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VALIDATED RP-HPLC METHOD FOR THE ESTIMATION OF TRAZODONE HYDROCHLORIDE IN MARKETED FORMULATION

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ABSTRACT

The present paper deals with the development of a rapid and feasible reveres phase high-performance liquid chromatographic method for the determination of trazodone in bulk and pharmaceutical dosage form. Chromatographic separation achieved isocratically on thermo C18 column (5 µm, 150mm x 4.60mm) and acetonitrile: methanol in the ratio of 50:50 (v/v) as the mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 256 nm. The retention times for TRZ was found to be 3.53 ±0.5 min. Parameters such as linearity, precision, accuracy, recovery, specificity and ruggedness are studied as reported in the ICH guidelines. The method was linear in the concentration range of 5-25 µg/ml with correlation coefficient of 0.999. The mean recoveries obtained for TRZ 99.78 % and RSD was less than 2. The correlation coefficients for all components are close to 1. Developed method was found to be accurate, precise, selective and rapid for estimation of TRZ.

K**eywords:** RP-HPLC, trazodone, Method development, Validation

INTRODUCTION

Trazodone (2-[3-[4-(3-chlorophenyl)-1-piperazinyl] propyl]-1, 2, 4-triazole [4, 3-a] pyridin-3(2*H*)-one, TRZ, Fig. 1) is a weak inhibitor of monoamine reuptake and its major mechanism of action seems to be the antagonism at serotonin 5-HT2/5-HT1C receptors [1]. TRZ is used for the treatment of major depression, sometimes in conjunction with selective serotonin reuptake inhibitors (SSRIs), like fluoxetine [2], or to control sleep disturbance symptoms when using serotonin and norepinephrine reuptake inhibitors (SNRIs) [3].TRZ is commercially available as tablets, long-acting tablets, oral solutions and solutions for injection [4]. Treatment should be started with a dose of 25-50mg daily, which may be increased slowly to a maximum of 300mg daily in ambulatory patients or to 600mg daily in hospitalised patients. TRZ is mainly metabolised in the liver by the cytochrome isoform CYP3A4. The most important metabolite thus formed is 3-(1- clorophenyl) piperazine[5], which is a serotonergic agonist with a long half-life [6]. Plasma levels in patients treated with TRZ at therapeutic doses usually range between 130ng/ml and 2µg/ml for the parent drug [7].

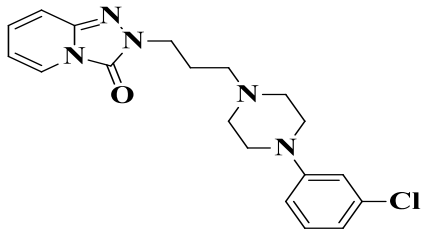


Figure 1 Chemical structure of trazodone

The main side effects associated with TRZ administration are: nausea, insomnia, agitation, dry mouth, constipation, headache, hypotension, blurred vision and confusion [8]. The drug was first synthesized in 1966, has minimal anticholinergic and cardiovascular effects, and a marked sedative action [9]. Analytical methods for the determination of trazodone in serum include spectroflurometry [10], gas chromatography with flame-ionization, nitrogen-phosphorous selective or mass spectrographic detection [11-14]. HPLC method with ultraviolet, fluorescence or electrochemical oxidation detection [15-20] and UV [21-25] methods.

These methods suffer certain limitations including:

a) The use of a lengthy extraction procedure
b) The addition of the internal standard after the extraction step is completed
c) The use of toxic or highly flammable extraction solvents
d) A limited linear concentration range or a poor limit of detection

In the present work, we are therefore focused on to achieve the optimum chromatographic conditions for the determination of TRZ in a formulation. The developed method could be applied to quality control of the tablet dosage form. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH guidelines, [26] which are mandatory also.

Materials and Methods

Instrument

Liquid chromatographic system from Waters model no 784 comprising of manual injector, water 515 binary pump for constant flow and constant pressure delivery and UV-Visible detector connected to software Data Ace for controlling the instrumentation as well as processing the generated data.

Reagents and chemicals

Trazodone HCl were obtained as pure sample from Sun Pharmaceutical Industries Ltd. Dewas, as gift samples along with their analytical reports. HPLC grade methanol and acetonitrile was obtained from Merck (India) limited. All other chemical used were of analytical grade. Triple distilled water was used

for whole experiment was generated in house. Tablet Trazodep 50mg Torrent Lab. Pvt. Ltd. Ahmedabad, India was purchased from local market.

Chromatographic conditions

The isocratic mobile phase consisted of Methanol: Acetonitrile in the ratio of (50:50 v/v), flowing through the column at a constant flow rate of 1.0 ml/ min. The mobile phase was filtered through nylon 0.22 µm membrane filters and was degassed before use (30 min). A Thermo (C-18) Column (5 µm, 250mm x 4.60mm) was used as the stationary phase. By considering the chromatographic parameter, sensitivity and selectivity of method for drugs, 256 nm was selected as the detection wavelength for UV-Visible detector.

Standard preparation

Standard stock solution

Accurately weighed 10 mg of Trazodone was transferred into 10 ml volumetric flask, dissolved in 5ml of methanol and volume was made up to 10ml with methanol to get concentration of solution 1000 µg/ml (Stock-A), 5ml of stock-A was taken and diluted up to 50ml to get concentration of 100µg/ml (Stock-B).

Working standard solution

Working standard solutions were prepared by taking dilutions ranging from 5, 10,15,20,25 µg/ml for TRZ.

Sample preparation

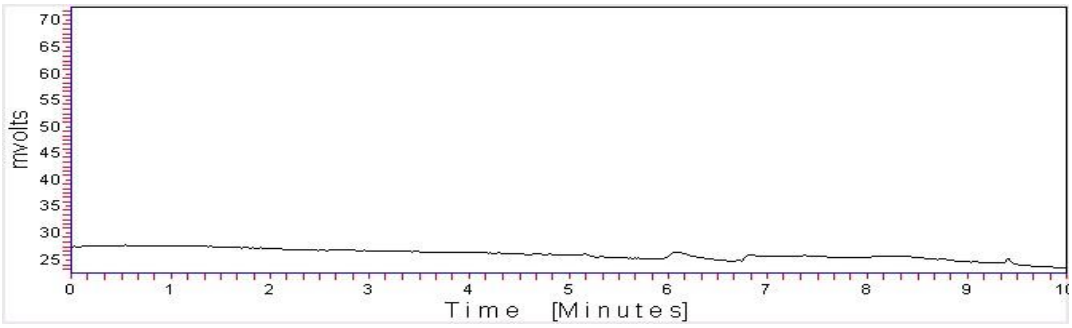
Commercial formulations trazodone of trazodep was selected for analysis. Twenty tablets of trazodep were weighed and powdered separately. Weight equivalent to 50 mg Trazodone was dissolved in 50 ml diluents and then sonicated for 15min. and filtered through watmann paper no. 41. Then different concentration of solution were prepared by serial dilution technique, as per standard and each dilution was analyzed.

Results and discussion

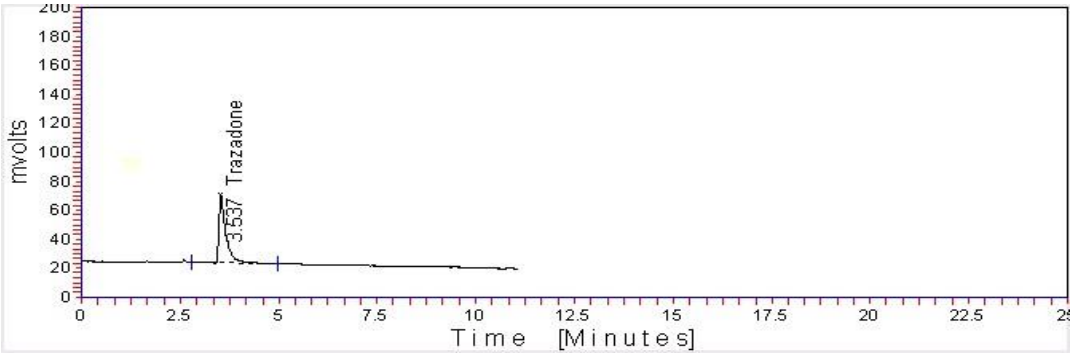
Chromatography

The mobile phase was chosen after several trials with methanol, isopropyl alcohol, acetonitrile, water, and buffer solutions in various proportions and at different pH values. A mobile phase consisting of acetonitrile/ methanol (50:50, v/v) was selected to achieve maximum separation and sensitivity. Flow rates between 0.5 and 1.5 min were studied. A flow rate of 1 ml/min gave an optimal signal-to-noise ratio

with a reasonable separation time. Using a reversed-phase C18 column, the retention times for TRZ was observed to be 3.53± 0.05 min. Total time of analysis was less than 6 min. The maximum absorption of TRZ was detected at 256 nm, and this wavelength was chosen for the analysis Fig. 2.



(A)



(B)

Figure 2 Chromatograms of (A) Blank mobile phase (B) TRZ (15µg/ml) as reference substances

System suitability

System suitability parameters such as number of theoretical plates, HETP, and peak tailing are determined. The results obtained are shown in Table 1. The number of theoretical plates for TRZ was 2948.54.

Table 1 Results of system suitability parameters

Parameters	Trazodone
AUC*	391.2
No. of Theoretical Plates*	2948.54 ± 25.05
Tailing Factor*	1.125
Retention time*	3.752
HETP*	0.252
Calibration range (µg/ml)	5-25

*Each value is the mean ± SD of six determinations

Linearity

The calibration curve was linear over the concentration range of 5-25 µg/ml for TRZ. The linearity was represented by a linear regression equation as follows:

Y (TRZ) = 25.97conc. + 3.857 (*r*2 = 0.999)

Accuracy

Method accuracy was performed by adding known amounts of TRZ to the preanalysed synthetic mixture solution and then comparing the added concentration with the found concentration. Three levels of solutions were made which correspond to 80%, 100%, and 120% of the nominal analytical concentration (10µg/ml for TRZ). Each level was made in triplicate table 2. The mean percentage recoveries obtained for TRZ was 99.78%, respectively, and RSD was less than 2.

Table 2 Results of recovery study

Statistical data	**Trazodone**		
	80%	**100%**	**120%**
% Mean*	**99.62**	**99.71**	**99.784**
SD*	**0.084**	**0.091**	**0.170**
%R.S.D*	**0.084**	**0.092**	**0.170**

*Mean of nine determinations (three replicates at three concentration level)

Precision

Repeatability

Five dilutions in three replicates were analyzed in the same day for repeatability and results were found within acceptable limits (RSD < 2) as shown in table 3.

Intermediate precision

Five dilutions in three replicates were analyzed on two different days and by two analysts for day-to-day and analyst-to-analyst variations, and results were found within acceptable limits (RSD < 2) as shown in table 3.

Table 3 Statistical data for precision

Statistical parameter	Trazodone		
	Mean*	S.D*	R.S.D*
Repeatability	98.41	1.076	1.09
Intermediate Precision (I) (A day to day)	98.60	1.10	1.10
(II) Analyst to Analyst	100.62	0.99	0.99
Robustness	98.87	0.64	0.64

***Mean of 15 determinations (three replicates at five concentration level)**

Robustness

As per ICH norms, small, but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was chain from, Methanol: ACN (50:50% V/V), to (55: 45% V/V) and method is found robust as RSD is again found < 2.0 table 3.

Specificity and selectivity

Commonly used excipients were spiked in to a preweighed quantity of drugs. The chromatogram was taken by appropriate dilution and the quantities of drug were determined. The specificity of the HPLC method is illustrated in Fig. 3. Where complete separation of TRZ in presence of tablet excipients.

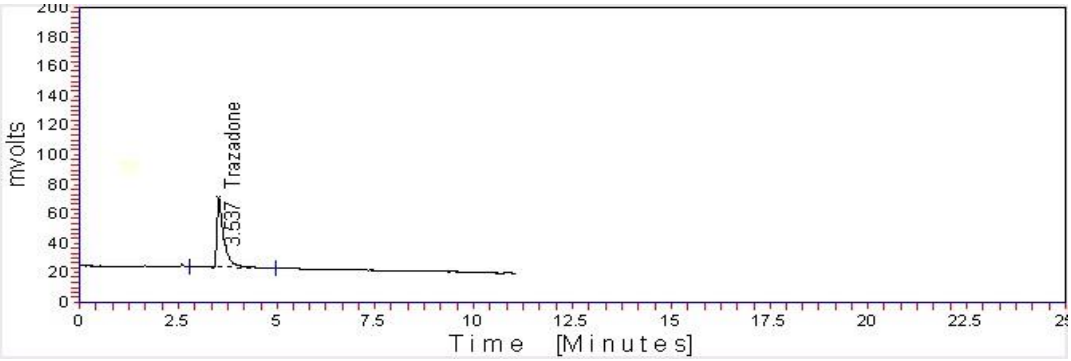


Figure 3 Chromatograms of TRZ (15µg/ml) in a tablet formulation

Analysis of Tablets

The concentration of TRZ in the tablet formulation was found to be 101%. The low values of % RSD indicate that the method is precise and accurate in table 4.

Table 4 Results of tablet analysis

S.NO.	Parameter	Sample
		Trazodone
1	% Found	101.00
2	S.D.	1.08
3	% R.S.D.	1.06
4	SEσ*	0.44

* Mean of nine determinations

CONCLUSION

The proposed HPLC method was validated as per the International Conference on Harmonisation (ICH) Q2B Guidelines, and was found to be applicable for routine quantitative analysis of trazodone by HPLC in pharmaceutical dosage form. The results of linearity, precision, accuracy and specificity, were proved to be within the limits. The method provides selective quantification of trazodone with no interference from other formulation excipients. The proposed method was highly reproducible, reliable, rapid, robust and specific. Therefore, a high percentage of recovery and the run time of less than six minutes allow its application for the routine determination of TRZ in the tablet dosage form.

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