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Analytical Method Development and Validation of Trazadone Hydrochloride in Bulk and Solid Dosage Form Using Uv Spectrophotometer and Uhplc Method

Chaudhari Payal Prakash*, Dr. Sunila A Patil and Dr . Sunil P Pawar

P.S.G.V.P Mandal College of Pharmacy, Shahada, Dist , Nandurbar , 425409 (M.S) India

Abstract

A new simple, accurate, rapid and precise isocratic, (ultra-high performance liquid chromatography), (UHPLC) method was developed and validated for determination of trazodone in bulk drug and tablet dosage form .analytical tech UV detector and column with 100 mm x 4.6 mm i.d . And 2.5 µm particle size methanol's 60 with OPA (60: 40 v/v) PH were used as the mobile phase for the method. The detection wavelength was 249 mm and flow rate were 0.7ml/min .in the developed method. The retention time of trazodone were found to be 4.641 min .the method was validated according to the regulatory guidelines with respect to specificity, precision, accuracy, linearity, and robustness, etc.

Keywords: Trazodone HCL; UHPLC; UV spectroscopy; Validation; Method development

Introduction

Trazodone, chemically designed as (2-3[4-(3-Chlorophenyl) piperazin -1-yl] propyl} 2h, 3h, -[1, 2, 4 triazolo [4, 3 - a] pyriclin -3 - one (fig: 1). category of anti- depressant [1]. (Figure 1) (Table 1)

According to the information collected from literature method the determination of trazodone HCL out of this method only one method is in UHPLC.

The determination of trazodone HCL in tablet dosage form we describe a simple accurate, sensitive and validated UHPLC method of trazodone with total run time 10 minutes for the determination of trazodone.

Materials and Methods

Standards Drugs

Trazodone HCL of purity 99% w/w procured from Zydus Cadila Health Care ltd. gift sample

Apparatus

The analysis of drugs was carried out on Agilent (110) software system UV detector. Equipped with reverse phase (Agilent) C18 column (4.6 id x 100 mm: 2.5 µm). A 20 µl injection loop and UV absorbance detector and running UV analyst software.

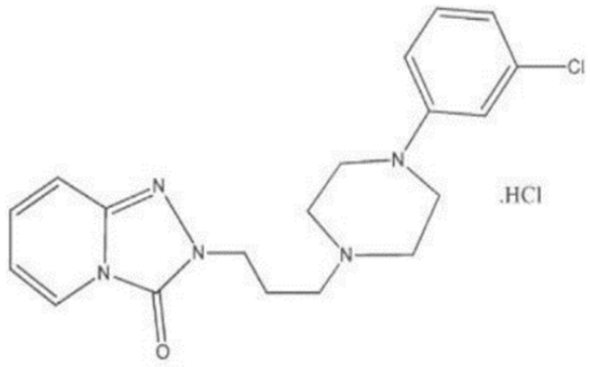

Figure 1: Chemical Structure of Trazodon.

Preparations of Standard Solutions

Accurately weight and transfer 5mg Trazodone working standard into 10 ml volumetric flask as about diluent Methanol completely and make volume up to the mark with the same solvent to get 500µg/ml standard (stock solution) and 15 min sonic ate to dissolve it and the resulting stock solution 0.1ml was transferred to 10 ml volumetric flask and the volume was made up to the mark with mobile phase Methanol: Water (0.1% OPA prepared in (MEOH 60+40 Water v/v) solvent.

Sample Solution Preparations

Weight 20 trazodone tablets and calculate the average weight, accurately weight and transfer the sample equivalent to 5mg of trazodone into a 10 ml volumetric flask and diluent was added to make up the volume solicited for 10 min with occasional swirling the above solution was filter through 0.45 ml of this solution diluted up to 100 ml with diluent.

Optimized chromatographic conditions

- Equipment: ultra-high performance liquid chromatography.
- Column: C18 (4.6 x 100mm) 2.5 µm
- Mobile phase: methanol: orthophosphoric acid (60: 40)
- Flow rate: 0.7 ml /min
- Wavelength: 249 nm
- Injection volume: 20 ml
- Run time: 10 min

***Corresponding author:** Prakash CP, P.S.G.V.P Mandal College of Pharmacy, Shahada, Dist, Nandurbar, 425409 (M.S) India, E-mail: Payalchaudhari71@Gmail.Com

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Table 1: Different Trials of Chromatographic Condition.

Fig No	Column used	Mobile phase, Flow Rateand Wavelength	Inj. Vol.	Observation	Conclusion
1	Agilent C18 (100 ×4.6mm, 2.5µ)	Methanol + 0.1% (OPA)Water (80+20 %v/v), 249nm, Flow rate 0.7mL	20µl	Sharp Peaks were not obtained	Hence rejected
2	Agilent C18(100 ×4.6mm, 2.5µ)	Methanol + (0.1% (OPA pH3.0 Water (50+50 %v/v), 249nm, Flow rate 0.7mL	20 µl	Sharp Peaks were not obtained	Hence rejected
3	Agilent C18 (100 ×4.6mm, 2.5µ)	Methanol + 0.1% OPA pH3.0)Water (60+40 %v/v), 249nm, Flo rate 0.7mL	20 µl	Sharp Peaks were obtained	Hence selected

Preparation of orthosphosphoric acid (PH: 3.2)

0.1% OPA (orthophosphoric acid) makes volume 100 ml volumetric flask, the volume was adjusted to PH 3.2

Method Validation

Analytical method validation was carried out as per ICH method validation guidelines to follow parameters such as precision, accuracy, linearity, robustness, LOD, and LQD, repeatability etc.

Precision

The method was established by analyzing various replicates standards of trazadone all the solutions was analyzed thrice in order to record any intra- day and inter-day variation in the result that concluded repeatability of the retention time and expressed as mean and % RSD calculated from the data obtained.

Accuracy

The accuracy of analytical method is the closeness of test results obtained by that method to the true value. Accuracy many often the expressed as percent recovery by the assay of known added amount of analyte the accuracy of an analytical method is determined by applying the method to analyzed samples to which known amounts of analyte have been added .

Linearity

The linearity of the analytical method is determined by mathematical treatment of test results obtained by analysis of samples with analyte concentrations across the claimed range. Area is plotted graphically as a function of analyte concentration.

Limit of detection and limit of quantification

Detection and quantification limits were determined through dilution method using S/N approach by injecting a 20 ml sample. LOD was considered as the minimum concentration with a signal to noise ratio of at least (3 S/N). LOQ was minimum concentration with signal to noise ratio of at least (10 S/N)

Robustness

The mobile phase composition was changed in (±1 ml/min) proportion in the mobile phase composition and flow rate was (±1 ml/min-1) the changed in detection wavelength.

Repeatability

Precision of the system was determined. Peak areas were measured and % RSD was calculate

Result and Discussion

UV Spectroscopy

UV absorption of 10 µg/mL solution of Trazodone in methanol was generated and absorbance was taken in the range of 200-400 nm. λ max [2]. (Figure 2)

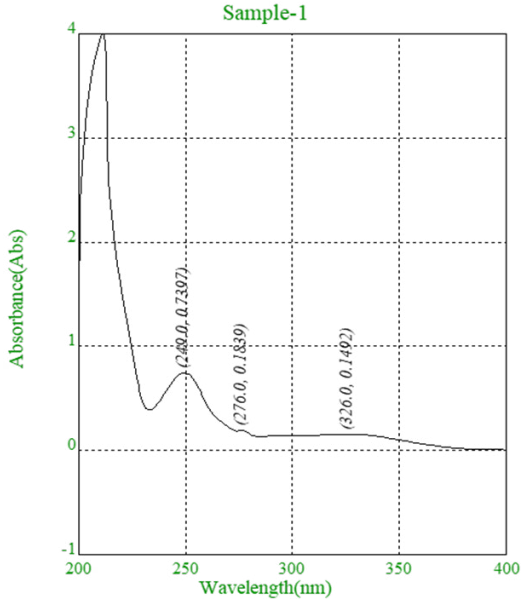

Figure 2: UV Spectrum of Trazodone.

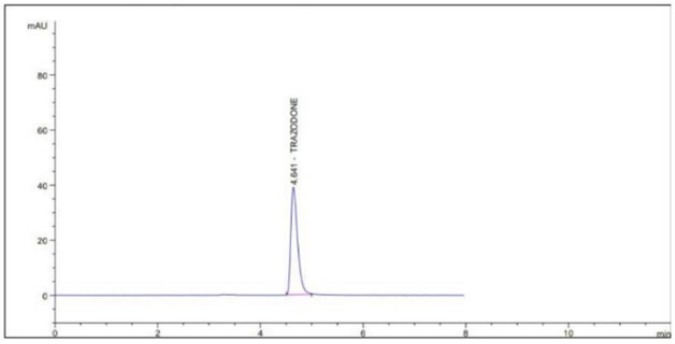

Figure 3: Chromatogram of Standard Trazodone.

Sample preparation

The mobile phase of MEOH + 0, 1% (OPA) water, PH =3.0, water in proportion of 60:40v/v) flow rate 1.0 ml gave adequate retention at 4.641min [3]. (Figure 3)

Method Validation

Precision

Intraday and interday precision for trazodone which shows the high precision % amount in between 5% to 30% indicates to analytical method that concluded [4]. (Figure 4) (Table 2, 3)

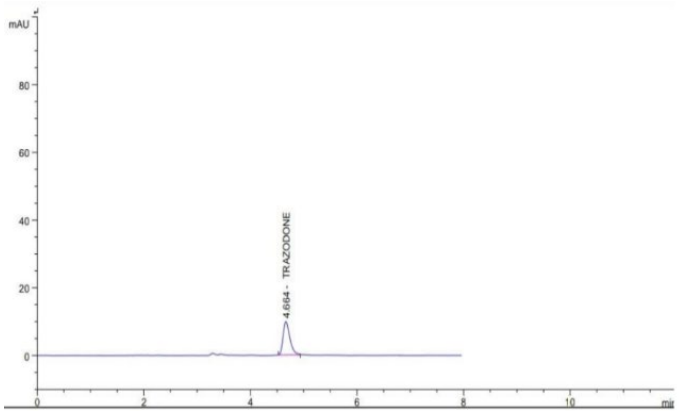

Figure 4: Chromatography of Precision.

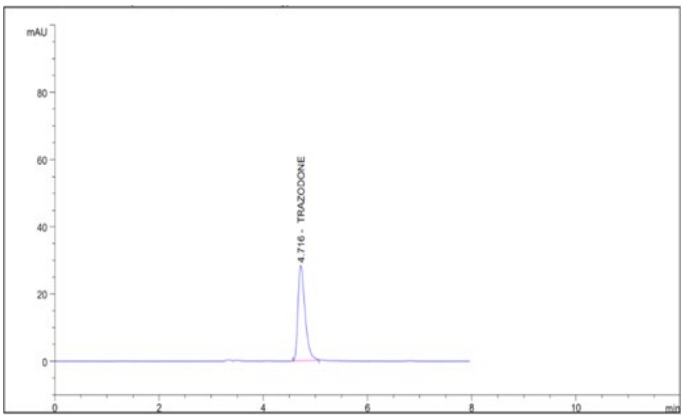

Figure 5: Chromatogram of Accuracy.

Table 2: Result of Intraday and Inter day Precision for Trazodone.

Drug	Conc n(µg/ml)	Intraday Precision			Inter day Precision		
		Mean± SD	%Amt Found	%RSD	Mean± SD	%Amt Found	%RSD
TRZ	5	82.01±0.63	5.14	0.76	80.83±0.97	5.08	1.20
	15	279.62±0.35	14.96	0.13	267.33±1.15	15.06	0.43
	25	465.82±5.31	25.68	1.14	463.77±3.46	25.57	0.75

Table 3: Result of Intraday and Inter day Precision for UV.

METHOD	Drug	Conc (µg/ml)	Interday Precision		Intraday Precision	
			Mean± SD	%Amt Found	Mean± SD	%Amt Found
UV	Trazodone HCL	20	0.2558±0.96	101.54	0.2585±0.96	102.40
		30	0.4084±1.26	66.77	0.4016±0.00	99.84
		40	513.1957±1.26	73.88	0.5492±0.06	73.13

Table 4: Result of Recovery Data for Trazodone.

Drug	Sr no	Level (%)	Amt taken (µg/ml)	Amt added (µg/ml)	Amt . found ±S.D	Amt recovered mean ±S.D	% recovery mean ±S.D
TRZ	1	80%	5	4	9.9 ± 0.038	4.09±0.038	102.23 ± 0.94
	2	100%	5	5	10.03 ±0.042	5.03 ± 0.042	100.58 ± 0. 83
	3	120%	5	6	11.08 ±0.015	6.08 ± 0.015	101.33 ± 0.25

Table 5: Recovery Studies Trazodone for UV method.

METHOD	Level (%)	Amt. taken (ug/ml	Amt. Added (ug/ml	Absorbance Mean* ± S.D.	Amt. recovered Mean *±S.D.	%Recovery Mean *± S.D.
	80%	10	8	17.92 ±0.04	7.92±0.04	98.95 ±0.47
UV	100%	10	10	19.82 ±0.02	20.58±0.02	98.18 ±0.25
	120%	10	12	21.94 ±0.02	11.94±0.02	99.97 ±0.13

Accuracy

Average recoveries studies preformat different level of concentration (80%, 100 %, 120%) the 5 recovery was formed to be within the limit 98 % to 100 %, the method is accurate [5]. (Table 4, 5)

Linearity

Linearity of trazodone was observed in the range of 5-25µg/ml, and UV 10-50µg/ml, detection wavelength used was 249 nm the calibration curve yielded correlation coefficient (r2) 0.9992 and 0.9992 for trazodone respectively [6]. (Figure 5) (Table 6, 7)

Table 6: Linearity of Trazodone.

Sr no	concentration (µg/mg)	Area of trazodone
1	5	80.9380
2	10	175.8961
3	15	262.8816
4	20	355.2099
5	25	458.8400

Table 7: Linearity of UV.

Sr. No.	CONC.	AVG ABS
1	10	0.1125
2	20	0.2667
3	30	0.4087
4	40	0.5202
5	50	0.6916

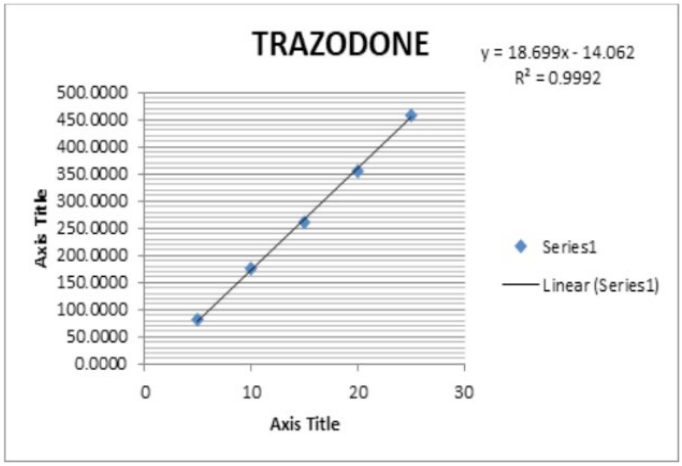

Figure 6: Calibration Curve of Trazodone.

Repeatability

Repeatability studies trazodone was found to be, the %RSD was less than 2, which shows high percentage amount found in between 100% to 102% indicates the analytical method that concluded [7]. (Figure 6) (Table 8)

LOD and LOQ

• The LOD of trazadone was founded to be 0.1605 (µg/ml) analytical methods concluded.

Table 8: Repeatability Studies on Trazodone.

Sr no	Concentration of trazodone (mg/ml)	Peak area	Amount found (mg)	% amount found
1	15	270.268	15.18	101.18
2	15	268.910	15.18	101.18
3	Mean	269.59	15.18	101.18
4	SD	0.02	0.02	0.02
5	%RSD	0.20	0.20	0.20

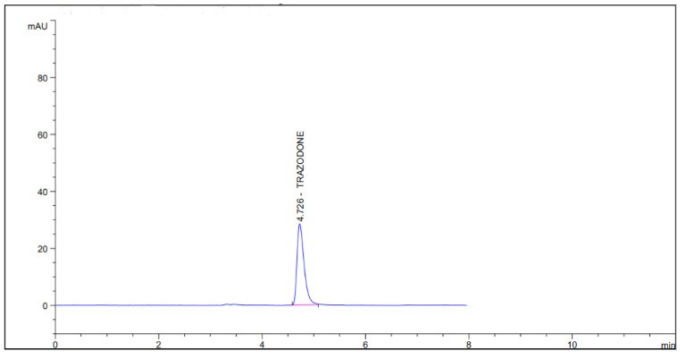

Figure 7: Calibration Curve of Trazodone.

Table 9: Result of robustness study of trazodone.

Parameters	Conc.(µg/ml)	Area (mean +SD)	%RSD
MP composition(59ml+41ml) Methanol + 0.1%water with OPA	15	342.9188+0.41953	0.12
MP composition(61ml+39ml) Methanol + 0.1% (OPA)water with OPA	15	343.89+02+0.94	0.27
Wavelength change 248nm	15	371.4+0.25	0.07
Wavelength Change 250nm	15	311,48+1.73	0.56
Flow rate change(0.6ml)	15	392.89+2.29	0.12
Flow rate change(0.8ml)	15	298.09+0.33	0.11

• The LOQ of trazodone was found to be 0.4866 (µg/ml) analytical methods that concluded.

Robustness

The changes were did flow rate (1ml/min -1) PH of mobile phase composition and wavelength % RSD for peak area calculated which should be less than 2% the result shown analytical method concluded [8]. (Figure 7) (Table 9)

Conclusion

The presented project work was planned to developed simple, accurate, precise, and cost effective UHPLC method or quantification of TRAZADONE HCL by ASSAY METHOD in husk as API and to explore its applicability for marked formulation. The above study gives the analysis of trazodone hydrochloride by using UHPLC methods and UV spectroscopic methods. Fig 1, Chemical Structure of Trazodon.

The proposed method was highly sensitive, reproducible, reliable, rapid, robust and specific therefore, this method can be employed in quality control to estimate the amount of trazodone, hydrochloride in bulk and in pharmaceutical dosage forms.

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