula probability was shown by Xcalibur software for 289.11963 were $C_{16}H_{13}N_6$. The error was calculated using formula [12]:

$$\text{Error in ppm for Molecular ion} = \frac{\text{Theoretical value – Actual Value}}{\text{Theoretical Value}} \times 10^6$$

$$= \frac{289.12017-289.11963}{289.12017} \times 10^6$$

$$= 1.87\text{ppm}$$

1.87ppm error is highly acceptable for characterization of $[M+H]^+$ of unknown structure using HR/AM-LCMS.

$$\text{Error in ppm for MS/MS} = \frac{\text{Theoretical value – Actual Value}}{\text{Theoretical Value}} \times 10^6$$

$$= \frac{272.09307-272.09298}{272.09307} \times 10^6$$

$$= 3.3\text{ppm}$$

3.3ppm error is highly acceptable for characterization of unknown structure using HR/AM- LCMS/MS.

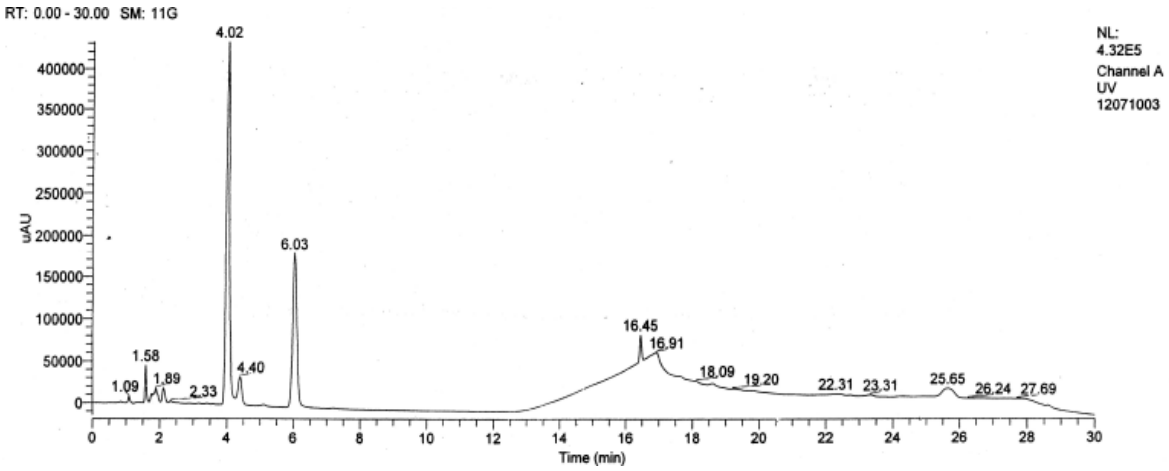

Fig. 5 A typical TIC chromatogram of unknown impurity at 6.03 minute

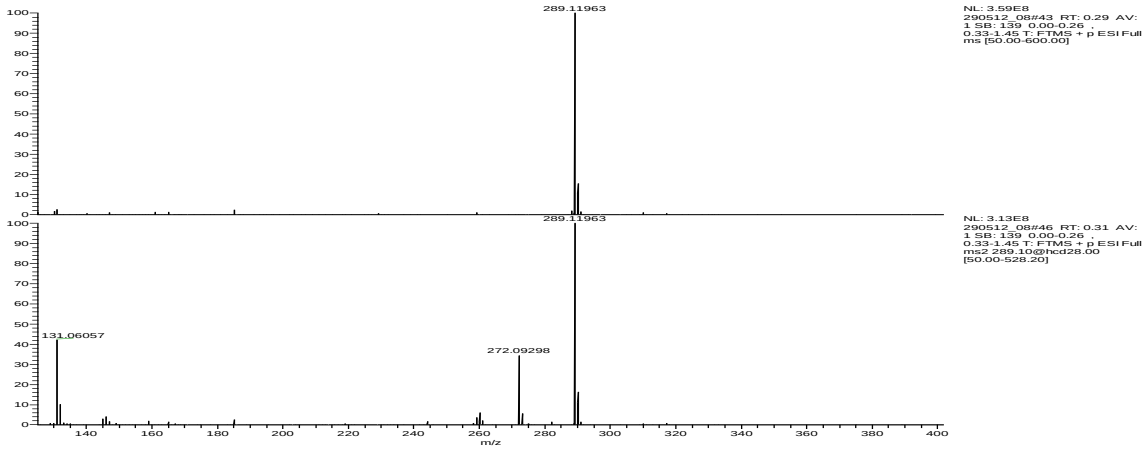

Fig.6 HR/AM- LC MS/ and MS/MS of Hazh Dimer impurity

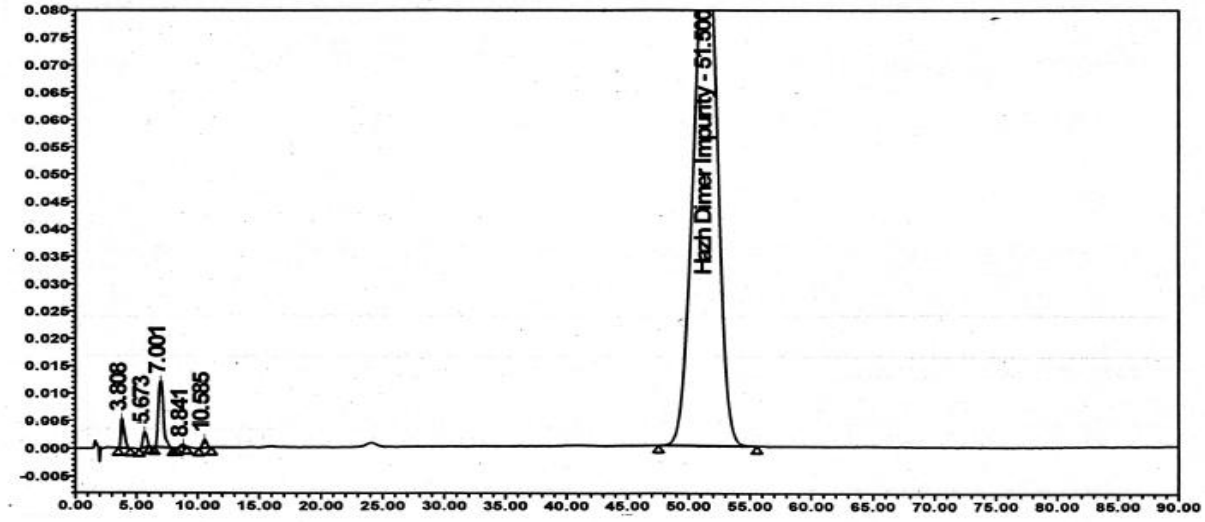

Fig.7 A typical chromatogram of preparative isolated Hazh Dimer impurity

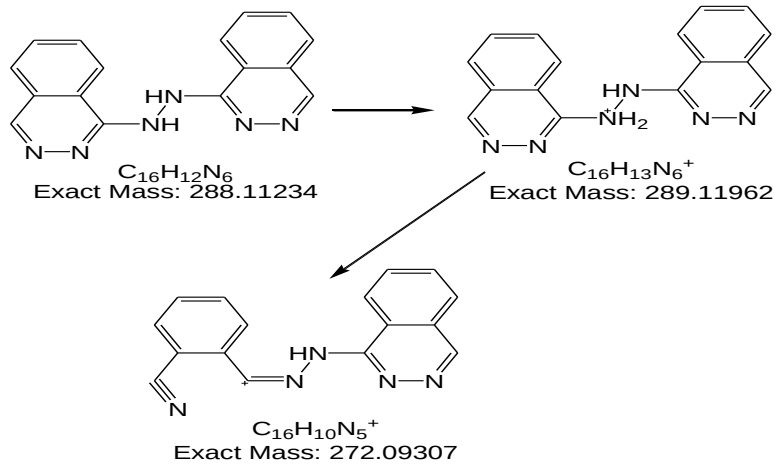

Fig.8 Plausible fragmentation for Hazh Dimer impurity Dimer

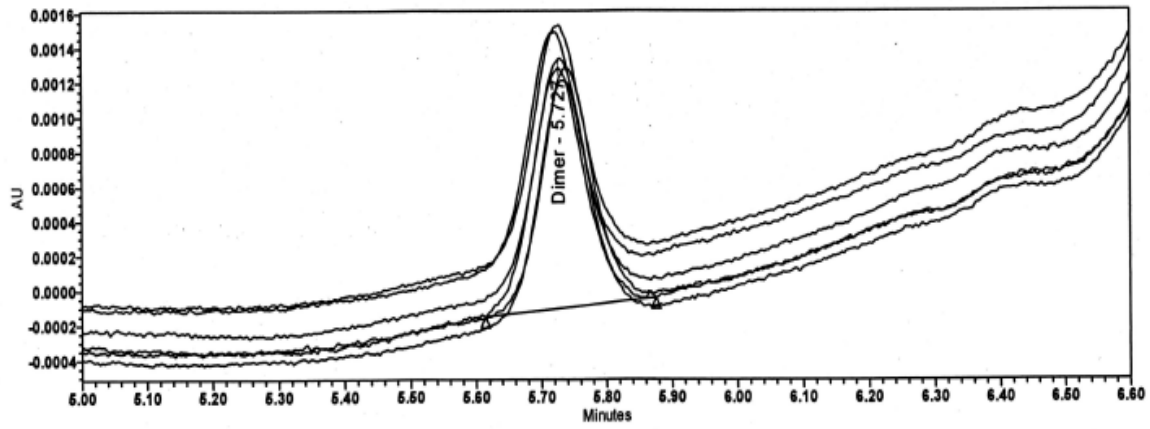

Fig.9 A typical chromatogram showing RSD for six replicate injections of Hazh Dimer

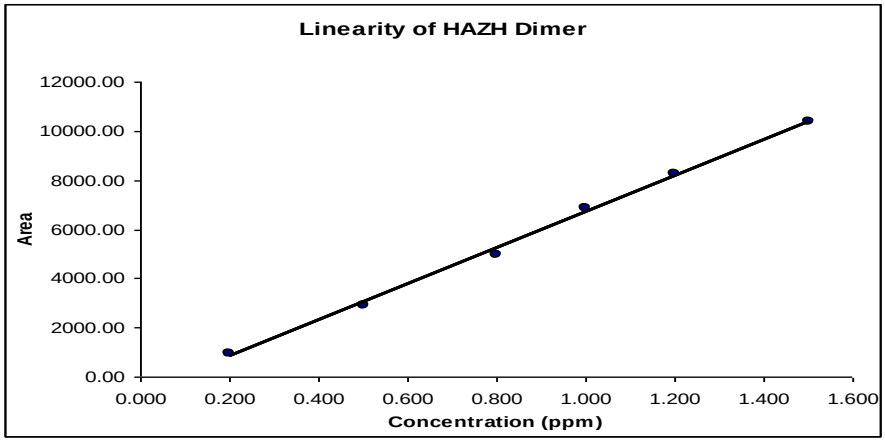

Fig.10 Linearity of Hazh Dimer

Isolation and structural confirmation of unknown impurity by NMR and FTIR: During the synthesis of Hydralazine hydrochloride the unknown impurity formed was isolated using preparative chromatography described. The chromatographic purity was checked and found to be 99%. ^{1}H and ^{13}C NMR spectral data confirmed for the determination of structure. The MS/MS spectrum obtained for

isolated compound of impurity using direct infusion mode was exactly same as MS/MS spectrum of 1-(2-phthalazin-1-ylhydrazino) phthalazine the plausible mass fragmentation is given in the infrared spectrum of Hazh Dimer was scanned from 400 to 4000cm^{-1} some of the absorption bands assigned are 3332 cm^{-} 1for N-H stretch secondary amine, 1590 for C=C stretch and -CH-N- for aromatic tertiary amine.

Analytical Method Validation by HPLC: The validation study allowed the evaluation of the method for its suitability for regular analysis. The newly developed method for Hydralazine hydrochloride and Hazh Dimer impurity was validated according to ICH guidelines [14]. A typical chromatogram showing Hydralazine Hydrochloride and Hazh Dimer impurity

Specificity: Specificity is the ability of analytical method to measure the analyte response in the presence of its potential impurities and degradants. The specificity of the Acquity UPLC H-class liquid method was determined by injecting individual impurity samples, wherein no interference was observed for any of the components. The chromatograms were checked for the appearance of any extra peak. Peak purity of these was verified using a PDA detector. The peak purity of the principle and other chromatographic peaks was found to be satisfactory.

Precision: The precision of the method was examined using six replicate injections of a standard solution. The relative standard deviation (RSD) was calculated for response (area) of Hazh Dimer impurity.

Accuracy: The accuracy of the method was determined for the related substances by spiking of known amounts of a Hazh Dimer impurity in Hydralazine hydrochloride at levels, LOQ, 80%, 100% and 120% of the specified limit. The recoveries of impurities were calculated.

Limit of detection and limit of quantification: Detection limit (DL) and quantization limit (QL) was estimated as per ICH Q2 (R1). The limit of detection established for Hazh Dimer impurity was found to be 0.0081% and limit of quantification was found to be 0.0246%.

Linearity*:* Linear calibration curve were obtained over the calibration range i.e. LOQ, 50%, 80%, 100%, 120% and 150% at six concentration levels in triplicate. The results showed excellent correlation between the peak area and concentration of Hazh Dimer impurity of about 0.9989.

Robustness: In all the deliberately varied chromatographic conditions (column temperature and flow rate), no significant changes in results were observed

Solution stability: The solution stability of Hydralazine sample and Hazh Dimer impurity was carried out by keeping both solutions in tightly capped HPLC vials at 25°C for 8 hrs in an Acquity UPLC H-class auto sampler no significant changes were observed in the peak area.

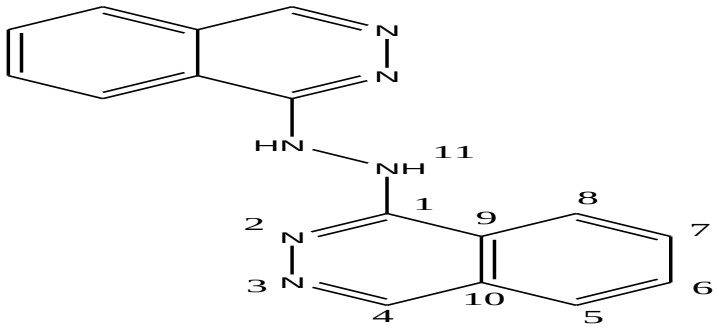

Table .1 [1]H NMR and C-13 assignment for Hazh Dimer

Position [a]	Integration	δ (ppm)	Multiplicity, J (Hz)[a]	13C δ (ppm)
1	**-**	**-**	**-**	**144.3**
2	**-**	**-**	**-**	**-**
3	**-**	**-**	**-**	**-**
4	**2H**	**7.86**	**s**	**136.9**
5	**2H**	**7.57-7.66**	**m**	**127.1**
6	**2H**	**7.57-7.66**	**m**	**131.7**
7	**2H**	**7.57-7.66**	**m**	**131.6**
8	**2H**	**8.64-8.66**	**m**	**126.3**
9	**-**	**-**	**-**	**124.8**
10	**-**	**-**	**-**	**128.1**
11	**2H**	**11.59**	**s**	**-**

[a] Refer the structural formula in Figure. [b] [1]H-[1]H coupling constants.

Table no. 2 Accuracy for Hazh Dimer

				% RSD		1.27			
				Concentration in ppm of HAZH Dimer (Cs)		1.0000			
				Area of HAZH Dimer in Parent test		0			
level	**Solution no.**	**Conc. in ppm(C)**	**Area counts in test added in ppm**	**Corrected area (A)**	**Recovery in ppm**	**% recovery**	**Mean % recovery**	**SD**	**%RSD**
LOQ	Test-1	0.250	1929	1929	0.2542	101.68	97.88	3.74	3.82
	Test-2		1787	1787	0.2355	94.19			
	Test-3		1855	1855	0.2444	97.78			
80%	Test-1	0.800	7179	7179	0.9460	118.25	117.0	1.49	1.28
	Test-2		7006	7006	0.9232	115.40			
	Test-3		7140	7140	0.9409	117.61			
100%	Test-1	1.000	8986	8986	1.1841	118.41	117.4	1.05	0.89
	Test-2		8827	8827	1.1632	116.32			
	Test-3		8916	8916	1.1749	117.49			
120%	Test-1	1.200	10631	10631	1.4009	116.74	116.6	1.10	0.95
	Test-2		10511	10511	1.3851	115.42			
	Test-3		10711	10711	1.4114	117.62			
Mean % Recovery (n=12)							**112.24**		
% RSD of % Recovery							**7.89**		
						Min	**97.88**		
						Max	**117.41**		

Table no. 3 Linearity of Hazh Dimer Linearity of HAZH Dimer

Level	**Conc.(ppm)**	**Area**	**Regression area**
Near LOQ Level	0.200	975.33	820
50%	0.500	2909.33	3032
80%	0.800	4994.00	5244
100%	1.000	6864.67	6718
120%	1.200	8266.33	8193
150%	1.500	10402.00	10405

Slope	7373.14
Intercept	-654.77

APPLICATIONS

This work will help bulk drug manufacturer globally to identify and quantify HAZh dimer impurity in there bulk drug. As a result of which they will be able to manufacture more pure Hydralazine hydrochloride drug, by controlling this impurity by the method prescribed in this article. Hence it would be helpful to the mankind when consumed pure drug which in turn will decrease the side effects on human.

CONCLUSIONS

A major process related unknown impurity of Hydralazine hydrochloride was Isolation by using semi-preparative method. The structural characterization of the isolated impurity was carried out by using HR/AM- LC MS/MS and other modern spectroscopic (NMR and FTIR) techniques. The combined result of HR/AM- LC MS/MS, NMR and FTIR confirmed the structure of unknown impurity as1-(2-phthalazin-1-ylhydrazino) phthalazine. A rapid Acquity UPLC H-class liquid chromatographic method developed was successfully validated for control of 1-(2-phthalazin-1-ylhydrazino) phthalazine in Hydralazine Hydrochloride API.

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