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IDENTIFICATION, METHOD DEVELOPMENT AND METHOD VALIDATION FOR THE PROCESS AND DEGRADATION IMPURITIES OF VARDENAFIL HCl BY RP-UPLC AND UPLC-TOF

Kaviraj Yarbagi [*1, 2], Nagaraju Rajana [1], J. Moses Babu[1], B. Venkateswara Rao [2] and Paul Douglas [2]

Analytical Research [1], Custom Pharmaceutical Services, Dr. Reddy's Laboratories Ltd., Bollaram road, Miyapur, Hyderabad-500049, Andhra Pradesh, India.
Department of Engineering Chemistry [2], Andhra University, Visakhapatnam - 530003, Andhra Pradesh, India.

Keywords:

Vardenafil HCl; RP-UPLC, HR-MS; Validation; Identification

Correspondence to Author:
Kaviraj Yarbagi

Analytical Research
Custom Pharmaceutical Services,
Dr. Reddy's Laboratories Ltd.,
Bollaram road, Miyapur, Hyderabad-500049, Andhra Pradesh, India.

E-mail: kaviraj@drreddys.com

ABSTRACT: This present work is on method development of assay and related substances of Vardenafil HCl and degradation of drug substance under acid, base hydrolysis and oxidation conditions. Identification of the impurities were done by the UPLC-TOF. Waters LCT Premier time of flight HRMS instrument with 5 ppm accuracy employed for degradation impurity identification. The validation of the method for its impurities was done in RP-UPLC chromatography as per the ICH Q2(R1) guidelines and the validation parameters such as specificity with all the process and degradation impurities, linearity, precision, accuracy, solution stability, mobile phase stability, robustness and ruggedness parameters met acceptance criteria as per ICH guidelines.

INTRODUCTION: Vardenafil is PDE5 inhibitor which is used for treating erectile dysfunction, the PDE5 shows degradative action of cGMP-specific phosphodiesterase type 5 (PDE5) on cyclic GMP in the smooth muscle cells lining the blood vessels supplying the corpus cavernosum of the penis. Erectile dysfunction (ED) [15] is sexual dysfunction characterized by the inability to develop or maintain an erection of the penis during activity. The chemical name of vardenafil is 4-[2-Ethoxy-5-(4-ethylpiperazin-1-yl)sulfonyl-phenyl]-9-methyl-7-propyl-3,5,6,8-tetrazabicyclo[4.3.0]nona-3,7,9-trien-2-one.

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Many HPLC and UPLC methods for determination of its process impurities and degradation impurities can be found in literature [13-14], Gas chromatography mass technique was used for determination of vardenafil in blood and human urea [21-22], LC-MS/MS-ESI was used to determine vardenafil with combination of others such as sildenafil, tadlafil and udenafils [16-19]. But these many process impurities and degradation impurities were not done by UPLC-TOF, LC-MS/MS, RP-UPLC [4, 5, 6, 7, 20, 23]. This RP-UPLC method was developed and validated for the vardenafil and five process related impurities and identification of degradation impurities by UPLC-TOF and characterized by LC-MS/MS.

RP-UPLC method for impurities was linear from LOQ to 200% and precise, the % RSD was less than 2.5% at 0.15% (at specification) levels and less than 5.1% at limit of quantification level for all

impurities and accuracy was in between 80 to 120%, the LOQ for all impurities were three times of LOD and LOQ was 30% of 0.15%. This method was robust and rugged. The total validation was done as per ICH Q2(R1) guidelines and other useful guidelines [10,1, 2, 3, 8, 9, 11, 12] .

Experimental:

1.0 Chemicals: All reference standards of Vardenafil HCl salt and process impurities received from Dr. Reddy's hyderabad. HPLC grade acetonitrile was purchased from Rankem, Mumbai. Analytical reagent grade Ammonium acetate was also purchased from Rankem. High purity water was prepared by using a Millipore Milli-Q plus water purification system. Analytical reagent grade Sodium hydroxide pellets were purchased from Rankem, 35% of HCl solution were purchased from Rankem, Hydrogen peroxide were purchased from Merck Millipore.

2.0 Equipment:

RP-UPLC: Reverse phase Ultra performance Liquid chromatography and stability chambers. The Method development validation and identification were done on RP-UPLC Waters Acquity system with a diode array detector. The data were collected and processed using empower software. The photolytic degradation was done by using Mack Pharmatech's Photo stability Chambers.

Mass spectrometry: Formula confirmation of vardenafil HCl and process impurities and degradant impurities confirmed by High resolution mass spectrometry and spectra were recorded on a Waters acquity ultra performance liquid chromatography system coupled with a time of flight mass spectrometer with masslynx software. Detection of both positive mode and negative mode ions were collected by using Electron spray ionization technique.

Fragmentation of each impurity was done by LC-MS/MS Agilent make 6410 model with triple quadrupole with mass hunter software.

Chromatographic Conditions:

The chromatographic column used was a ACQUITY UPLC BEH130 C18 1.7µm x 2.1 x 100 mm. The buffer was having 10 mM Ammonium acetate adjusted pH at 7.5 with NH_4OH solution the mobile phase A was buffer and Acetonitrile with 70:30 % v/v ratio and mobile phase B was acetonitrile and water with 70:30 %v/v ratio, column temperature was maintained at 25°C throughout the analysis, the flow of RP-UPLC pump was maintained with 0.2 mL/min with gradient of 0.0/15, 2.0/15, 6/70, 10/15, 11/15 as Time versus percent of mobile phase B and load of injection volume was 1.0 µL and the detection was done at 240 nm. Diluent of this method was mobile phase B.

Mass spectrometry conditions:

LC-MS conditions: Mass conditions were optimized to get the molecular ion by positive mode ionization with single quadrupole of triple quadrupole with gas flow: 10 L min^{-1}, 4000 psi pressure, in the range of 50 m/z to 1600 m/z and the MS/MS fragmentation was done by keeping product ion information. Optimized of fragmentor as 135 and collision energy as 50 s.

HR-MS conditions: HR–MS conditions were optimized as capillary voltage 2500 volts, cone voltage 80 volts, desolvation gas flow:300 L/min, cone gas flow:50 L/min, data analysis was done by Mass lynx soft ware.

Method development and optimization for RP-UPLC: Initial method development trials tried with HPLC, for determination for all process impurities and degradation impurities with mobile phase potassium dihydrogen orthophosphate and acetonitrile as mobile phase and C18 column but the run time was 60 min and the time taken for analysis was more, Potassium di hydrogen phosphate was not LC-MS compatible.

Method developed with HPLC technique has drawbacks like solvent consumption is more, time taken for completion of analysis was more and non-compatibility of buffer with LC-MS/MS. To overcome from these problems,the method was developed by using advanced separation technique, RP-UPLC method was to determine process impurities and degradation impurities and assay of vardenafil HCl, the analysis time taken for the preparation of mobile phase and solution can be reduced. The initial study was done in ACQUITY UPLC BEH130 C18 1.7µm x 2.1 x 100 mm column because all impurities are having long carbon chain and they are neutral in nature hence

initially water and acetonitrile were taken for mobile phase A & B respectively. water, acetonitrile, aqueous TFA, phosphate buffer, ammonium acetate buffer were also attempted, Among all the mobile phases, peak shape and separation found good with combination of ammonium acetate and acetonitrile,

Specificity: Specificity is the ability to assess unequivocally the analyte in presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

[ICH Q2], The specificity of Vardenafil HCl and other impurities were examined by RP-UPLC and UPLC-TOF and this study was done for all degradation conditions such as acid, base hydrolysis, oxidation with hydrogen peroxide, thermal condition at 105°C and photolytic degradation with visible fluorescence light of 1.2 million lux hours, 200 watt hrs per square meter, the characterization of degradent impurities and process impurities, was done by UPLC TOF.

Method validation:

Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered in three categories repeatability, intermediate precision and reproducibility.

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements [ICH Q2 R1].

In this present work repeatability, intermediate precision and reproducibility were done at 0.15% level of all process impurities such as VAR4, VAR5, VAR7, VAR8 in presence of Vardenafil HCl with respect to 0.5 mg/mL concentration, all the method precision parameters % RSD of content met the pre defined criteria, the intermediate precision was done with different instrument, different day.

Limit of detection and Limit of quantitation: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. In this work established LOD and LOQ values by fixing the test concentration and based on the signal to noise levels of 3:1 for LOD and 10:1 for LOQ, precision at LOQ performed for VAR4, VAR5, VAR6, VAR7, VAR8 and Vardenafil HCl and calculated the % RSD for all impurities and Vardenafil HCl and accuracy study was done for all these impurities and the recovery was calculated and the range of recovery meet the ICH Q2 R1.

Linearity: The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. The linearity study of the VAR4, VAR5, VAR6, VAR7, VAR8 and Vardenafil HCl was done by LOQ to 0.3% w/w with respect to analyte test concentration (LOQ, 0.05, 0.10, 0.15, 0.20 and 0.30 % w/w), blend solution of all five impurities used for linearity, the regression line was plotted with area versus concentration. The value of the slope, % Y-intercept of the calibration curves were calculated. The relative response factor (RRF) of each impurity was determined by dividing the slope of the each impurity with slope of Vardenafil HCl

Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. In this present work the accuracy study was done by placing the 50%, 100%, 150% recovery solution by spiking in test concentration (0.5 mg/mL of the Vardenafil HCl) as 0.15% (100% level), the concentration of Accuracy solution were 0.075%w/w, 0.15%w/w, 0.225%w/w, the recovery of each impurity were calculated against respective impurity such as VAR4, VAR5, VAR6, VAR7, VAR8 in presence of analyte (0.5 mg/mL) the range of recovery was between 80 and 120% and meets the ICHQ2 R1.

Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness study was done by changing the temperature of column compartment, flow and gradient change of mobile phase %B, %RSD meets the ICHQ2 R1 for all the impurities and Vardenafil HCl.

Solution stability and mobile phase stability: The solution stability and mobile phase stability gives the information of the method's reliability in normal usage during the storage of the solutions using, the vardenafil HCl solution at analyte level spiked with 0.15 % w/w and injected 6 hrs, 12 hrs and 48 hrs, the quantification was done against the fresh standard solution of impurities, calculated the % RSD and % difference, both met the ICH Q2 R1.

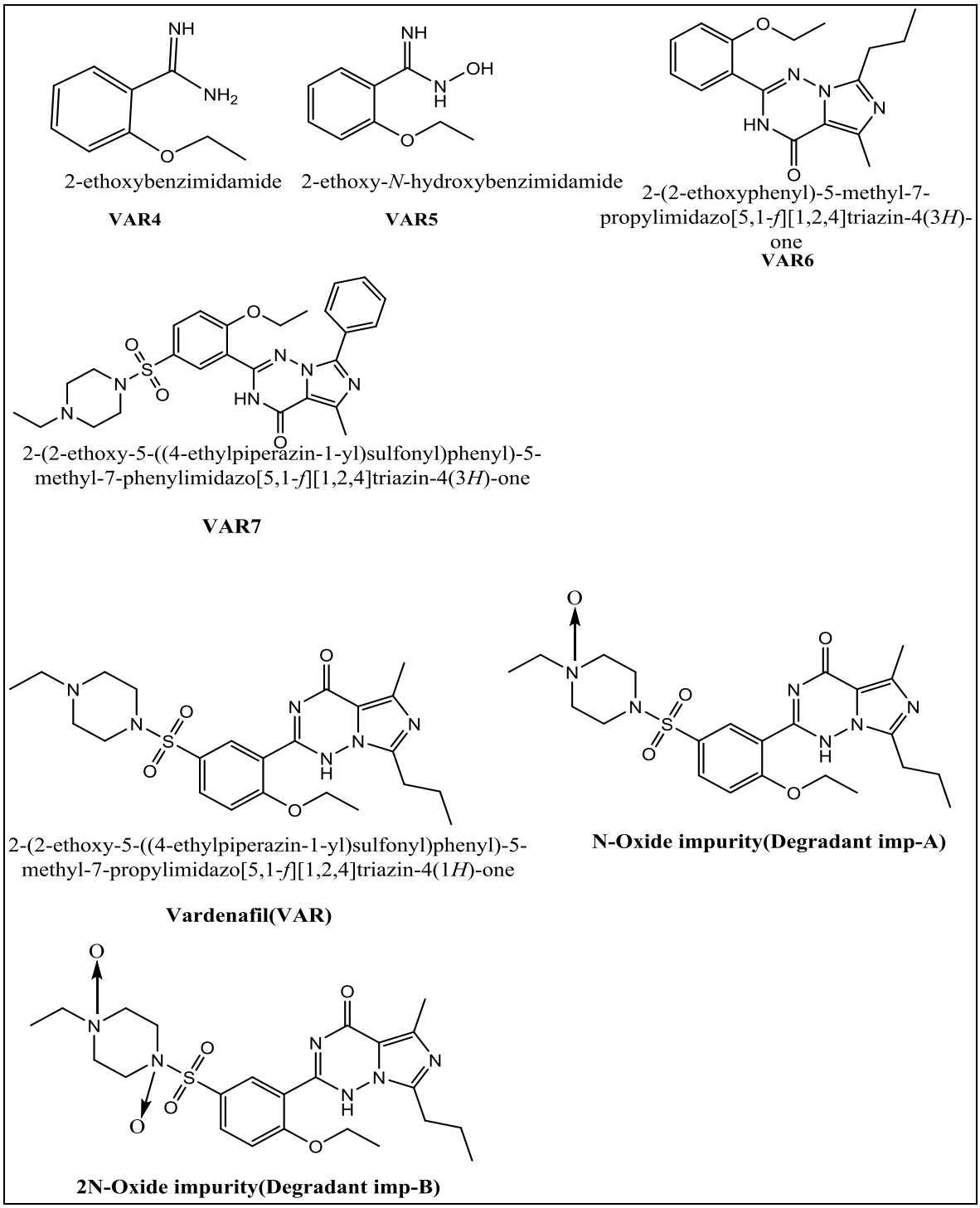

FIG. 1: STRUCTURE OF PROCESS AND DEGRADATION IMPURITIES

RESULTS AND DISCUSSIONS:

Results of forced degradation: Forced degradation study was done in 3% H_2O_2 solution, 0.5% NaOH solution, 0.5% HCl solution, thermal degradation at 105°C, photo degradation in visible fluorescence light of 1.2 Million lux hours, and 200 watt hrs/meter square. In 3% H_2O_2 Vardenafil HCl solution was kept in room temperature up to 4 days. The 0.38 RRT, 0.76 RRT unknown impurities were formed and that impurities were characterized by using UPLC-TOF, where generated the formula by Mass lynx soft ware, confirmed that N-Oxide impurity (**Fig. 2**) these impurities were showing specificity in this present method and all process impurity also confirmed their structure by using the UPLC-TOF. They were eluting away from the unknown impurities, along with 0.76 RRT, 0.84 RRT, 0.93 RRT impurities were formed along with N-Oxide impurity they were characterized by using UPLC-TOF. The impurity profile of degradation study was presented at **Table 1, Table 2** and **3.**

In the Thermal degradation at 105°C and on 8 day, the 0.19 RRT, 0.20 RRT, 0.32 RRT, 0.33 RRT & 0.93 RRT were identified as 0.02%, 0.25%, 0.74%, 0.02%, 0.04% levels respectively, all RRT impurities were characterized by using UPLC-TOF, impurities were presented in the **Table 1** and **Table 2** and **3.** In the Photo degradation study, the fluorescence light was used for the visible light of 1.2 million lux hours, the impurities of 0.26 RRT, 0.33 RRT, 0.87 RRT were identifies at 0.01%, 0.004%, 0.02% levels respectively, the UV light of 200 watt per meter square were used for UV Degradation, the 0.33 RRT, 0.87 RRT were identified at 0.02%, 0.01% respectively. The impurities were characterized by using UPLC-TOF, the degradation of Visible and UV were extended up to 2 months. The % vardenafil HCl purity was decreased to 84.07 % w/w in case of visible and 81.6 % w/w in case of UV, the drastic change of impurity profile in photo degradation happened at second month. The impurity profile was presented in the **Table 1, 2** and **3.**

In 0.5% NaOH vardenafil HCl solution was kept at room temperature upto 12 days and 0.5% HCl vardenafil HCl solution was kept at room temperature upto 12 days. No impurity was formed in both conditions. The impurity profile was presented in the **Table 1** and **2.** In assay method all the impurities of VAR is specific with all impurities no change of assay in presence of process impurities. Variation of assay and mass balance observed in the presence of oxidation condition and long term photo stability study, but variation not observed of assay in acid and base hydrolysis. Peak homogeneity of Vardenafil HCl was done by PDA and UPLC-TOF.

TABLE 1: HR-MS DATA OF FORCED DEGRADATION STUDY IMPURITIES OF VARDENAFIL HCL

S. No	Name of the product	Mass $[M+H]^+$	Formula	ppm	DBE	Impurity identified in study
1	VAR	489.2307	$C_{23}H_{33}N_6O_4S$	4.7	10.5	
2	N-Oxide impurity(impurity-A)	505.2256	$C_{23}H_{33}N_6O_5S$	4.6	10.5	H_2O_2
3	Unknown impurity-1	521.2175	$C_{23}H_{33}N_6O_6S$	-1.3	10.5	Degradation
4	Unknown impurity-2	487.2121	$C_{23}H_{31}N_6O_4S$	-1.4	11.5	
5	Unknown impurity-3	408.2071	$C_{19}H_{30}N_5O_3S$	0.5	7.5	
6	Unknown impurity-4	393.1235	$C_{17}H_{21}N_4O_5S$	0.5	9.5	Thermal
7	Unknown impurity-5	461.1979	$C_{21}H_{29}N_6O_4S$	1.7	10.5	degradation at
8	Unknown impurity-6	517.2607	$C_{25}H_{37}N_6O_4S$	1.9	10.5	105°C
9	Unknown impurity-7	366.1609	$C_{16}H_{24}N_5O_3S$	2.5	7.5	
10	Unknown impurity-8	408.2071	$C_{19}H_{30}N_5O_3S$	0.5	7.5	
11	Unknown impurity-9	410.1497	$C_{17}H_{25}N_5O_5S$	-0.2	8.5	UV 2 months
12	Unknown impurity-10	436.1660	$C_{19}H_{26}N_5O_5S$	1.1	9.5	
13	Unknown impurity-11	524.2237	$C_{25}H_{30}N_7O_6$	4.0	14.5	
14	Unknown impurity-12	540.2130	$C_{23}H_{35}N_5O_8S$	0.4	9.5	
15	Unknown impurity-13	366.1612	$C_{16}H_{24}N_5O_3S$	3.3	7.5	
16	Unknown impurity-14	408.2071	$C_{19}H_{30}N_5O_3S$	0.5	7.5	
17	Unknown impurity-15	410.1497	$C_{17}H_{25}N_5O_5S$	-0.2	8.5	Visible 2 months
18	Unknown impurity-16	436.1660	$C_{19}H_{26}N_5O_5S$	1.1	9.5	
19	Unknown impurity-17	524.2237	$C_{25}H_{30}N_7O_6$	4.0	14.5	

Precision: The VAR4, VAR5, VAR6, VAR7, VAR8 and vardenafil HCl precision values at method precision level were 1.1% to 2.4%, at 150% level the impurity precision values were 0.6% to 3.5%, at 50% level, the impurities were 1.4% and 4.1% and at LOQ level the precision level were 0.6% to 5.1% the range of the impurities in related substance method should not be less than 15% for impurities and vardenafil HCl. The range of % RSD for method precision and intermediate precision and LOQ precision were 0.6 to 5.1. The percentage RSD values of related impurities and Vardenafil HCl were reported in **Table 2**. The %RSD at analyte concentration (0.2 mg/mL) in assay method was 0.5, 0.6 and 0.2 for precision at 50% level, 100% level and 150% level respectively. The Assay values for method precision and intermediate precision were 100.5 and 101.0. The precision values were provided in **Table 2**.

Limit of quantification and Limit of detection: The limit of detection of VAR5, VAR6, VAR7 and VAR8 were less than 0.02 %w/w or equal to 0.02 % with respect to test sample concentration 0.5 mg/mL with 1.0µL injection volume, The LOQ for VAR4 and vardenafil HCl were 0.05% w/w with respect to test sample concentration 0.5 mg/mL with 1.0µL injection volume. The limit of quantitation of VAR5, VAR6, VAR7 and VAR8 were 0.07% w/w, 0.05% w/w, 0.05 %w/w and 0.05%w/w respectively, the limit of detection for VAR4 and vardenafil HCl were 0.016 %w/w and 0.015 % respectively. The LOQ and LOD values were established based on Signal to noise ratio method by Empower-3 software and no interference was found with blank .At LOQ levels all impurities were precise and accurate, the precision at LOQ was less than 5.1% RSD and recovery values were in between 80% to 120%. Hence this method is useful to quantify at all known and unknown impurities and vardenafil HCl can be quantified by using this related substance method. The LOQ and LOD values of all impurities were provided in **Table 2**.

Linearity: The correlation coefficient for the VAR4, VAR5, VAR6, VAR7, VAR8 and vardenafil HCl studied from LOQ to 200% with respect to specification level (0.15%). The correlation coefficient was more than 0.995 for VAR4, VAR6 and vardenafil HCl and more than 0.999 for the VAR5, VAR7 and VAR8, the RRF values established by using calibration plot method, The %*Y*-intercept of each plot was below 1.9% of the response at 0.15 % *w/w* level of the corresponding impurity. RRF values were very close to the Vardenafil HCl response. The correlation coefficient of VAR for assay was 0.999 in assay. The linearity results and RRF results were tabulated in **Table 2** and **5**.

Accuracy: The percentage recovery values at 0.15%w/w values were in the range of 83.70 % to 90.68 % for all process impurities in vardenafil HCl and the percent recovery values at 0.075 % w/w values were 85.4 % to 98.4% for all process impurities in vardenafil HCl and the percent recovery values at 0.225% w/w in the range of 81.3% to 92.9% for all impurities in vardenafil HCl. The recovery values were reflects that quantification of all known and unknown impurities can be determined accurately. The accuracy study for assay method w.r.t 0.2 mg/mL was in the range of 99.8, 100.0 and 100.9 of 50%, 100% and 150% respectively. The flow of the accuracy values were presented in **Table 4**.

Robustness: The robustness study was done on by changing the column oven temperature, flow, wavelength, ratio of mobile phase B, the retention time of all impurities and vardenafil HCl were not changed, the content of spiked impurities matching the initial values. The assay values in the all robustness parameters viz temperature change from 25°C to 30°C and 20°C the assay range was 100.1 to 100.4%, flow of 0.15 mL/min and 0.25 mL/min the assay range was 100.3 and 99.8 % respectively, The change of 10% acetonitrile in gradient, the assay values were 100.0% and 100.2 ratio in low and high ratio mobile phase B. The change wavelength from 240 nm to 235nm and 245 nm assay were 100.1 and 99.96. The results were presented in the **Table 5.**

Solution stability and Mobile phase stability: The solution and mobile phase stability were studied up to 48 hours, it helps to reduce the time consumption of sample preparation and standard preparations. This study was performed by spiking impurities at 0.15% w/w with respect to test concentration (0.5 mg/mL), the recovery values

were in the range of 91.1 %w/w to 106.4 %w/w. The recovery values in quantification levels were acceptable. The recovery study extended up to 48 hrs. Assay study of VAR was done with respect to 0.2 mg/mL. The mobile phase and solution stability were done up to 48 hrs, there was no variation in assay from initial to 48 hrs.

TABLE 2: LOD, LOQ AND PRECISION DATA

Parameter	VAR	VAR-4	VAR-5	VAR-6	VAR-7	VAR-8
LOQ % w/w (w.r.t analyte conc.)	0.05	0.05	0.021	0.014	0.014	0.014
LOD % w/w (w.r.t analyte conc.)	0.015	0.016	0.007	0.005	0.005	0.005
slope (b)	207597	141589	85977	247182	258859	259528
Intercept(c)	-4973	-1224	1348.5	-306.171	126.5	-383.4
Correlation coefficient	0.995	0.998	0.999	0.996	0.999	0.999
LOQ precision (% RSD)	4.0	5.1	2.9	1.7	0.6	2.9
Method precision (% RSD)		2.5	2.4	1.9	1.1	1.3
Intermediate precision (% RSD)		1.6	0.9	0.3	0.4	0.3
Precision at 50 (% RSD)		1.8	4.1	2.5	1.4	1.8
Precision at 150 (% RSD)		1.6	3.5	1.9	0.6	1.0

TABLE 3: ACCURACY RESULTS FOR RELATED SUBSTANCE

Compound	Level (%)	Recovery in %
		Mean
VAR-4	LOQ	97.3
	0.075	84.5
	0.15	90.7
	0.225	81.3
VAR-5	LOQ	95.3
	0.075	98.4
	0.15	83.7
	0.225	85.6
VAR-6	LOQ	102.6
	0.075	96
	0.15	87.8
	0.225	89.9
VAR-7	LOQ	90.5
	0.075	94.0
	0.15	90.8
	0.225	92.9
VAR-8	LOQ	90.2
	0.075	96.0
	0.15	86.9
	0.225	90.0

TABLE 4: SPECIFICITY RESULTS

Stress condition	Duration	Purity of Vardenafil HCl after forced degradation	Content of major degradant	Remarks
Acid hydrolysis	4 Days	99.9	0.1	No degradation products formed
Base hydrolysis	4 Days	99.9	0.1	No degradation products formed
3% H_2O_2 Oxidation	4 Days	92.1	7.9	Significant degradation product formed
Thermal degradation (105ºC)	4 days	98.9	1.1	No degradation products formed
Visible light	8 days	99.9	0.1	No degradation products formed
UV light	8 days	99.8	0.2	No degradation products formed
Visible light	2 months	84.0	15.9	degradation products formed
UV light	2 months	81.6	18.4	degradation products formed

TABLE 5: ASSAY RESULTS

Parameter	Results
Correlation coefficient	0.997
Method precision (% RSD)	0.6
Intermediate precision (% RSD)	0.3
Precision at 50 (% RSD)	0.5
Precision at 150 (% RSD)	0.2
% Accuracy (50%)	99.8
% Accuracy (100%)	100.0
% Accuracy (150%)	100.9
Solution stability after 48 hrs	0.2% difference from initial
Mobile phase stability after 48 hrs	0.2% difference from initial

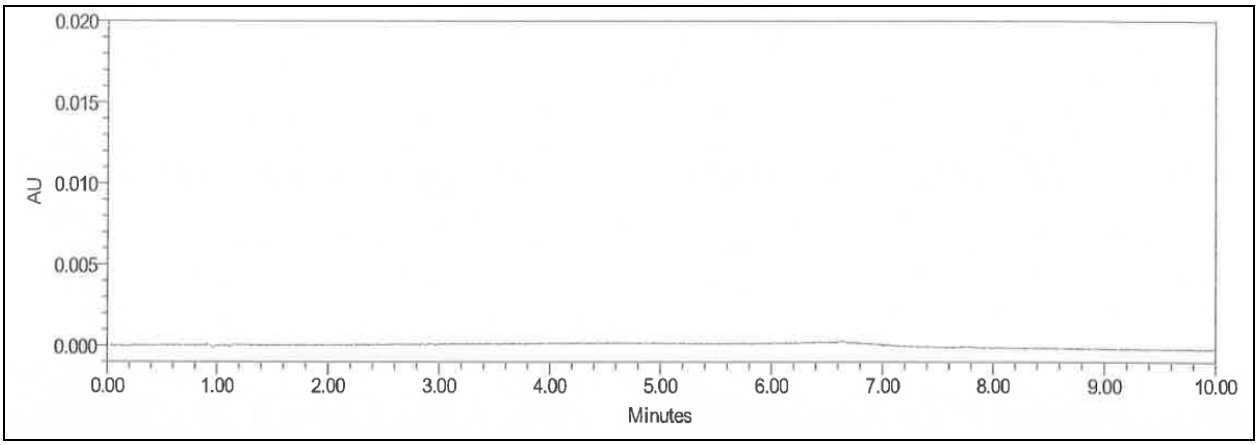

FIG. 2: BLANK CHROMATOGRAM.

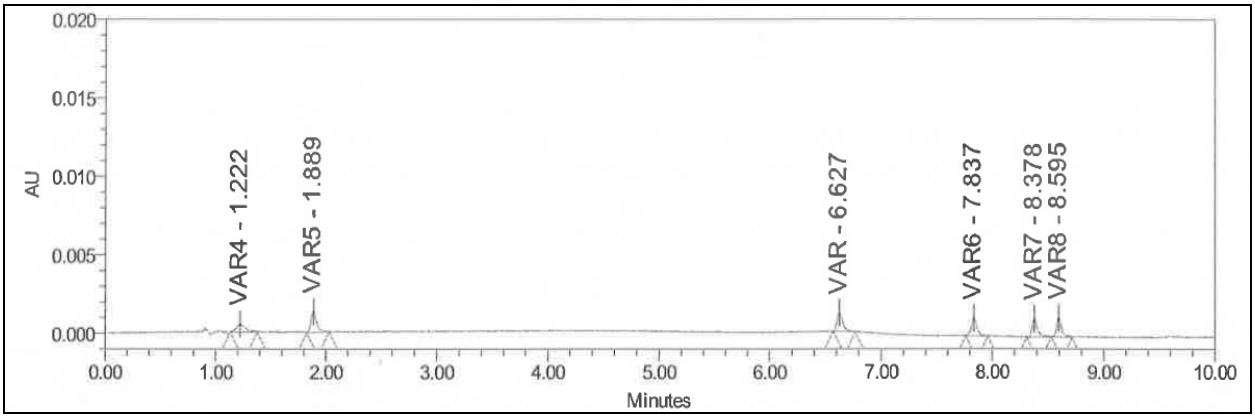

FIG. 3: LOQ CHROMATOGRAM.

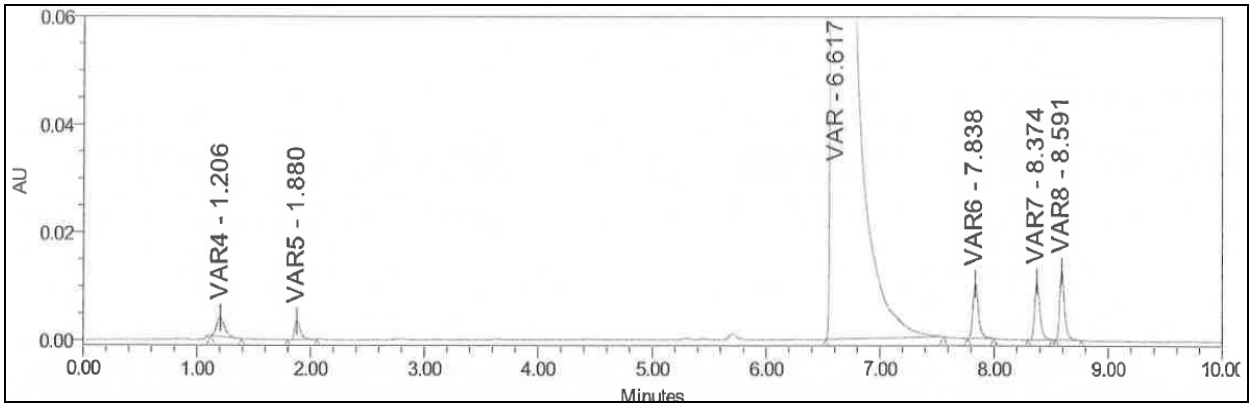

FIG. 4: RELATED SUBSTANCE CHROMATOGRAM VARDENAFIL HCL.

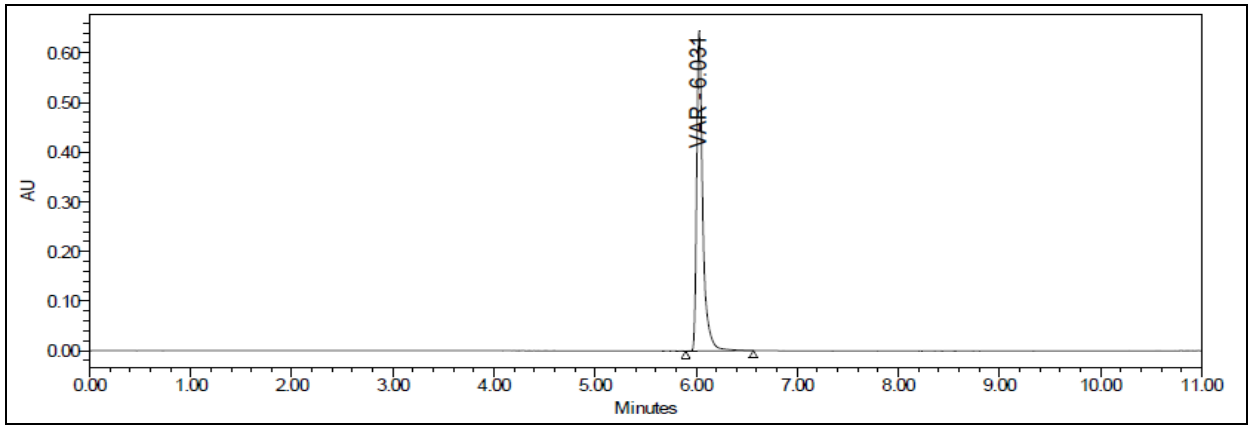

FIG. 5: ASSAY CHROMATOGRAM OF VARDENAFIL HCL.

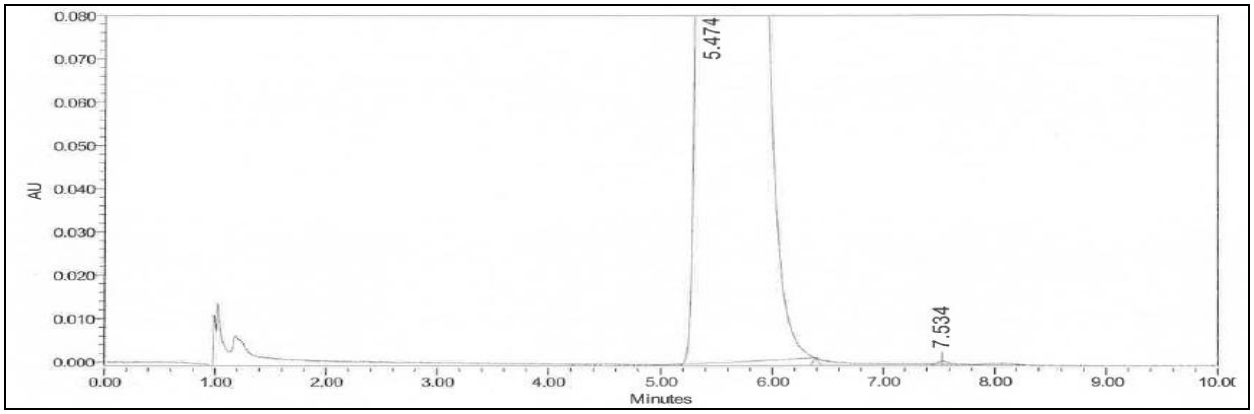

FIG. 6: ACID DEGRADATION CHROMATOGRAM

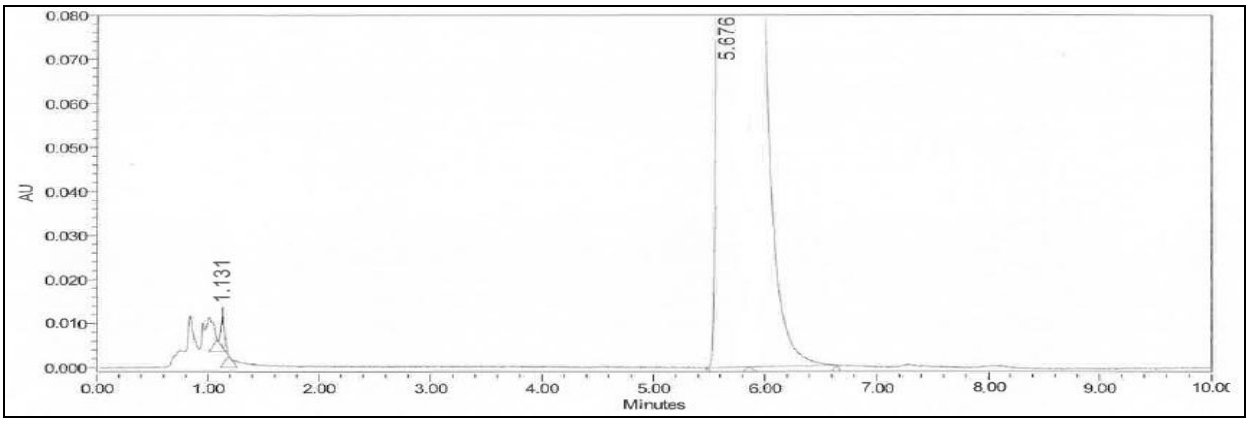

FIG. 7: BASE DEGRADATION CHROMATOGRAM

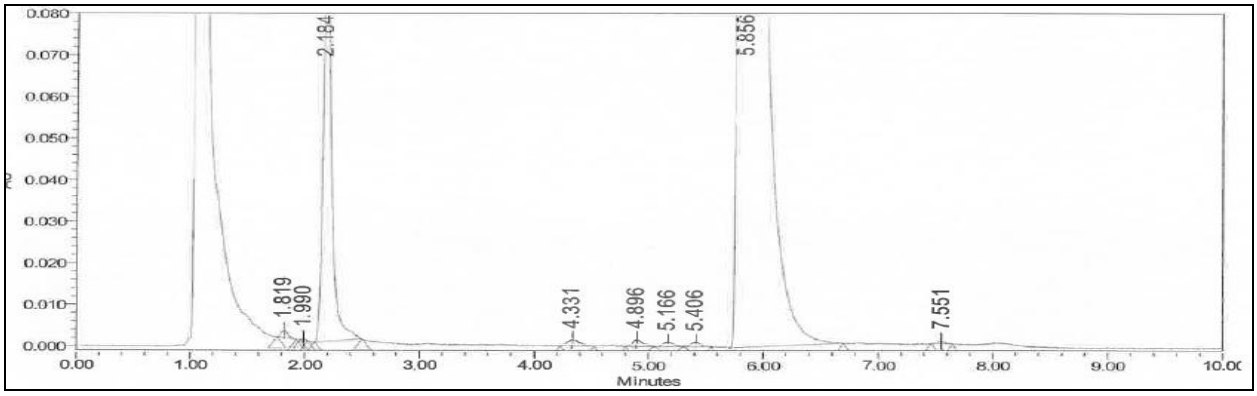

FIG. 8: PEROXIDE DEGRADATION CHROMATOGRAM

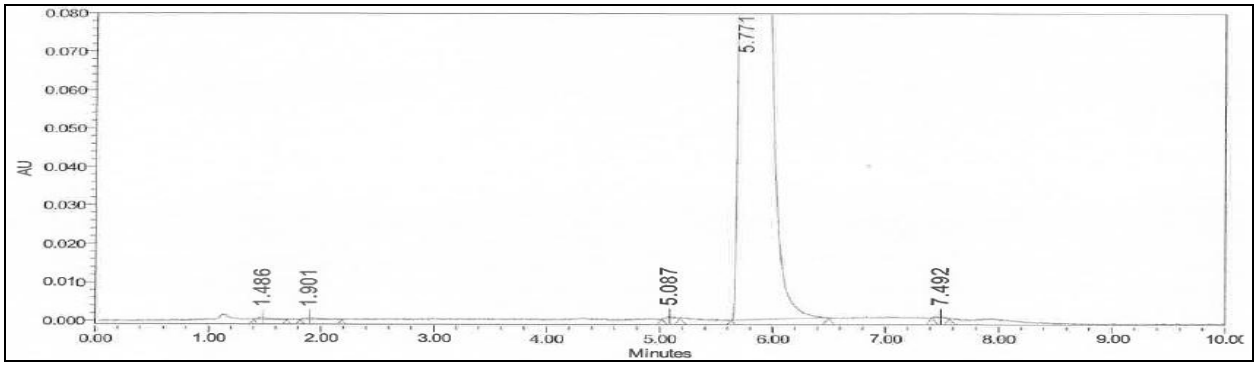

FIG. 9: UV LIGHT DEGRADATION CHROMATOGRAM

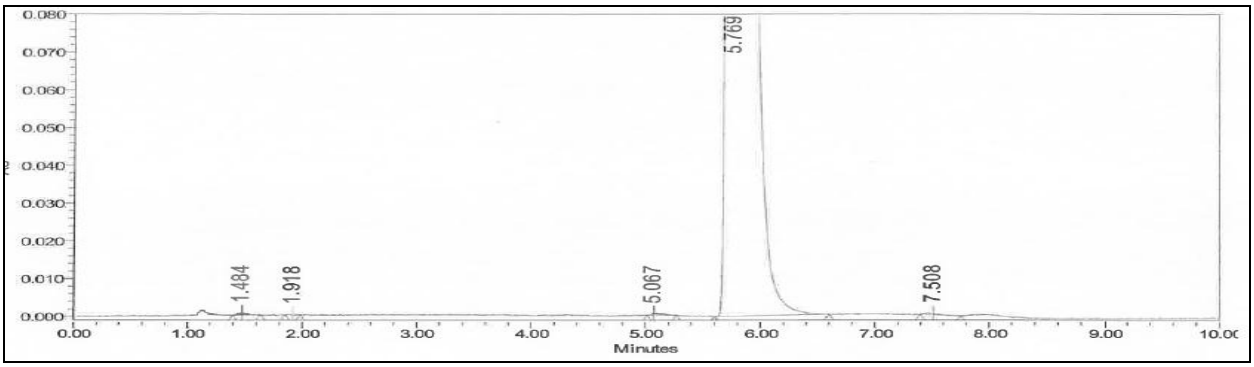

FIG. 10: VISIBLE LIGHT DEGRADATION CHROMATOGRAM

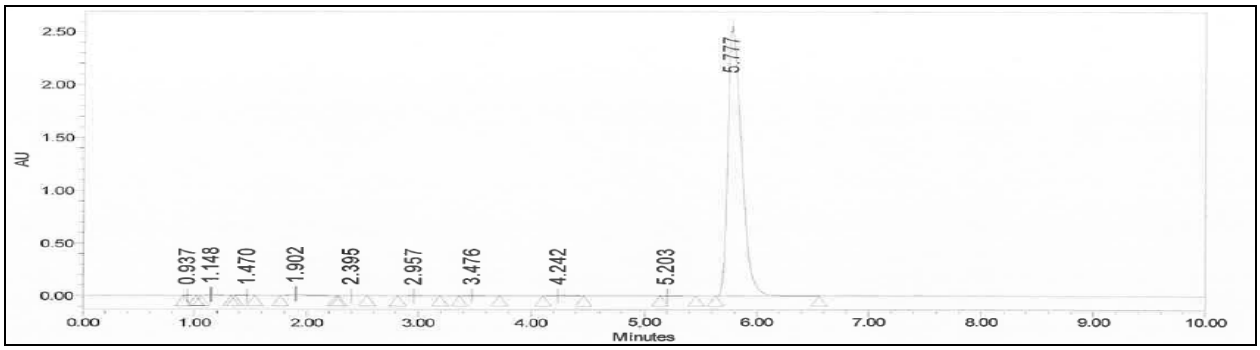

FIG. 11: THERMAL DEGRADATION CHROMATOGRAM

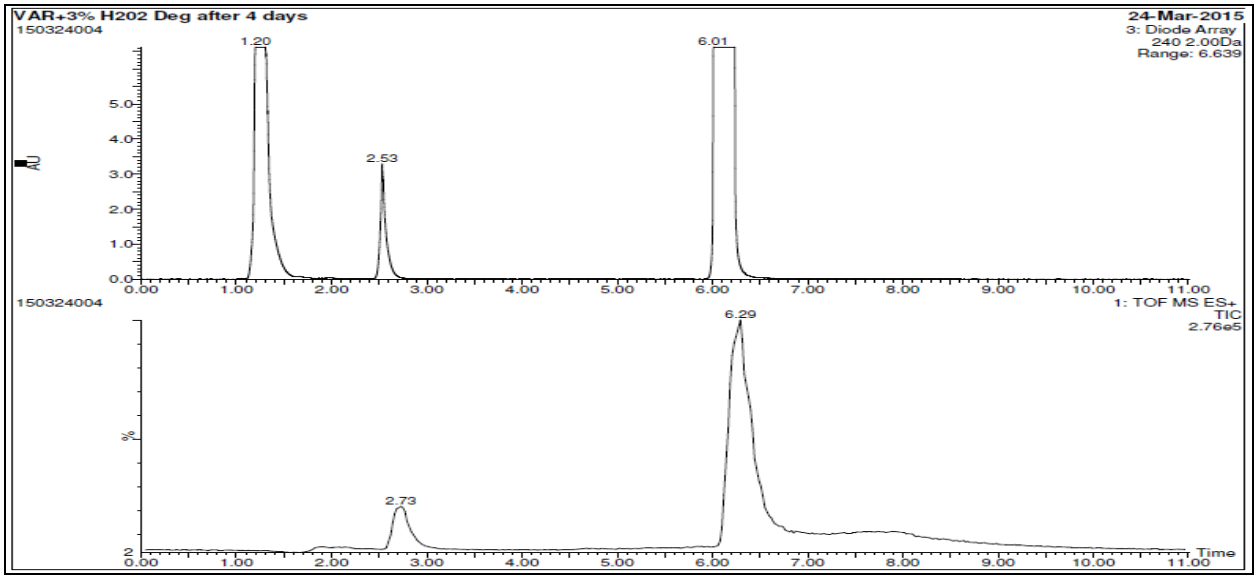

FIG. 12: PDA CHROMATOGRAM AND TOTAL ION CHROMATOGRAM OF H2O2 DEGRADATION OF VARDENAFIL HCL.

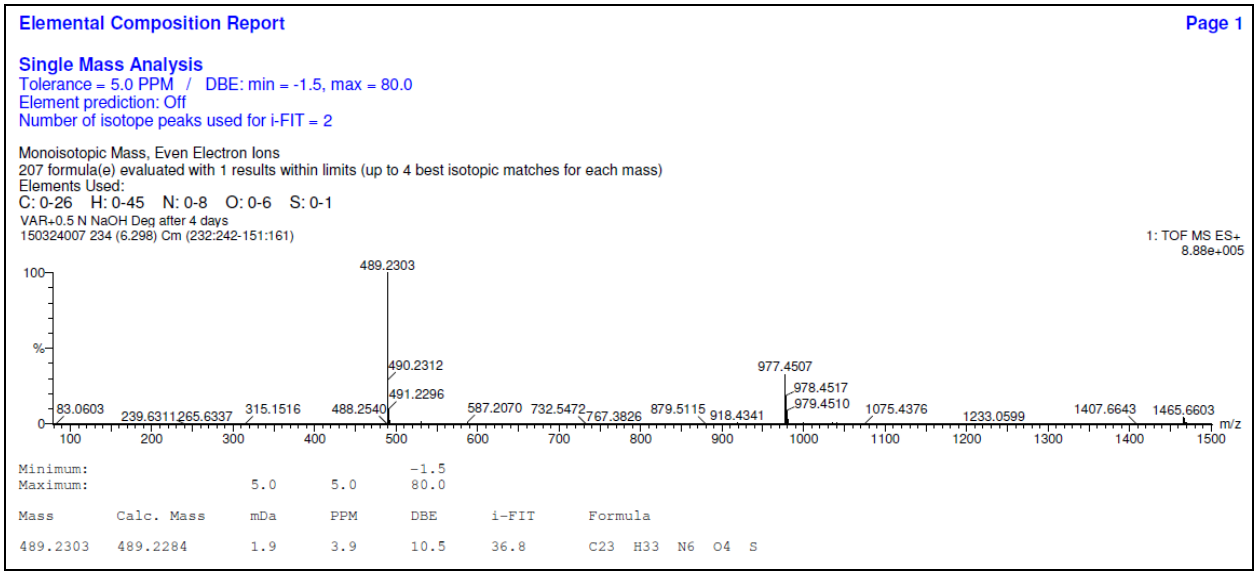

FIG. 13: VARDENAFIL HR-MS SPECTRUM

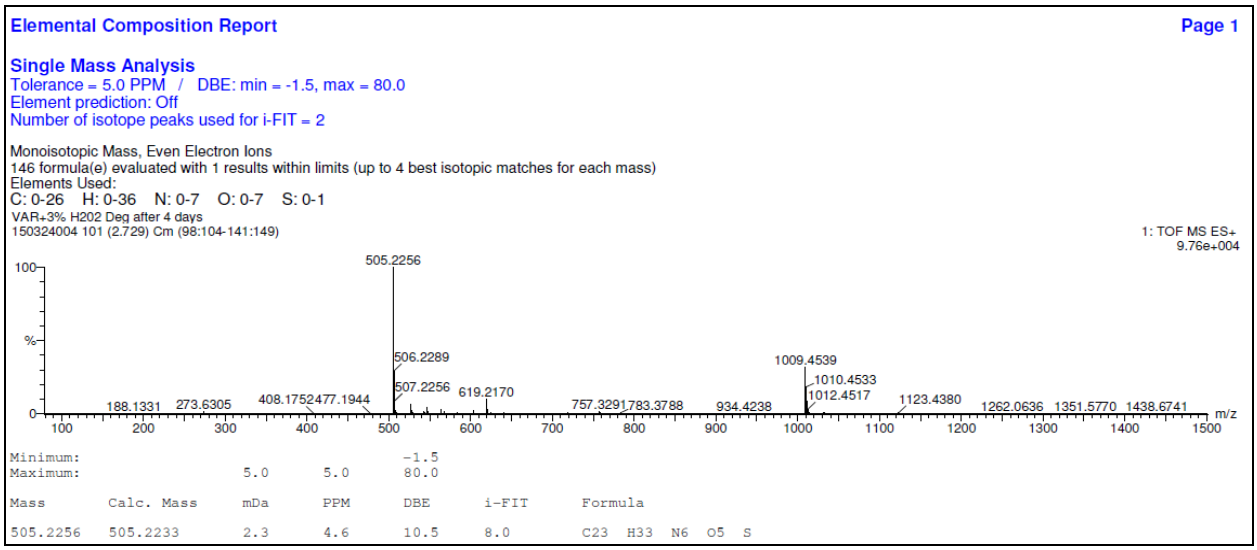

FIG. 14: 0.38 RRT N-OXIDE IMPURITY (IMPURITY-A) HR-MS SPECTRUM

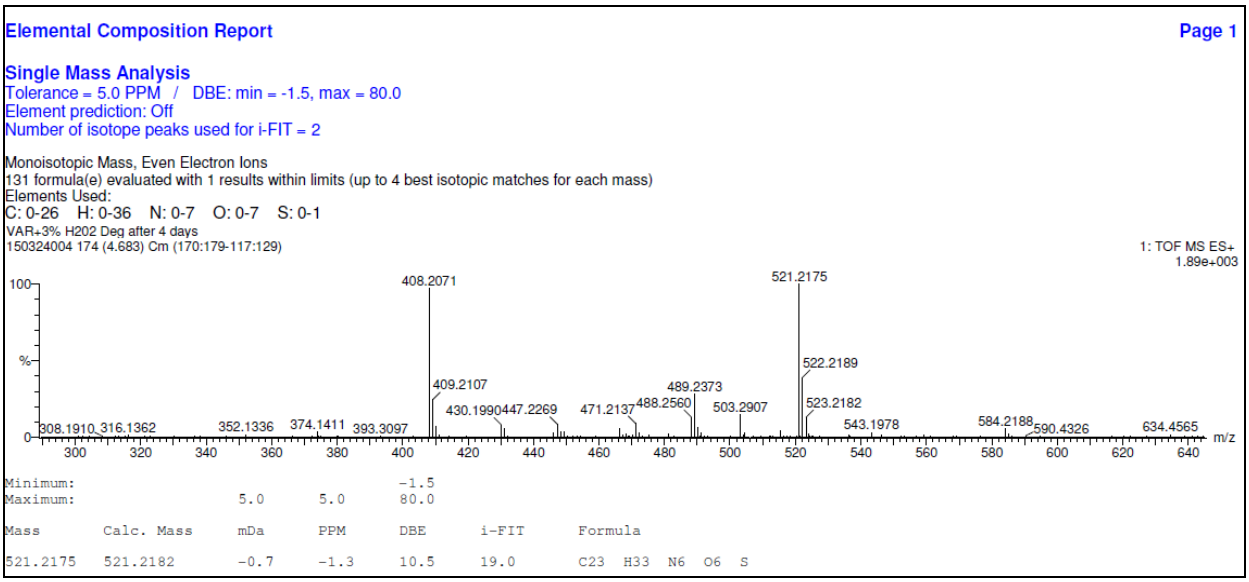

FIG. 15: 0.76 RRT (IMPURITY-B) HR-MS SPECTRUM

CONCLUSION: The present RP-UPLC method is with less runtime, high specificity with all process and degrading impurities, the mobile phase used in this method is ammonium acetate and acetonitrile, this is LC-MS compatible, the unknown impurities identification and characterization of all impurities were done by using UPLC-TOF, this high resolution spectrometry gives the formula for all unknown and known impurities, the fragmentation pattern of the all impurities were done by LC-MS/MS, All above information of this method gives complete study of method and unknown and known impurities in RP-UPLC, the quality control lab person can perform analysis easily and understand the chemistry of process and analytical method.

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