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Development and validation of liquid chromatographic method for trazodone hydrochloride

Nandini R. Pai * and Deeptaunshu Atul Pusalkar

Department of Chemistry, D.G. Ruparel College, Mahim, Mumbai 400016, Maharashtra, INDIA

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 determination of related substance in Trazodone hydrochloride using C8 ODS (150X4.6mm) column by keeping the flow rate of 1ml/min and having sensitivity of 1.The elution is carried out by using mobile phase of 300ml acetonitrile,50ml THF,400ml water and 250 ml methanol and pH was adjusted to 11 by TEA. The detection is carried out at 255nm with injection volume of 10 microlitre. Specificity, system suitability, linearity, precision, ruggedness, robustness has been carried out for Trazodone hydrochloride. Limit of quantification and limit of detection has been carried out for the impurities of Trazodone hydrochloride. Forced degradation study of Trazodone hydrochloride been carried out .Impurity profiling is also been carried out.

Keywords: HPLC, Trazodne Hydrochloride, related impurities, validation

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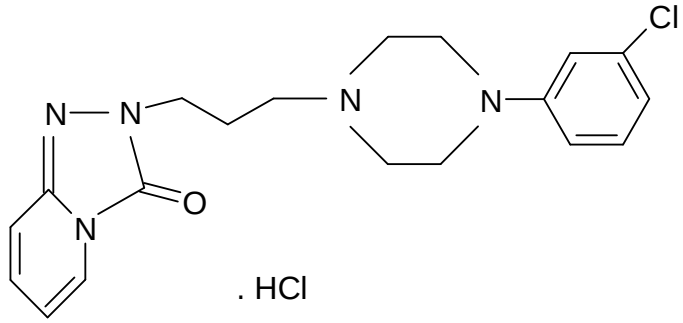
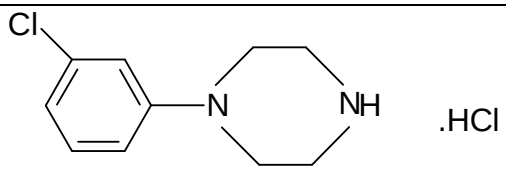
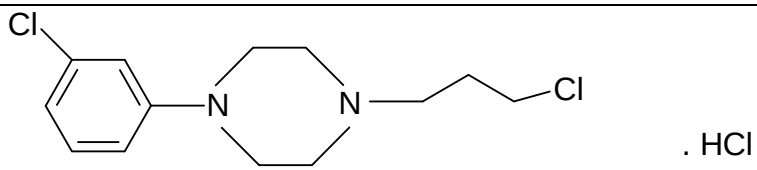
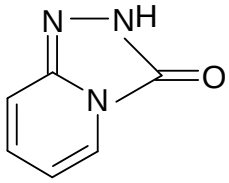
INTRODUCTION

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 an 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[2-5]. Some of these methods are applicable for the analysis of Trazodone HCL drug substance[5].

For the development and validation for liquid chromatographic method of Trazodone hydrochloride following parameters were evaluated: Specificity, system suitability, accuracy, linearity, precision, ruggedness, robustness, limit of Quantification and Detection according to USP and ICH guidelines. Also forced degradation study has been done for this API drug molecule. The impurities or un-reacted precursors in Trazodone hydrochloride are following.

Compound	Structure
Trazodone hydrochloride	
Imp A	
Imp B	
Imp C	

MATERIALS AND METHODS

Reagents and Chemicals

Trazodone Hydrochloride 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, 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, 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. Spectroscopic Data of Trazodone Hydrochloride and related impurities are given in table no.1 and 2.

Preparation of Solutions, Chromatographic Conditions and System Suitability Parameter-

Chromatographic Conditions-

Elution is carried out using C8 ODS (150X4.6mm) column by keeping the flow rate of 1ml/min and having sensitivity of 1.The elution is carried out by using mobile phase of 300ml acetonitrile,50ml THF,400ml water and 250 ml methanol and pH was adjusted to 11 by TEA. The detection is carried out at 255nm with injection volume of 10 microlitre.

Standard solution Preparation-

About 10 mg of Trazodone hydrochloride 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 required ppm solutions.

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

(1) Specificity-
By injecting diluent & individual components into the chromatograph Diluent, Trazodone Hydrochloride and related impurities namely Impurity A, Impurity B, Impurity C 100 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 time. Diluent, Trazodone Hyderochloride, and its impurities show different retention times. 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 10 mg of accurately weighed sample is taken in 50ml 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 for Trazodone Hydrochloride and its process related impurities.

Linearity is mentioned 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, 100ppm solution (from stock solution) of Trazodone Hydrochloride and its impurities were injected 5 times. 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.

(4) Ruggedness-
Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst. Its a degree of exactness of a measurement to its true value. Rugdness of Trazodone hydrochloride and its impurities are given in table no 7. The individual area is found to be within 98.0 to 102.0% indicates that analytical system is well precise.

(5) Accuracy-
The accuracy of an analytical method is the extent to which test results generated by the method and the true value agree. Accuracy can also be described as the closeness of agreement between the value that is adopted, either as a conventional, true or accepted reference value, and the value found. From stock solution of 200 ppm further dilutions are for the analysis. Accuracy of Trazodone hydrochloride and its impurities are given in table no 8.

(6) Robustness-
Robustness can be described as the ability to reproduce the (analytical) method in different laboratories or under different circumstances without the occurrence of unexpected differences in the obtained results. It was carried out by change in flow rate, change in mobile phase composition, change in wavelength and change in pH. It is observed that method is unaffected by small changes in experimental conditions complies the robustness. Results are mentioned in table no 9.

(7) 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. Results are mentioned in table no 10.

(8) 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.. It was observed that signal to noise ratio must be 3:1 as given in ICH guideline. Results are mentioned in table no 10.

(9) Forced Degradation Studies results-

To demonstrate the specificity and stability indicating characteristics of the method, samples of Trazodone hydrochloride were subjected to various stress conditions such as 0.1M HCl acid, 0.5 NaOH base, 10% v/v H2O2, Heat (105 0C), UV light (254 nm, 24 h). Results are mentioned in table no 11.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions, the main objective of the chromatographic method is to separate Trazodone hydrochloride from Imp-A, Imp-B, Imp-C Impurities were co-eluted using different stationary phases such as C18, C8 and cyano as well as different mobile phases. Effective chromatographic separation was achieved with using C8 ODS (150X4.6mm) column by keeping the flow rate of 1ml/min and having sensitivity of 1. Elution is carried out using C8 ODS (150X4.6mm) column by keeping the flow rate of 1ml/min and having sensitivity of 1.The elution is carried out by using mobile phase of 300ml acetonitrile,50ml THF,400ml water and 250 ml methanol and pH was adjusted to 11 by TEA. The detection is carried out at 255nm with injection volume of 10 microlitre.

Table 1-I.R spectra of Trazodone Hydrochloride and its process related impurities

Compound	I.R spectra KBr cm^{-1}
Trazodone Hydochloride	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
Imp A	3050 (Aromatic C-H stretching), 2900(aliphatic C-H str), 1589 (Aromatic C=C),1300(C-N str), 750(C-Cl str),
Imp B	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),
Imp C	3106(aromatic C-H str),1637 (C=N str), ,1721.(C=O str), 1541(aromatic C=C str) ,1353 (C-N str)

Table 2-^{1}HNMR of Aripiperazole and its process related impurities

Compound	Solvent	^{1}H NMR
Trazodone Hydrochloride	$CdCl_3$	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)
Imp 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)
Imp B	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

Concentration	Sample	Retention time(min)
100 ppm	Trazodone Hydrochloride	3.43
100 ppm	Imp A	4.21
100 ppm	Imp B	6.84
100 ppm	Imp C	1.21

Table 4- Specificity of Trazodone Hydrochloride

Sr. No	Component	Retention Time
1	Diluent	1.85, 2.62
2	Trazodone Hydrochloride	3.43
3	Imp A	4.21
4	Imp B	6.84
5	Imp C	1.21

Table no 5- Linearity of Trazodone Hydrochloride and its process related impurities

Sr no	Concentration (ppm)	Trazodone Hydrochloride	Imp A	Imp B	Imp C
1	80	2884.753	3364.484	2783.276	1380.796
2	90	3202.722	3759.107	3142.701	1563.058
3	100	3615.813	4196.654	3467.373	1726.353
4	110	3946.990	4620.484	3848.728	1907.636
5	120	4308.497	5050.434	4182.268	2060.609

Table 6-Precision for Trazodone Hydrochloride and process related impurities

Sr no	Concentration (ppm)	Trazodone Hydrochloride	Imp A	Imp B	Imp C
1	100	3615.813	4196.654	3467.373	1726.353
2	100	3601.118	4179.017	3476.272	1707.646
3	100	3617.355	4158.638	3434.634	1710.864
4	100	3609.342	4182.129	3473.433	1711.444
5	100	3619.009	4183.728	3465.915	1711.948
	MEAN	3612.5274	4180.033	3463.525	1713.651

Table 7-Rugdness for Trazodone Hydrochloride and process related impurities

Sr no	Concentration (ppm)	Trazodone Hydrochloride	Imp A	Imp B	Imp C
1	120	4404.873	5050.434	4186.614	2065.237
2	120	4434.669	5063.971	4173.243	2071.481
3	120	4412.581	5078.001	4198.887	2085.967
4	120	4430.768	5039.630	4196.174	2093.183
5	120	4403.560	5071.103	4190.441	2087.984
	MEAN	4417.2902	5081.2071	4189.071	2080.770

Table 8-Accuracy for Trazodone Hydrochloride and process related impurities.

(8.1) Trazodone Hydrochloride

Level	Concentration (ppm)	Area	Amount recovered	% Recovery
80%	80	2884.753	79.85	99.81
100%	100	3612.5274	_	_
120%	120	4417.2902	122.27	101.89

(8.2) Imp A

Level	Concentration (ppm)	Area	Amount recovered	% Recovery
80%	80	3364.484	80.48	99.40
100%	100	4180.033	_	_
120%	120	5081.2071	120.60	100.5

(8.3) Imp B

Level	Concentration (ppm)	Area	Amount recovered	% Recovery
80%	80	2783.276	80.35	100.43
100%	100	3463.525	_	_
120%	120	4182.268	120.75	100.62

(8.4) Imp C

Level	Concentration (ppm)	Area	Amount recovered	% Recovery
80%	80	1380.796	80.57	100.71
100%	100	1713.651	_	_
120%	120	2060.609	120.24	100.2

Table 9-Robustness for Trazodone Hydrochloride and process related impurities.

(9.1) change in flow rate (I) Trazodone Hydrochloride (200 ppm)

flow	R.T(min)	Area
0.8ml/min	4.89	5275.245
	4.86	5363.469
	4.86	5299.816
1.2 ml/min	3.28	3670.433
	3.30	3611.055
	3.29	3606.216

(ii) Imp A (200 ppm)

flow	R.T(min)	Area
0.8ml/min	5.19	6303.897
	5.16	6358.302
	5.16	6308.974
1.2 ml/min	3.45	4293.782
	3.46	4299.761
	3.47	4282.374

(ii) Imp B (200 ppm)

flow	R.T(min)	Area
0.8ml/min	12.21	4215.296
	12.19	4249.459
	12.21	4220.847
1.2 ml/min	8.22	2823.560
	8.11	2820.204
	8.18	2801.404

(ii) Imp C (200 ppm)

flow	R.T(min)	Area
0.8ml/min	1.61	2265.548
	1.62	2263.722
	1.61	2269.749
1.2 ml/min	1.09	1516.604
	1.09	1550.029
	1.09	1542.012

(9.2) change in wavelength-

(i) Trazodone Hydrochloride (200 ppm)

Wavelength(nm)	R.T(min)	Area
253	3.77	4316.4034
	3.68	4200.7351
	3.500	4091.1732
257	3.57	4503.5343
	3.54	4512.4798
	3.54	4540.1640

(ii) Imp A (200 ppm)

Wavelength(nm)	R.T(min)	Area
253	4.97	5140.6260
	4.96	5177.9613
	4.84	5077.2476
257	5.13	5064.2281
	5.16	5092.9203
	5.20	5035.1219

(ii) Imp B (200 ppm)

Wavelength(nm)	R.T(min)	Area
253	6.98	2248.8689
	6.99	2940.6033
	7.06	2953.5856
257	6.49	1879.0569
	6.57	1912.0312
	6.49	1960.3500

(ii) Imp C (200 ppm)

Wavelength(nm)	R.T(min)	Area
253	1.50	3162.4918
	1.52	3282.5069
	1.53	3239.5153
257	1.49	3378.5324
	1.41	3385.9091
	1.48	3391.4814

(9.3) change in pH of mobile phase-
Trazodone Hydrochloride (200 ppm)
(I)

pH	R.T(min)	Area
10.80	3.03	3828.838
	3.04	3835.624
	3.04	3833.647
11.20	3.41	5189.1575
	3.39	5191.0000
	3.31	5188.8065

(i) Imp A (200 ppm)

pH	R.T(min)	Area
10.80	3.48	5421.154
	3.45	5435.390
	3.46	5434.632
11.20	3.73	6272.3465
	3.65	5940.8035
	3.70	6240.1390

(ii) Imp B (200 ppm)

pH	R.T(min)	Area
10.80	5.81	3943.371
	5.81	3928.200
	5.81	3924.271
11.20	5.96	1602.5209
	6.02	1638.0730
	6.02	1598.6052

(iii) Imp C (200 ppm)

pH	R.T(min)	Area
10.80	1.43	2696.978
	1.43	2702.387
	1.42	2714.518
11.20	1.700	3442.6250
	1.700	3454.3211
	1.67	3436.3575

(9.4) change in mobile phase composition-

(i) Mobile phase 1- 270ml ACN+ 50ml THF + 430ml water +250ml methanol pH adjusted to 11 by TEA

Compound	R.T (min)	Area
Trazodone Hydrochloride	6.50	3763.514
	6.44	3775.401
	6.49	3798.463
IMP A	5.98	4005.449
	5.94	4013.113
	5.89	3992.891
IMP B	16.12	3059.321
	16.10	3016.243
	16.14	3001.052
IMP C	1.15	2141.643
	1.11	2131.396
	1.12	2136.595

(ii) Mobile phase 2- 330ml ACN+ 50ml THF+370ml water +250ml methanol pH adjusted to 11 by TEA

Compound	R.T (min)	Area
Trazodone Hydrochloride	4.27	3383.637
	4.32	3277.284
	4.24	3227.714
IMP A	5.21	3437.002
	5.21	3463.795
	5.20	3465.692
IMP B	9.07	2636.876
	9.05	2642.120
	8.96	2602.570
IMP C	1.14	1591.118
	1.10	1626.792
	1.10	1568.781

(iii) Mobile phase 3- 270 ml ACN+ 50ml THF+ 40 ml water + 280 ml methanol pH adjusted to 11 by TEA

Compound	R.T (min)	Area
Trazodone Hydrochloride	2.54	3629.074
	2.59	3555.373
	2.55	3609.203
IMP A	3.55	4077.048
	3.57	4063.196
	3.53	4070.180
IMP B	6.42	3138.413
	6.21	3188.597
	6.46	3181.867
IMP C	1.30	2162.348
	1.30	2131.771
	1.31	2168.987

Table 10- LOQ and LOD for impurities of Trazodone Hydrochloride

Compound	LOQ (ppm)	LOD (ppm)
Imp A	0.5	0.15
Imp B	0.3	0.1
Imp C	0.2	0.06

Table 11- Forced degredation study of Trazodone Hydrochloride

Condition	% degredation
Temperature 105^{O} C	23.94
UV	14.77
moisture	26.37
0.05M HCl	5.00
0.05 M NaOH	35.53
10% H_2O_2	22.28
1M NaOH	11.00
1M HCl	35.35

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 process 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 its related impurities is found to be Simple, rapid and sensitive.

The method is completely evaluated for its specificity linearity, precision, accuracy, robustness, ruggdness, limit of quantification and detection

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