Research Article

Cost Effective Analytical Method Validation For Hydralazine Hydrochloride Related Substances Method-II By Reverse Phase High Performance Liquid Chromatography In Drug Substances

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ABSTRACT

A simple, rapid, precise, accurate and cost effective stability-indicating reversed phase (RP) HPLC related substance method-II was validated for Hydralazine Hydrochloride (HYD HCl) in Active pharmaceutical ingredient. All the analytical parameters were determined as per ICH Q2B guidelines. Good chromatographic separation was achieved with Cosmosil MS-II, C18 column (4.6 mm x 250 mm, 5 µm particle size) at a wavelength of 230 nm using phosphate buffer pH 3.0 as mobile phase A and Methanol as mobile phase B with gradient programming with a flow rate of 1.0 ml/ min. From the statistical treatment of the linearity data of Hydralazine HCl, it is clear that the response of Hydralazine HCl is linear between 50 % to 150 % level. The correlation coefficient is greater than 0.998. The developed method showed good linearity, Accuracy, reproducibility, precision and robustness and can be suitably applied for the routine quality control analysis in the estimation of commercial formulations.

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INTRODUCTION:

Hydralazine HCl is chemically 1-hydrazinylphthalazine. With molecular formula- $C_8H_8N_4$ and 160.17 mg molecular weight. It is freely soluble in water and sparingly soluble in methaline chloride. Hydralazine is a direct-acting smooth muscle relaxant. It is used as an antihypertensive agent in cases like preeclampsia (a condition in pregnancy characterized by high blood pressure). Hydralazine HCl acts by increasing cyclic guanosine mono-phosphate (cGMP) levels which causes an increase in the activity of protein kinase G (PKG). This results in blood vessel relaxation and causes dilation of arteries and arterioles[1-3].
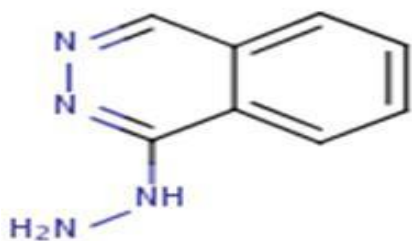
Figure 1: Chemical structure of Hydralazine HCl

Objective of Study

Literature survey revealed that Methods for the determinations of Hydralazine HCl include HPLC, Gas chromatography, simultaneous spectrophotometric determination and other methods. Literature survey reveals that different assay methods like spectrophotometry, spectrofluorometry, oxidimetry, and HPLC are available for the validation of Hydralazine hydrochloride in drug substances, but none of these methods are found suitable for routine quality control studies due to the following reasons like poor sensitivity, longer run time, using costly solvent, suitable at higher concentration only, extraction procedure involved in sample preparation [4-6]. Based on this, it was felt necessary to develop a validated simple, selective and sensitive HPLC method for the determination of Hydralazine hydrochloride in drug substances. The proposed method has been demonstrated superior to the existing procedures due to its sensitivity, speed, accuracy and it is suitable for routine quality control analysis. This proposed method can be successfully employed for quality control during manufacture and for assessment of the stability of drugs in drug substances [6-10].

EXPERIMENTAL WORK:

Chromatographic Conditions:

Column : Cosmosil MS-II C18, 250 x 4.6mm, 5.0µm

Detector wavelength : UV at 230 nm

Flow rate : 1.0mL / min.

Column Temperature : 30°C

Sample temperature : 10°C

Injection volume : 10µL

Run time : 35 minutes

Diluent : Acetonitrile: Methanol (1:1) v/v

Rinsing solution : Acetonitrile: Methanol (1:1) v/v

Buffer Preparation: Weigh and transfer about 1.36 g of potassium dihydrogen phosphate in 1000mL Water, sonicate to dissolve and adjust the pH to 3.0 with dilute Orthophosphoric acid, filter thorough 0.45µ.

Mobile phase A: Buffer

Mobile phase B: Methanol

Gradient Program:

Time (minutes)	Mobile phase-A (%)	Mobile phase-B (%)
0	60	40
10	60	40
15	40	60
25	40	60
27	60	40
35	60	40

Standard Stock solution-A: Weigh and transfer accurately 15.0 mg of Impurity-E reference standard into a 100mL volumetric flask, add 10 mL of diluent sonicate to dissolve and make up to volume with diluent and mix.

Standard Stock solution-B: Weigh and transfer accurately 10.0 mg of Impurity-F reference standard into a 100mL volumetric flask, add 10 mL of diluent sonicate to dissolve and make up to volume with diluent and mix.

Standard Stock solution-C: Pipette out 5.0 mL of each standard stock solution-A and standard stock solution-B into a 50mL volumetric flask and dilute to mark with diluent.

Standard solution: Transfer 5.0 mL of standard stock solution-C in to a 50mL volumetric flask and dilute up to mark with diluent.

Test Sample solution: Weigh and transfer accurately 50.0 mg of sample in to 50 mL volumetric flask add diluent, sonicate to dissolve and dilute up to the mark with diluent.

Procedure:

Equilibrate the column for 1hr minimum with mobile phase. Run the sequence as follows

Name of the Solution	No. of Injections
Blank	2
System suitability solution	1
Standard solution	6
Test solution Preparation	2

Retention table:

Name of the component	Retention time (RT)
Impurity-E	9.1
Impurity-F	22.1

Evaluation of System suitability: The system is suitable for analysis, if and only if,

%RSD for area of six replicate injections of standard solution for each component should be not more than 5.0

Calculation: Integrate the peaks due to Impurity E and Impurity F only in test solution and standard solution. Calculate %Impurity E and Impurity F by following formula,

$$\% \text{ Impurity E /Impurity F} = \frac{\text{Area of impurity in sample X Wt.of impurity.std (mg) X 5 X 5X 50 X P}}{\text{Avg.area of Impurity Std. X100 X 50 X 50 X Sample Weight (mg)}}$$

Where,

P=Potency of Impurity E /Impurity F Reference standard

Table 1: Specification limit of impurities

Specification Limit:		
Sr.No	Name of the Component	Specification
1	Impurity E	0.15%
2	Impurity F	0.10%

Table 2: Standard and Sample details

Standard and sample details:		
Name of the standard/sample	**Batch no/Lot No/reference No**	**Potency (%)**
Impurity-A(Phthalazine)	IRS/HLZ/IMP-A/001/17	98.8
Impurity-B	IRS/HLZ/IMP-B/002/17	96.7
Impurity-C	IRS/HLZ/IMP-C/003/17	99.6
Impurity-D	IRS/HLZ/IMP-D/004/17	96.6
Impurity-E	IRS/HLZ/IMP-E/005/17	94.3
Impurity-F	IRS/HLZ/IMP-F/006/17	94.2
EDTA Disodium salt	2489310118	98.2
Hydralazine Hydrochloride sample	PD/HLZ-III/Exp-274/17	NA
Hydrazine dihydrochloride	A0352647	100.0

VALIDATION PARAMETERS:

Specificity: Blank (diluent), system suitability solution, diluted standard solution, all known impurity solutions individually, sample solution and sample solution spiked with all known impurities at specification level were prepared and injected into the

.

HPLC equipped with a photodiode array detector and analysed. Peak purity passed for Hydralazine and its related impurities in control sample and spiked sample. Data is reported in Table 3 to table 5 and Figure 2 to figure 5
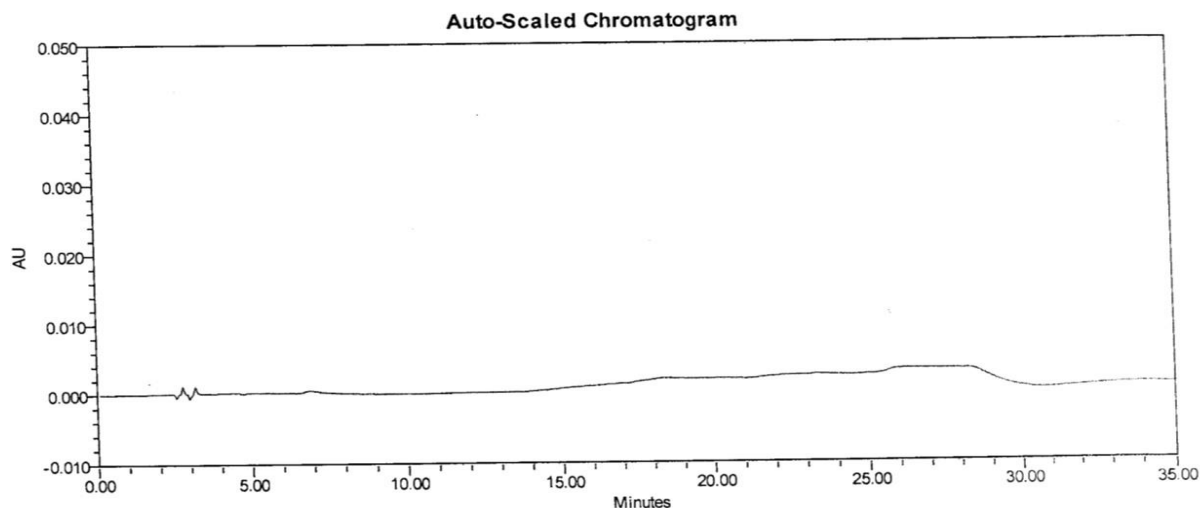

Figure 2: Blank chromatogram

Peak Results

	Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	Purity1 Flag	Int Type
1	Impurity E	9.14	62478	64.76	0.479	0.843	No	BB
2	Impurity F	22.25	33999	35.24	0.490	0.812	No	BB
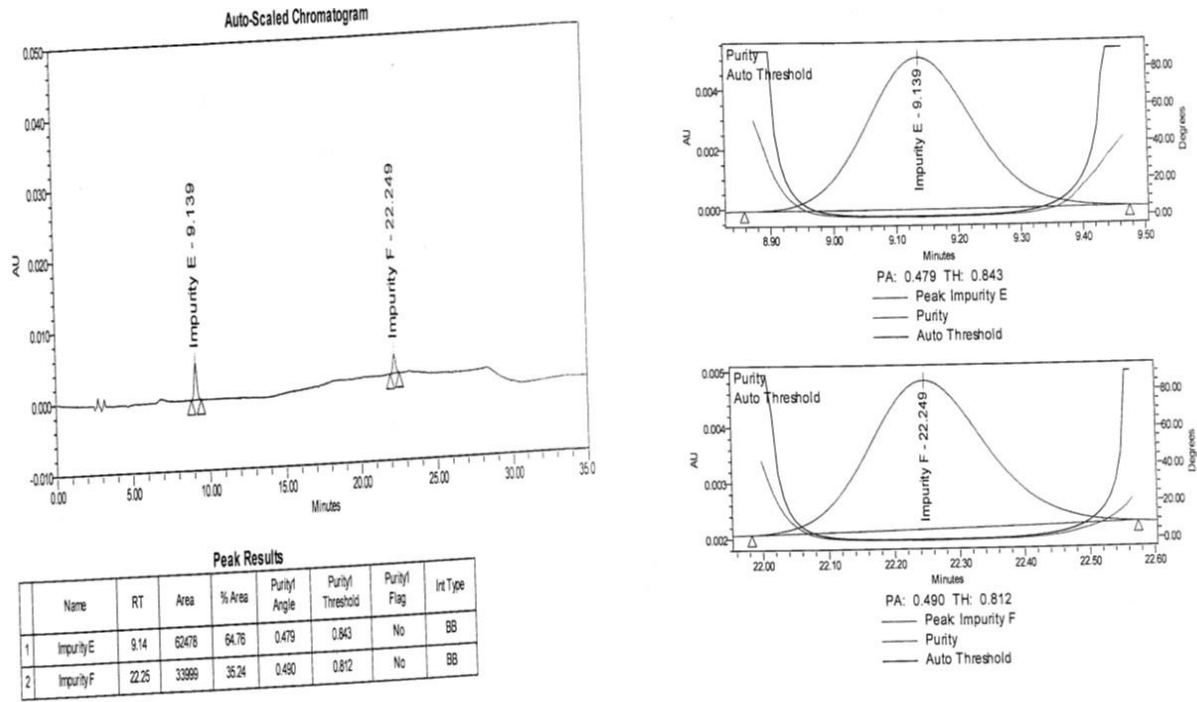
Figure 3: Standard Solution with peak purity chromatogram

Peak Results

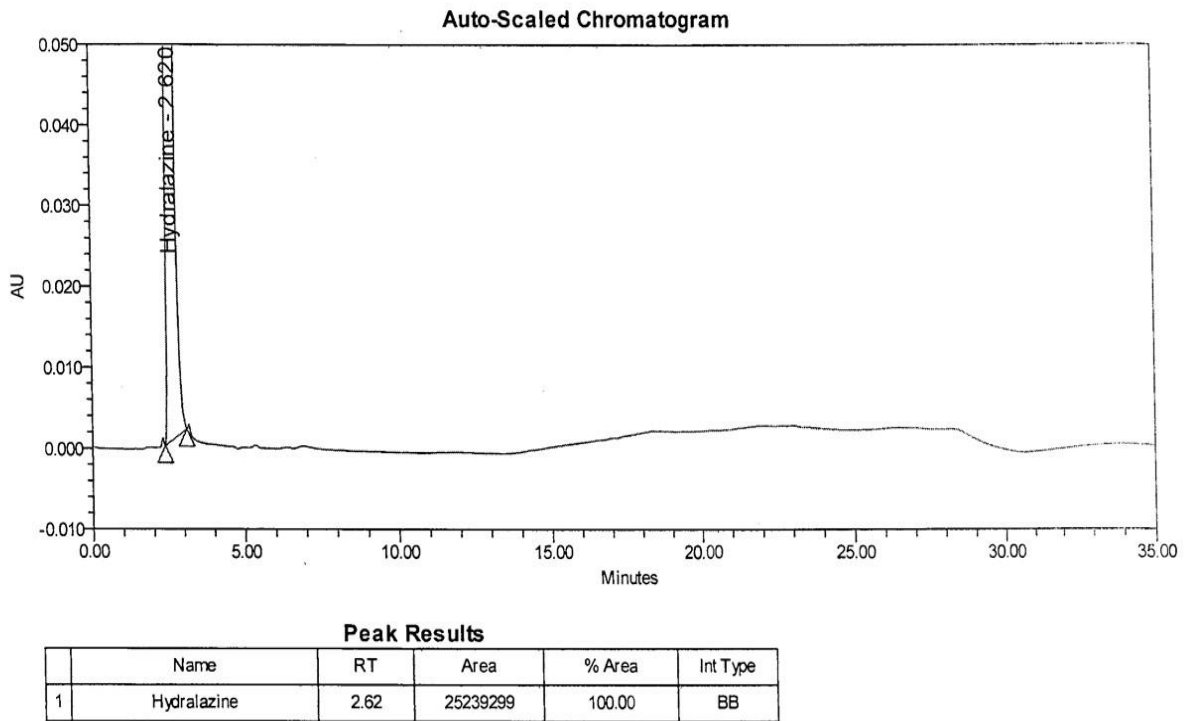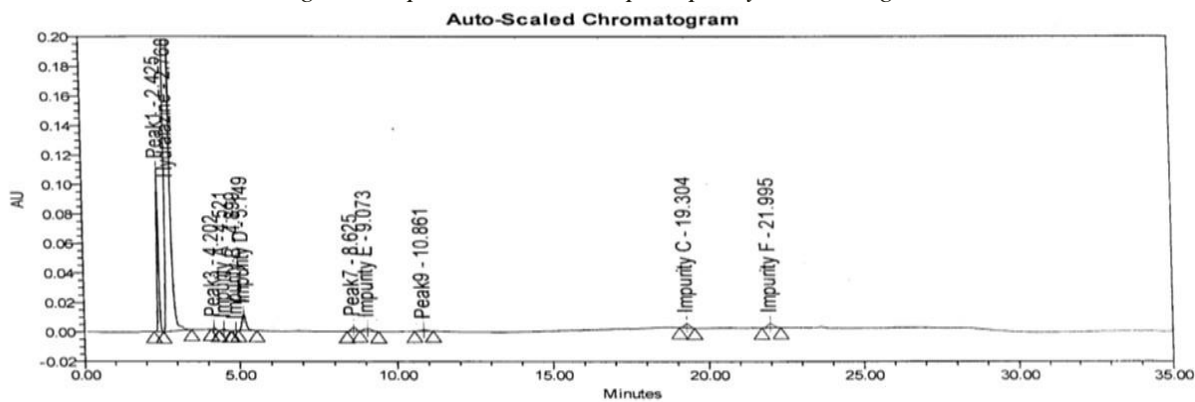|	Name	RT	Area	% Area	Int Type
1	Hydralazine	2.62	25239299	100.00	BB

Figure 4: Sample Solution chromatogram

Figure 5: Spike Solution with peak purity chromatogram

Peak Results

	Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	Purity1 Flag	Int Type
1	Peak1	2.42	385584	2.09	0.559	0.243	Yes	BB
2	Hydralazine	2.77	17857015	96.58	5.046	7.455	No	BB
3	Peak3	4.20	6857	0.04	15.017	6.276	Yes	BB
4	Impurity A	4.52	8109	0.04	3.678	2.834	Yes	BB
5	Impurity B	4.90	992	0.01	13.999	26.031	No	BB
6	Impurity D	5.15	98258	0.53	1.541	0.712	Yes	BB
7	Peak7	8.63	26133	0.14	4.588	3.856	Yes	BB
8	Impurity E	9.07	28547	0.15	3.267	3.877	No	BB

Peak Results

	Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	Purity1 Flag	Int Type
9	Peak9	10.86	11525	0.06	2.063	1.909	Yes	BB
10	Impurity C	19.30	30717	0.17	0.824	0.639	Yes	BB
11	Impurity F	22.00	36015	0.19	0.617	0.905	No	BB

Table 3: Specificity table for individual and spiked solution

Name of the compound	RT Obtained in individual solutions	RT Obtained in Spiked solution	
		Retention time	RRT
Hydralazine	2.62(Test solution)	2.78	1.00
Impurity E	9.13	9.09	3.27
Impurity F	22.10	22.03	7.95

Table 4: Peak purity information (For spiked solution)

Name of the compound	Purity angle	Purity Threshold	Peak purity
Impurity E	1.428	2.199	Pass
Impurity F	0.589	1.032	Pass

Table 5: Peak purity information (For Standard solution)

Name of the compound	Purity angle	Purity Threshold	Peak purity
Impurity E	0.479	0.843	Pass
Impurity F	0.490	0.812	Pass

From the above data, it is clear that, Impurity-E and Impurity-F are well separated from each other and Hydralazine peak. There is no interference of Blank at the retention time of all known impurities and unknown Impurities. Peak Purity is passes for Hydralazine peak and all known impurities. Based on the above data method is Specific.

Solution Stability: From the below given data it is clear that, test Solution and spiked test solution are stable upto 24hrs at room temperature. Acceptance criteria the % difference in response obtained from each individual component with respect to initial at each time interval should not be more than ±5.0%. Data reported in table no. 6.

Table 6: solution stability data for Spiked solution at 10°C Sample cooler temperature:

Sr. No	Sample ID	Impurity E area	% Diff with initial	Impurity F area	% Diff with initial
1	Initial	70278	---	39747	---
2	after 6hrs	68835	2.1	38342	3.5
3	after 12hrs	69924	0.5	39949	0.01
4	after 18hrs	70315	0.1	40992	3.1
5	after 24hrs	70465	0.3	40362	1.5

Limit of Detection and Limit of Quantification:
Slope of Impurity E the linearity calibration curve (S) is 456683.814 and Standard Deviation of response (σ) is 283.652. Hence Limit of detection of Impurity E is 0.002% with respect to Test concentration and Limit of Quantitation of Impurity E is 0.006% with respect to test concentration.
Slope of Impurity F the linearity calibration curve (S) is 364644.656 and Standard Deviation of response

(σ) is 382.764. Hence Limit of detection of Impurity F is 0.003% with respect to test concentration and Limit of Quantitation of Impurity F is 0.010% with respect to test concentration.
These values shall be further confirmed by precision and accuracy studies. Details summarized in the given Table 7.

Table 7: for LOD and LOQ Establishment

Solution name	Concentration (%)			
	Impurity E	Avg Area	Impurity F	Avg Area
Linearity at 5% solution	0.007	3185	0.005	2194
Linearity at 10% solution	0.015	5735	0.010	3184
Linearity at 15% solution	0.022	9431	0.015	5242
Linearity at 20% solution	0.029	12630	0.020	6777
Linearity at 25% solution	0.036	16036	0.025	8935
Linearity at 30% solution	0.044	19308	0.029	10329
Linearity at 35% solution	0.051	23004	0.034	12887
Slope of calibration curve(S)	283.652		382.764	
STEYX (σ)	456683.814		364644.656	
LOD (in %)	0.002		0.003	
LOQ (in %)	0.006		0.010	

LOD Confirmation and LOQ Precision: The %RSD for area of each component in standard solution is complies (NMT 5.0). System suitability parameter Complies. From the below given results, it is concluded that method is precise at LOQ Level. All individual known impurities were detectable at LOD level concentration. Data reported in table no 8 to table no 10.

Table 8: Standard solution area of individual known impurities:

Inj.No	Area of Impurity-E	Area of Impurity-F
1	72558	34444
2	72088	34090
3	73123	33448
4	73276	34559
5	72715	34212
6	72447	34259
Avg.	72701	34169
STDEV	440.372	390.834
%RSD	0.6	1.1

Table 9: LOQ Precision:

Inj.No	Impurity E Area	Impurity F Area
1	3531	2907
2	3485	3042
3	3498	2954
4	3462	3114
5	3650	2848
6	3551	3251
Avg.	3530	3019
STDEV	67.10	147.91
%RSD	1.9	4.9

Table 10: Area for LOD Level standard solution:

Sample ID	Area obtained with LOD Level standard solution	
	Impurity E	Impurity F
LOD Solution-1	1303	753
LOD Solution-2	1414	536
LOD Solution-3	1198	758
Average	1305	682

Linearity & Range:

A series of Standard preparations (minimum of five preparations) in triplicate of Impurity E and F working standards were prepared over a range of the LOQ to 150% of specification limits. The Correlation coefficient for Impurity E and F is more than 0.99.

Therefore, HPLC Method-II for the determination of related substances of hydralazine hydrochloride is

linear. Linearity reported in Table 11 and graph in figure no 6.

Table 11: Linearity of Impurity E and Impurity F

Linearity levels	Impurity-A		Impurity-B	
	Conc. in %	Avg. Area	Conc. in %	Avg. Area
Linearity at LOQ	0.007	3505	0.005	2968
Linearity at 50%	0.072	36607	0.048	16437
Linearity at 80%	0.114	59263	0.077	26973
Linearity at 100%	0.143	73248	0.096	33403
Linearity at 120%	0.172	88386	0.115	41090
Linearity at 150%	0.215	112560	0.144	52277
STEYX		797.548	957.477	
Slope		522779.090	354506.874	
Correlation coefficient		1.000	0.999	

Impurity E $y = 518462x$
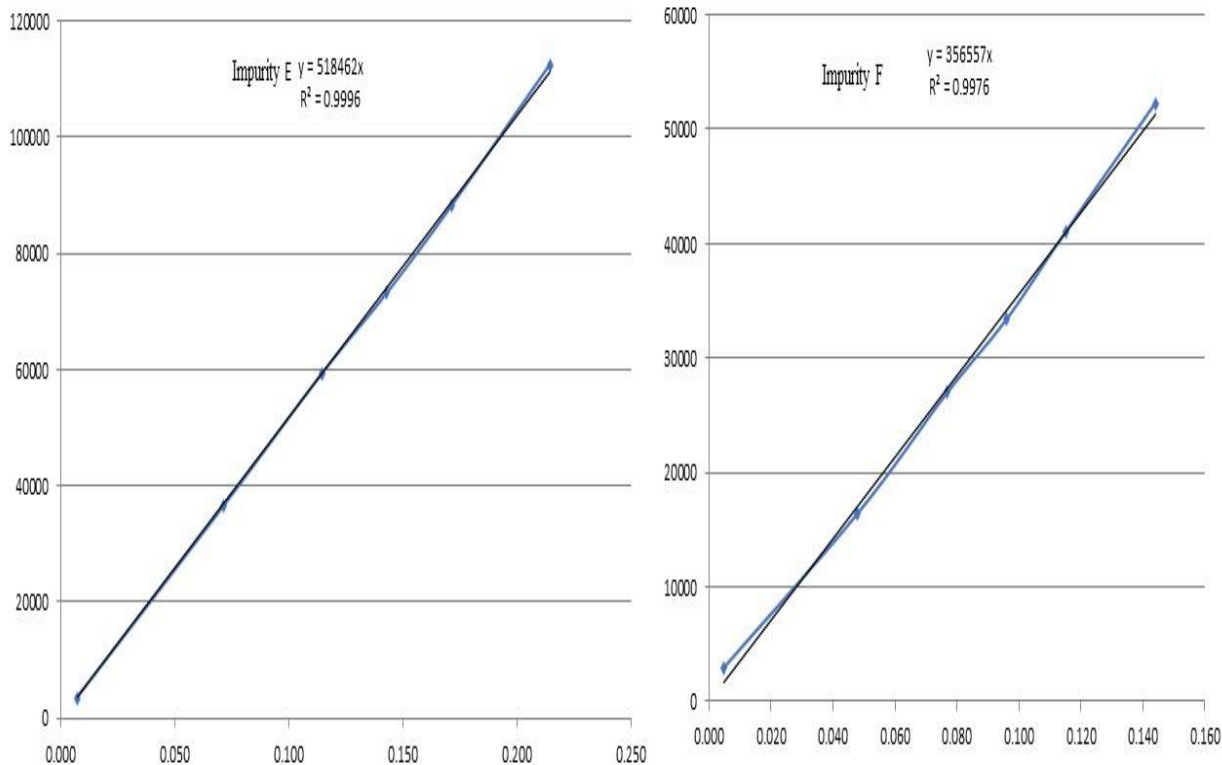$R^2 = 0.9996$

Impurity F $y = 356557x$
$R^2 = 0.9976$

Figure 6: Linearity Graph of Impurity E and Impurity F

Accuracy: Sample of Hydralazine hydrochloride drug substances, were spiked with Impurities E and F at four different levels: LOQ, 50%, 100%, and 150% of specification limits (in triplicate (in total twelve determinations) and analysed. The Mean Recovery for

known impurities is within limits. Therefore, the HPLC Method for the determination of related substances method-II of Hydralazine hydrochloride in Hydralazine hydrochloride drug substances is accurate. Accuracy reported in Table 12.

Table 12: Accuracy of Impurity E and Impurity F at LOQ to 150%

Name of the component	%Recovery			
	LOQ	50%	100%	150%
Impurity-E	91.3	96.0	97.9	99.0
Impurity-F	97.0	104.7	106.9	110.8

System precision Method precision and intermediate precision:

System Precision: Six replicate injections of the standard solution were made & injected. RSD should not be more than 5.0%.

Method Precision: Six Sample solutions of hydralazine hydrochloride spiked with Known impurities was prepared and injected into the HPLC, along with standard solution. RSD should not be more than 10.0%. RSD is less than 10.0%, therefore, the HPLC Method for the determination of related substances of hydralazine hydrochloride (Method-II) is precise.

Ruggedness (Intermediate Precision): Six Sample solutions of the same lot of hydralazine hydrochloride, spiked with known impurities was made by a different analyst and analysed using different column on a different day and injected into a different HPLC, along with Standard solution. Overall RSD is less than 10.0%. Therefore, the HPLC Method for the determination of related substances of hydralazine hydrochloride (Method-II) is rugged. Based on the above data it is clear the method is Precise &Rugged. Precision and ruggedness data summarized in Table 13.

Table 13: Overall RSD for method precision and intermediate precision:

Sample ID	Impurity-E (% w/w)	Impurity-F (% w/w)
Method precision-1	0.15	0.10
Method precision-2	0.15	0.10
Method precision-3	0.15	0.10
Method precision-4	0.15	0.10
Method precision-5	0.15	0.10
Method precision-6	0.15	0.10
Intermediate precision-1	0.15	0.12
Intermediate precision-2	0.15	0.12
Intermediate precision-3	0.15	0.12
Intermediate precision-4	0.15	0.12
Intermediate precision-5	0.15	0.12
Intermediate precision-6	0.15	0.12
Average	0.15	0.11
STDEV	0.0010	0.0076
% RSD	**0.7**	**6.9**

Robustness: System suitability results meet as per specification. The % RSD for content of each impurity in as such condition and changed condition should not be more than 10.0. The % RSD for

Contents of each impurity in spiked sample under test with each variable condition (mentioned in below table) along with as such condition is complies. Robustness data reported into table no14.

Table 14: Robustness of different variable conditions

Conditions	Impurity % w/w	
	Impurity E	**Impurity F**
Spiked solution (Low pH)	0.15	0.10
Spiked solution (Method precision)	0.14	0.10
Average	**0.15**	**0.10**
STDEV	**0.0036**	**0.0015**
% RSD	**2.5**	**1.5**
Spiked solution (High pH)	0.15	0.10
Spiked solution (Method precision)	0.14	0.10
Average	**0.15**	**0.10**
STDEV	**0.0030**	**0.0034**
% RSD	**2.0**	**3.3**
Spiked solution (Low column temp)	0.15	0.10
Spiked solution (Method precision)	0.14	0.12
Average	**0.14**	**0.11**
STDEV	**0.0050**	**0.0085**
% RSD	**3.5**	**7.8**
Spiked solution (High column temp)	0.15	0.10
Spiked solution (Method precision)	0.14	0.12
Average	**0.14**	**0.11**
STDEV	**0.0078**	**0.0109**
% RSD	**5.5**	**9.8**
Spiked solution (1.1 mL flow)	0.15	0.10
Spiked solution (Method precision)	0.14	0.11
Average	**0.15**	**0.11**
STDEV	**0.0030**	**0.0054**
% RSD	**2.0**	**5.0**
Spiked solution (Method precision)	0.14	0.11
Spiked solution (0.9 mL flow)	0.15	0.10
Average	**0.15**	**0.10**
STDEV	**0.0022**	**0.0024**
% RSD	**1.5**	**2.3**

CONCLUSION:

The Analytical Method for determination of Related substances (Method-II) by HPLC of Hydralazine Hydrochloride is validated as per method described in experimental section. The validated method is found Specific, Linear, Precise, Accurate, Robust and Rugged for determination of Related substances (Method-II) by HPLC. Hence it is concluded that determination of Related substances (Method-II) for Hydralazine Hydrochloride by HPLC can be used for Routine release analysis of API at Quality control department.

ACKNOWLEDGEMENTS:

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