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Development and validation of liquid chromatogrphic method for Trazodone hydrochloride

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Abstract

A simple, isocratic, rapid and sensitive high performance liquid chromatographic method has been developed for quantitative determination of Trazodone Hydrochloride and its three process related impurities. The method has been validated for the determination of related substances in Trazodone Hydrochloride using a C18, ODS (4.6mm×250mm×10µm) column by keeping the flow rate of 1.5ml / min and having sensitivity of 0.2. The elution is carried out using a mobile phase consisting of methanol l80 ml, Acetonitrile 180 ml, Tetrahydrofuran 40 ml, and Trifluroacetic acid (0.5%) 600 ml. The detection is been carried out at 252 nm with injection volume of 10 µL. The run time is 15 minutes for estimation of related substance. The precision, Linearity and accuracy of the method are demonstrated for Trazodone Hydrochloride. Specificity of the method is also been studied. Limit of Detection and Limit of Quantification is also carried out. The method is found to be stability indicating and useful for the analysis of related substances of Trazodone Hydrochloride .Impurity profiling is also been carried out.

Keywords: HPLC, Trazodone Hydrochloride, related impurities

Introduction

Trazodone Hydrochloride is Antidepressant agent. In clinical use the compound has proven to be an antidepressant equivalent in effectiveness to imipramine[1]. Trazodone HCL is a off-white crystalline powder having as a chemical name 2-[3-{4-(3-chlorophenyl)-1-piperazinyl} propyl]-1,2,4-triazolo [4,3-a] pyridine-3- (2H)-one hydrochloride. Several liquid chromatographic

methods are reported for estimation of Trazodone HCL in various matrix systems[2-6]. Some of these methods are applicable for the analysis of Trazodone HCL drug substance[5-6].

For the development and validation for liquid chromatographic method of Trazodone Hydrochloride the following parameters were evaluated: Linearity, Precision, Relative Response Factors (RRF), Accuracy, linearity, Specificity, Precision, Chromatographic purity, Quantification and Detection Limit, Stability in analytical solution and Robustness of lead molecule i.e Trazodone Hydrochloride according to USP and ICH guidelines.

The impurities or unreacted precursors in Trazodone Hydrochloride (fig 1) are following:

Figure 1: Trazodone Hydrochloride

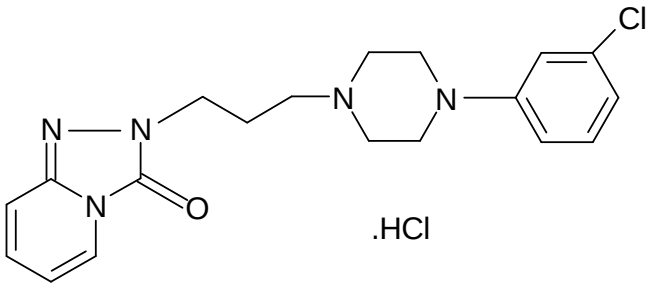


Figure 2: Impurity A: 1-(3-chlorophenyl) piperazine hydrochloride

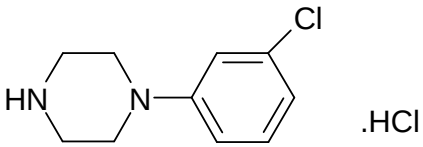


Figure 3: Impurity B: 1, 2, 4-triazolo [4, 3-a]-pyridine-3(2H)-one sodium salt

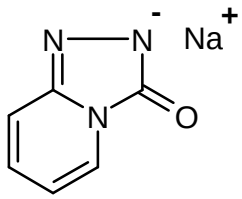
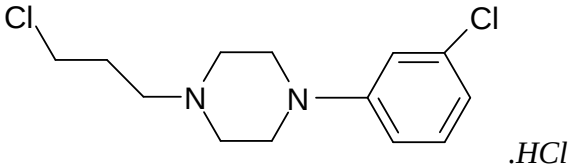


Figure 4: Impurity C: 1-(3-chlorophenyl)-4-(3-chloropropyl) piperazine hydrochoride



The structure of all above impurities along with lead molecule is been determined by spectral (NMR, MASS Spectra, I.R Spectra) analysis.

Materials and Methods

Reagents and Chemicals

Trazodone HCL drug substance was prepared and well characterized with the help of various spectroscopic and chromatographic techniques. This was used as reference standard for further work. The reference standard samples of impurity A, B and C which are intermediates are obtained from respective lab experiments after characterization using various spectroscopic and chromatographic techniques and are taken as standards for further experiment.

Analytical reagent grade Trifluroacetic acid was purchased from merck Chemicals and HPLC grade methanol, Acetonitrile, Tetrahydrofuran and from S.D Fine chemicals.

A chromatographic system is Agilent 1100 series equipped with a quaternary gradient pump, photodiode array detector. All the data was acquired using Chemstation data acquisition and integration software. A Bruker 300MHz NMR spectrometer was used for recording the ^1H spectrum. A Shimadzu UV spectrophotometer was used for recording the UV spectrum. An FTIR Spectrum One from Perkin Elmer was used for Infra Red analysis. Shimadzu Qp-2010 was used for mass spectroscopic analysis. Spectroscopic Data of Trazadone Hydrochloride and related impurities are given in table no.1 and 2.

Preparation of Solutions, Chromatographic Conditions and System Suitability Parameters:

Chromatographic Conditions-

The separation is carried out by using C18 ODS (4.6mm×250mm×10µm) column. The mobile phase was prepared by mixing of methanol l80 ml, Acetonitrile 180ml, Tetrahydrofuran 40 ml, and Trifluroacetic acid (0.5%) 600ml. The detection was carried out at 252 nm with the injection volume of 10 µL. The run time is 15 min for estimation of related substances.

Standard solution Preparation-

About 50 mg of Trazodone HCl Reference standard, accurately weighed was transferred in 50 mL volumetric flask, dissolved in sufficient mobile phase and diluted to the mark. This solution was further diluted with mobile phase to obtain a solution having a known concentration of about 1.0 µg/ mL.

Sample solution Preparation-

About 50 mg of Trazodone HCl sample, accurately weighed was transferred in 50 mL volumetric flask, dissolved in sufficient mobile phase and diluted up to the mark. A portion of this solution was loaded on the HPLC for analysis. Retention times of Trazodone Hydrochloride and its process related impurities are tabulated in table. no 3.

System suitability parameters-

3-chloro aniline and Trazodone Hydrochloride was dissolved in mobile phase to obtain a solution of 0.1mg per ml of 3-chloro aniline and 0.01 mg per ml of Trazodone Hydrochloride. The relative retention time of 3-chloro aniline is 2.66 min and that of Trazodone hydrochloride is 5.71 min. The resolution between Trazodone Hydrochloride and 3-chloro aniline is NLT 12.0. Mean area of 3-chloro aniline is 2769.180 and that of Trazodone Hydrochloride are 2093.28. The

% RSD for replicate injections of standard preparation should not be more than 10.0%.Theoritical plates and tailing factor should be NLT 10000 and NMT 2.0 respectively.

Results and discussion

Three related substances have been described as a process intermediates. The authentic standards of these impurities are obtained from respective lab experiments and based on data from various spectroscopic and chromatographic techniques their structures were elucidated as presented above.

Method Validation:
The proposed method for estimation of related substances of Trazodone HCl is validated as per the guideline of United States Pharmacopoeia and ICH guidelines Q2B.

(1) Specificity-

By injecting diluent & individual components into the chromatograph
Diluent, Trazodone Hydrochloride and related impurities namely Impurity A, Impurity B, Impurity C 10 ppm each are injected individually and in combination into the chromatograph. Retention times of all the components are given in table no.4. From retention time, it can be seen that, all the components have different retention timeDiluent shows peak at 1.6, 1.7and at 2.2 minutes, which is different from retention time of all other components. Thus all the components are well separated from each other indicating specificity of the analytical method.

(2) Linearity-
The linearity of an analytical method is its ability to elicit test results that are directly, or by well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. A graph of concentration (on X-axis) Vs Area (on Y-axis) is plotted.

About 50 mg of accurately weighed sample is taken in volumetric flask and dissolved in sufficient amount of mobile phase and diluted up to the mark. This is taken as stock solution. From stock solution serial dilutions are made of different concentration level and injected. Retention time and area are given in table no 5.

The graph of concentration (on X-axis) Vs Area (on Y-Axis) is linear in nature passing through origin.

(3) Precision-
The precision of analytical method is the degree of agreement among the individual test results when method is applied repeatedly to multiply sampling of homogenous sample.

To ensure analytical system is working satisfactorily and giving precise results, 10ppm solution (from stock solution) of Trazodone Hydrochloride was injected 5 times. The retention time and area are noted. RSD for retention time and area are calculated and tabulated in table no. 6.

Limit RSD: +/-2.0% [98.0% to 102.0%]

The individual area is found to be within 98.0 to 102.0% indicates that analytical system is well precise.

% Area is calculated on the basis of formula: (Area× 100)/ (mean area)

(4) Chromatographic purity-

Preparation of test solution:
Low load- The accurately weighed quantity of Trazodone hydrochloride is dissolved in mobile phase and diluted quantitatively with mobile phase having known concentration about 2µg/mL. Retention time and area of low load is given in tabular in table no.7:

High load- 50mg of accurately weighed quantity of Trazodone hydrochloride is transferred in standard volumetric flask dissolved and diluted with mobile phase up to the mark. Retention time and area of Trazodone hydrochloride and related substances are given below in tabular form in table no.8.

Calculation: Percentage of each peak [impurity] other than Trazodone hydrochloride is calculated by taking the formula:

$$100(c_s/c_r)\ (r_v/r_s)$$

Cs: Concentration in mg per mL of Trazodone hydrochloride in test solution of low load.
Cr: Concentration in mg per mL of Trazodone hydrochloride in test solution of high load.
r_v : Response of each peak other than Trazodone hydrochloride obtained from test solution of high load.
rs: Response of each peak other than Trazodone hydrochloride obtained from test solution of low load should not be not more than 0.4%for single impurity and not more than 1.0% of total impurity. [According to USP]

% Area of each impurity is less than 0.4%

Total % area of impurities is 0.061 [NMT 1% of total impurity area]

(5) Limit of quantification and limit of detection-
Limit of quantification:
Limit of quantification is lowest amount of analyte present in sample that can be determined with acceptable precision and accuracy under stated experimental conditions. Limit of quantification is calculated from signal to noise ratio. To determine limit of quantification, sample blank is injected first and noise is integrated at different intervals at different retention time near the peak of interest.

Eight injections of impurities of known concentration were injected. It was observed that signal to noise ratio must be in range of 10:1 as given in ICH guideline.

Limit of detection:
The detection limit is characteristic of limit test. It is lowest amount of analyte present in sample that can be detected but not necessarily quantities, under stated condition. Limit of detection is calculated from signal to noise ratio. To determine limit of detection, sample blank is injected and noise is integrated at different retention time near the peak of interest. Eight injections of impurities of known concentration were injected. It was observed that signal to noise ratio must be 3:1 as given in ICH guideline.

Sample solution preparation:
About 0.01g of accurately weighed impurities ('A','B','C') were transferred in 100mL volumetric flask in sufficient amount of mobile phase and diluted up to the mark. This solution was further diluted to get solution of concentration of 2 ppm. This is taken as stock solution.

Test solution preparation:
From test solution serial dilutions of different concentration are prepared. Limit of quantification and limit of detection of known impurities of Trazodone hydrochloride are given in tabulated form in table no. 9.

(6) Stability in analytical solution-
Stability in analytical solution study is carried out to know the stability of sample in analytical solution (diluent) over a period of time during routine analysis.

Stability in analytical solution study is carried out on six sample solutions prepared under Method Precision are reanalyzed after 24 hrs against freshly prepared standard solution. Results obtained are then compared with the method precision results.

Difference between '0' hr and '24' hr results is not more than 10% indicating that, the sample prepared in diluent is stable for at least '24' hrs.

(7) Robustness-
The robustness of an analytical method 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.

Table 1: MS and IR spectral data of Trazodone Hydrchloride and it's impurities

compound	MS (m/z)	IR(KBr) cm^{-1}
Trazodone Hydrochloride	372	: 3000(Aromatic C-H str), 2954 (aliphatic C-H str), 1704(C=O str) ,1650 (C=N str), 1600(Aromatic C=C str), 1350(C-N str),750(C-Cl str)
Impurity A	196	3050 (Aromatic C-H stretching), 2900(aliphatic C-H str), 1589 (Aromatic C=C),1300(C-N str), 750(C-Cl str),
Impurity B	136	3106(aromatic C-H str),1637 (C=N str), ,1721.(C=O str), 1541(aromatic C=C str) ,1353 (C-N str)
Impurity C	273	3062.75 (Aromatic C-H str),2916.17 (aliphatic C-H str), 1500 (Aromatic C=C str) ,1334.65(C-N str) , 775(C-Cl str),

Table 2: ^{1}H NMR data of Trazodone hydrochloride and it's realated impurities

compound	solvent	^{1}H NMR (ð)
Trazodone Hydrochloride	Cdcl3	2.16-2.12 (m, 2H,) ,2.64-2.60 (t, 2H),2.73 (s, 4H,), 3.09 (s, 4H,), 4.12-4.07 (t, 2H,), 6.51-6.46 (m, 1H, ArH), 7.02-6.93 (m, 2H, ArH), 7.09-7.08 (d, 2H, Ar H), 7.26-7.17 (m, 1H, ArH), 7.34-7.31 (d, 1H, ArH), 7.76-7.45 (d, 1H, ArH)
Impurity A	DMSO	3.54-3.41 (m, 4H,), 3.93 (s, 4H**),** 6.68-6.81 (d, 1H, ArH), 6.95-6.94 (d,1H,ArH), 7.02-7.01 (s,1H, ArH) , 7.26-7.22 (t, 1H, ArH)
Impurity B	DMSO	6.24-6.21 (t,1H,ArH), 6.68-6.65 (t,1H,ArH), 6.97-6.95 (d,1H,ArH), 7.58-7.56 (d,1H,ArH)
Impurity C	DMSO	2.28-2.21(m, 2H), 3.27-3.06 (m,6H,), 3.57-3.54 (t,2H,), 3.77-3.73 (m,2H,),3.89-3.86 (t,2H,),6.97-6.85 (m,2H,Aromatic H) ,7.05-7.04 (s,1H,Aromatic H),7.28-7.22 (t,1H,Aromatic H) ,10.87(S,1H,N-H)

Table 3: Relative retention time of the Trazodone hydrochloride and its Process related impurities

Conc.	sample	Retention time (min)
10 ppm	Trazodone Hydrochloride	5.5
10 ppm	Impurity A	4.6
10 ppm	Impurity B	6.8
10 ppm	Impurity C	1.9

Table 4: Specificity of Trazodone Hydrochloride

Sr. No	Component	Retention Time (min)	RRT
1	Diluent	1.6,1.7, 2.2	-
2	Trazodone Hydrochloride	5.5	1
3	Impurity A	4.6	0.83
4	Impurity B	6.8	1.23
5	Impurity C	1.9	0.34

Table 5: Linearity for Trazodone hydrochloride

Sr.No	Concentration(ppm)	Retention time(min)	Area
1	5	6.08	973.37
2	10	5.95	1889.334
3	15	5.93	2793.123
4	20	5.92	3699.358
5	25	5.87	4681.062

The robustness study was carried out by changing the Mobile phase composition, pH, and column temperature and flow rate. Samples of Trazodone Hydrochloride were analysed for

estimation of related substances under this changed experimental conditions. It is observed that method is unaffected by small changes in experimental conditions.

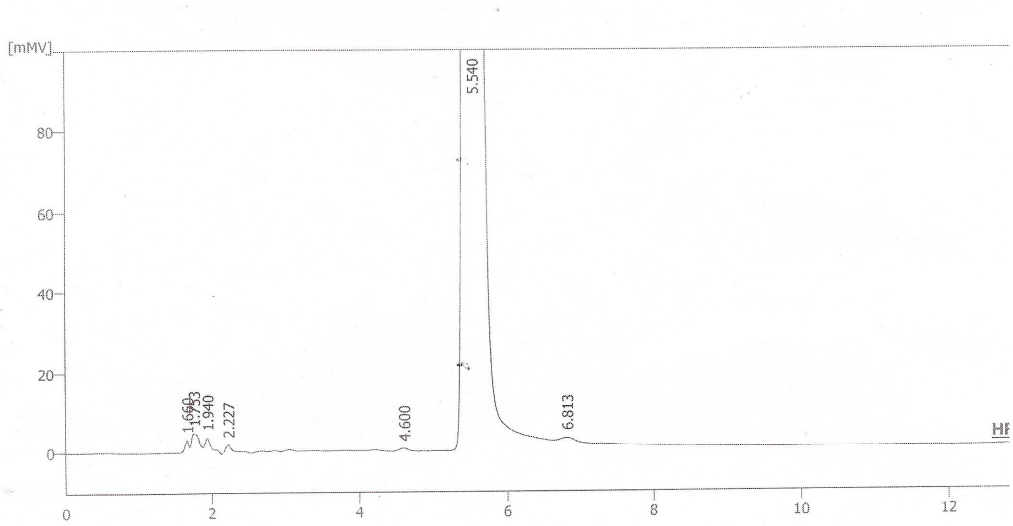
Table 6:Precision for Trazodone Hydrocloride

No. of injection	Retention time	Area	% Area
1of 5	6.167	1943.699	99.94
2 of 5	6.127	1942.450	99.88
3 of 5	6.167	1951.241	100.33
4 of 5	6.193	1939.775	99.74
5 of 5	6.160	1946.260	100.08
MEAN	6.162	1944.685	99.99

Table 7: Chromatographic purity of Trazodone Hydrochloride (Low load)

Sr.No	Retention time (min)	Area	%Area
1	5.64	37.081	100.000

Chromatogram 1: Typical chromatogram of sample solution preperation



Result Table - Calculation Method ISTD2

Reten. Time	Area [mV.s]	Height [mV]	Area % [%]	Height % [%]	W05 [min]
1.660	4.795	1.942	0.040	0.2	0.05
1.753	20.220	3.407	0.168	0.3	0.11
1.940	10.126	2.646	0.084	0.2	0.07
2.227	9.192	1.943	0.076	0.2	0.08
4.600	6.428	0.768	0.053	0.1	0.14
5.540	12000.788	1073.806	99.489	98.9	0.18
6.813	10.919	0.937	0.091	0.1	0.19
Total	12062.467	1085.449	100.000	100.0	

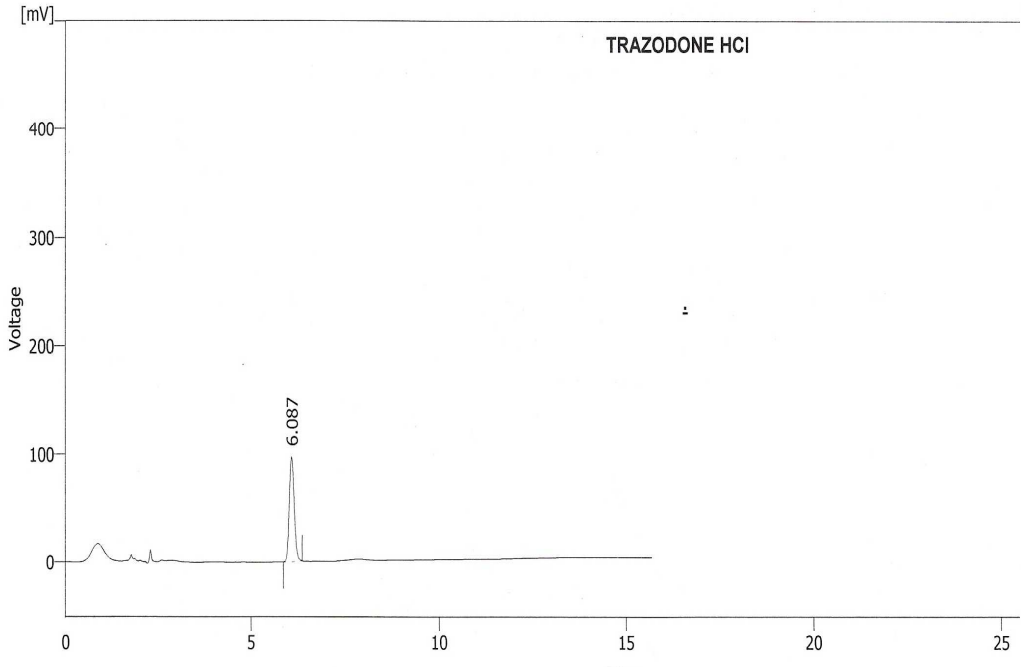
Table 8:Chromatographic purity of Trazodone Hydrochloride (High load)

Sr.No.	Retention time (min)	Area	% Area
1	1.900	3.546	0.020
2	4.607	0.112	0.001
3	6.827	7.211	99.940
4	5.547	18135.129	0.040
Total			100.00

Table 9:Test results of LOQ and LOD of Trazodone Hydrochloride

Sr.No	Impurity	LOQ (ppm)	LOD (ppm)
1	A	0.1	0.004
2	B	0.1	0.004
3	C	0.1	0.02

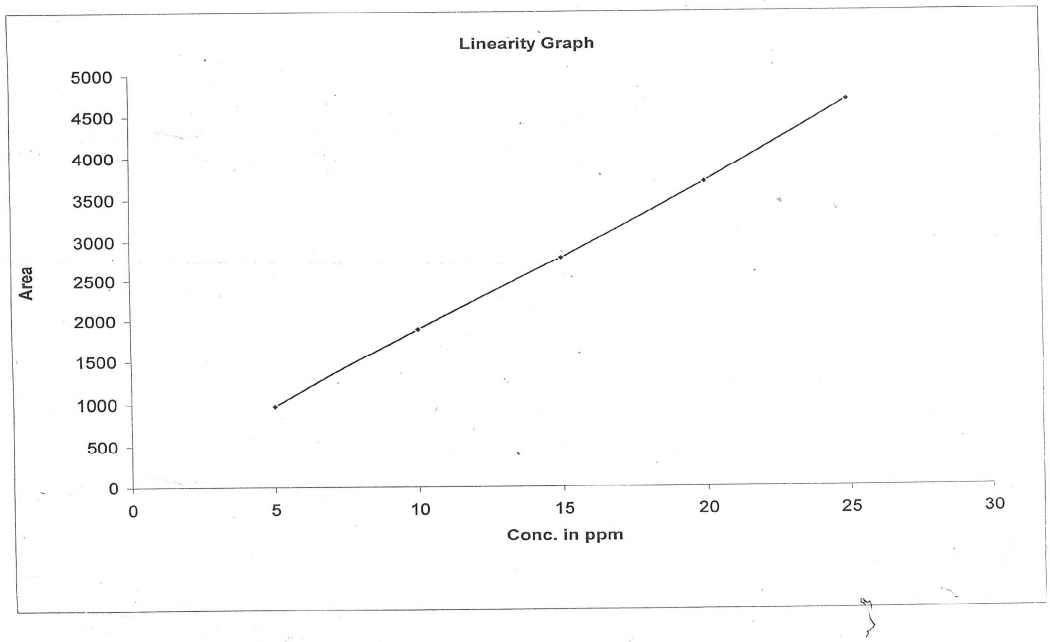
Chromatogram 2: Typical Chroamtogram of linearity of Trazodone Hydrochloride.



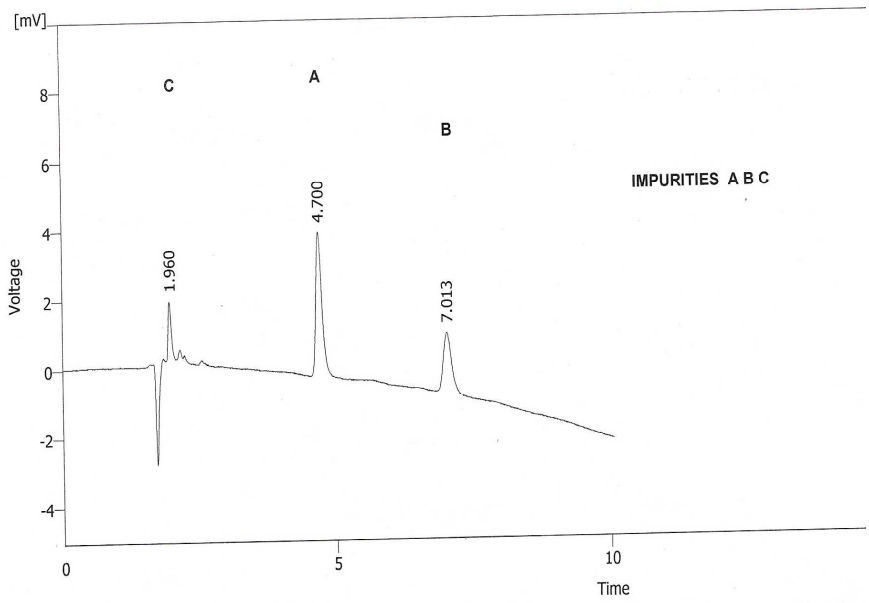
Result Table - Calculation Method ISTD2

Reten. Time	Area [mV.s]	Height [mV]	Area [%]	Height [%]
6.087	973.370	107.628	100.000	100.000
Total	973.370	107.628	100.000	100.000

Linear graph of Trazodone Hydrochloride



Chroamtogram 4 Typical Chromatogram For LOQ & LOD of Trazodone Hydrochoride



Conclusion

A. Analytical method is found to be specific as proved by injecting known amount of component into the chromatogram.
B. Limit of quantification and limit of detection for Trazodone Hydrochloride and its related impurities has been established and it is found to be within the range.
C. Analytical method is found to be linear over a specific range.
D. Analytical method is found to be précised and accurate.
E. Analytical method is found to be robust.
F. Sample prepared in analytical solution is found to be for at least 24 hrs.

The above mentioned isocratic method for the analysis of Trazodone Hydrochloride and it's related impurities is found to be Simple, rapid and sensitive. The method facilitates the separation of three of known related substances of Trazodone Hydrochloride with a resolution of minimum 2.0. The method is completely evaluated for its linearity, precision, accuracy, robustness, limit of quantification and detection and stability in the solution.

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