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RESEARCH ARTICLE

RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF TRAMADOL HYDROCHLORIDE IN BULK FORM BY ION-PAIR LIQUID CHROMATOGRAPHY

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ABSTRACT:

A simple, selective, accurate and precise high-performance liquid chromatographic (HPLC) method for estimation of Tramadol HCl in bulk form was developed & validated. The estimation was carried out on Phenomenax luna C-18 (250x4.6 mm,5µ) column using a mobile phase consisting of 20 mM potassium dihydrogen phosphate, 1.75mM 1-octane sulfonic acid sodium salt, 2% isopropanol: Methanol (25:75 v/v) of pH 4.0 adjusted with orthophosphoric acid, at a flow rate 1 ml/min. The UV detection was carried out at 272 nm. Method validation was performed as per the ICH guidelines. The method was validated for the precision, accuracy, linearity and robustness.

Key words: RP-HPLC estimation, Validation and Tramadol.

INTRODUCTION:

IUPAC name of Tramadol Hydrochloride is (lRS,2RS)-2-[(dimethylamino)methyl]- 1-(3 methoxyphenyl) cyclohexanol hydrochloride. Tramadol is a synthetic codeine analogue that is a weak µ-opioid receptor agonist. It is used as an oral non-steroidal anti-inflammatory drug with good analgesic and tolerability profile in various painful conditions.[1] In literature survey several methods like spectrophotometry[2-7], HPTLC[8-9] and HPLC[10-14] are reported for estimation of Tramadol HCl either alone or with other drugs.

Tramadol HCl is an official drug in Indian Pharmacopoeia 2010[15], British Pharmacopoeia 2009[16] and United State Pharmacopoeia[17]. Most of the methods were on estimation of Tramadol HCl in biological samples. As per literature survey there is no method for the estimation of Tramadol HCl by ion pair liquid chromatography. The use of ion pair reagent in the estimation of Tramadol with comparatively more hydrophobic conditions enhanced the scope of the work in simultaneous estimation of Tramadol HCl with those drugs, which are more hydrophobic in nature. The developed method is fast, simple, precise and reliable method for routine analysis. The present RP-HPLC method was validated as per the ICH guidelines.

MATERIAL AND METHODS:

Reagents and Chemicals: HPLC grade Methanol, Triple distilled water, Potassium dihydrogen phosphate, 1-Octane sulfonic acid sodium salt and Isopropanol were used in the study.

Chromatographic conditions: Shimadzu prominence UFLC 2000 equipped with SPD-20A UV detector and Phenomenax Luna C18 (250×4.6 mm i.d) column. The mobile phase consisting of 20 mM potassium dihydrogen phosphate, 1.75mM 1-octane sulfonic acid sodium salt, 2% isopropanol : Methanol (25:75 v/v) of pH 4.0 adjusted with orthophosphoric acid.

Preparation of standard solution:

Preparation of stock solution of TRA: Weigh accurately 10 mg of TRA and transferred into 10 ml volumetric flask add 5ml of methanol and sonicated for 10 min and diluted up to mark with methanol to get a stock solution having strength 1000 µg/ml.

Preparation of working Standard solution of TRA: 100 µg/ml solution of TRA was prepared by diluting 1ml stock solution to 10 ml with methanol and further diluted with methanol to get the concentration range of 10, 20, 30, 40, 50 µg/ml of TRA.

Method Validation: The developed method was validated for linearity and range, accuracy, precision, Limit of detection, Limit of quantitation and robustness as per ICH guidelines[18].

RESULT AND DISCUSSION:

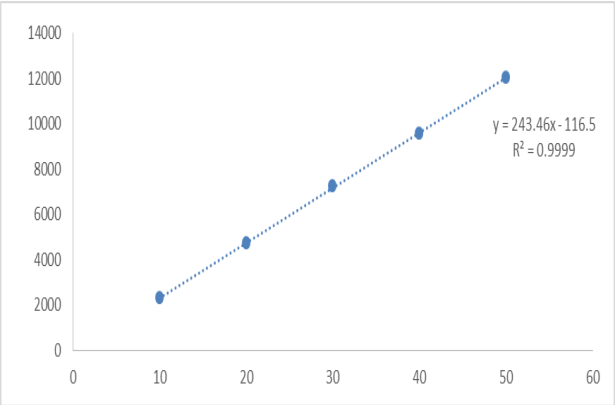

Figure 1: Calibration curve of Tramadol HCl

Linearity: Linearity of the method was evaluated by constructing calibration curves at five concentration levels over a range of 10-50 µg/ml. The calibration curve was linear and regression equation found to be y =

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243.6x – 116.5 with correlation coefficient (r^2) 0.999 as shown in fig. 1.

Inter-day and intra-day precision: The inter-day precision study was performed on three different days i.e. day1, day2 and day3 at three different concentration levels, (n=3). The intra-day precision study was performed on the same day at 3 intervals of time at three different concentration levels (n=3). The % RSD of the inter-day precision was found to be 1.91 % and intra-day precision was found to be 1.34 % (Table1).

Table1: Intra-day and inter-day precision studies of Tramadol Hydrochloride

Sr. No.	Concentration (µg/ml)	Intra-day precision*	% RSD*	Inter-day precision*	% RSD*
		Mean peak area ±SD		Mean peak area ±SD	
1	20	4724.66 ± 94.22	1.99	4643.66 ± 37.03	0.79
2	30	7261.33 ± 31.00	0.42	7204.33 ± 97.72	1.35
3	40	9573.66 ± 109.52	1.14	9895.00 ± 20.70	0.20

Mean of three replicates (n=3)

Accuracy: The accuracy of the method was evaluated triplicate at three concentration levels (80, 100 and 120%), and the percentage recoveries were calculated. The recovery% in accuracy study was found to be between 98-102% and %RSD was less than 2% (Table 2).

Table 2: Accuracy–recovery study of Tramadol Hydrochloride by standard-addition method

Sr. No.	Amt. of Sample(µg/ml)	Spiked Concentration (µg/ml)	Recovery* (%)	% RSD*
1	30	80%	98.40%	0.41
2	30	100%	98.19%	
3	30	120%	102.04%	

Mean of three replicates (n=3)

Robustness: The robustness of method was performed by small changes in flow rate (0.9 and 1.1 ml/min.). Robustness was studied using three replicates of concentration level at 100%. The % RSD in robustness study was less than 2%, his indicates that the method is precise, accurate and robust.

LOQ and LOD: The LOQ and LOD were based on the standard deviation of the response and the slope of the constructed calibration curve (n=3), as described in International Conference on Harmonization guidelines Q2 (R1). The LOD was found to be 0.377 µg/ml, and the value LOQ was found to be 1.144 µg/ml.

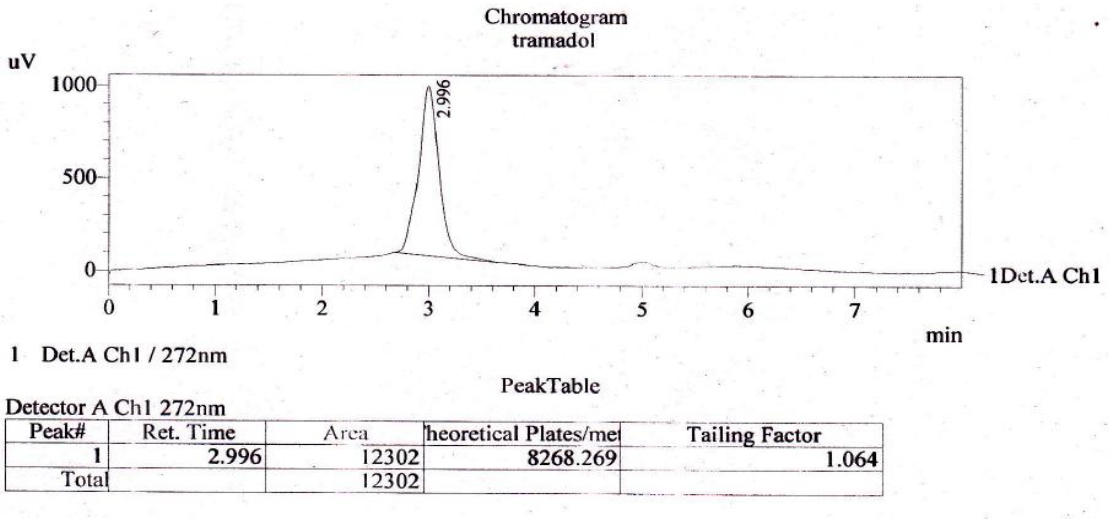

Figure 2: Chromatogram of Tramadol HCl

CONCLUSION:

The developed method was novel, simple, accurate, precise and reproducible, which would be used to estimate Tramadol HCl in bulk form for routine analysis. This method may also be useful for the simultaneous estimation of Tramadol HCl with other hydrophobic drugs.

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