



# Analytical Method Development and Validation of Tramadol Hydrochloride by Pharmaceutical Dosage Form by Ultraviolet Spectroscopy

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## Abstract

Tramadol could be a painkiller just like an opioid. It acts within the central system (CNS) to alleviate pain. Tramadol is employed to treat moderate to severe pain in adults widely used for clinical purposes. During this respect, we aimed to develop an easy and economic UV spectrophotometric method for estimation of Tramadol Hydrochloride in bulk, injection, and tablet dosage form and validate as per ICH guidelines. Analytical research and development may be a process that assures quality attributes and internal control of the products. Tramadol shows the most absorbance at wavelength 270nm. Tramadol showed the linearity range 20-160 $\mu$ g per ml for this procedure with correlation ( $R^2$ ) was 0.9998. The current methods were found to be simple, linear, precise, accurate, and sensitive and may be used for routine internal control analysis for the estimation of Tramadol Hydrochloride bulk and tablet dosage form.

**Keywords:** Validation; Tramadol; 0.1N HCL; UV spectrophotometric

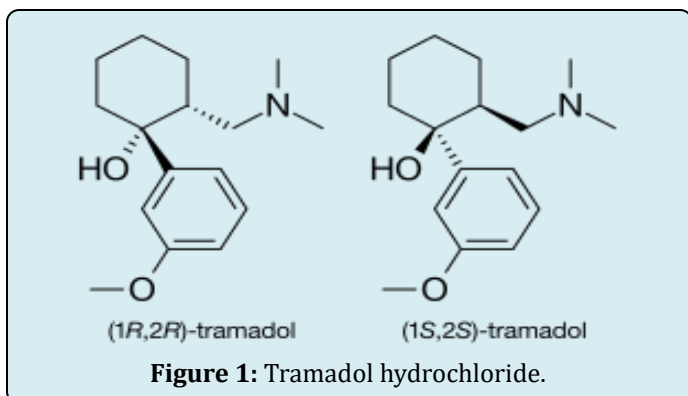
## Introduction

Tramadol hydrochloride first time was discovered and synthesized in 1962 by German company (Grünenthal GmbH) for the treatment of pain. After a protracted gap this drug was introduced into the market with the Tramadol name in 1977 [1]. Tramadol is 2-(dimethyl amino)-methyl)-1-(3'-methoxyphenyl) cyclohexanol hydrochloride. Its formula is  $C_{16}H_{25}NO_2$  with a relative molecular mass of 299.8 and contains a pKa of 9.41 [2]. It's 4-phenyl-piperidine analogue of opioid drug codeine. In US it absolutely was made available after 1995 [3]. It's available under brand name ULTRAM® [4].

It is a centrally acting synthetic analgesic accustomed to treat moderate to moderately severe pain. The drug incorporates a wide selection of applications, including treatment

of arthritis, syndrome, motor neurone disease and fibromyalgia [5,6]. Tramadol hydrochloride may be a centrally acting synthetic opioid analgesic binding to specific opioid receptors. It's a non-selective, pure opioid agonist at  $\mu$ , delta and kappa opioid receptors with a better affinity for  $\mu$  receptors [7,8].

There are different analytical procedures for the determination of Tramadol hydrochloride are stated in the literature supported UV spectrophotometry, RP-UPLC and LC-MS alone or together with other drugs [9-20]. Hence the most objective of this work is to present most accurate, precise and sensitive analytical method and its validation is also further used for determination of Tramadol during routine pilot manufacturing in bulk and pharmaceutical dosage form as per ICH guidelines. The chemical structure of Tramadol hydrochloride is shown in figure 1.



## Materials and Methods

### Materials

Tramadol Active Pharmaceutical Ingredient was provided as gift tester by Windlass Biotech Dehradun Uttarakhand. Tramadol (Marketed formulation) by Conserr Pharma limited.

### Instruments

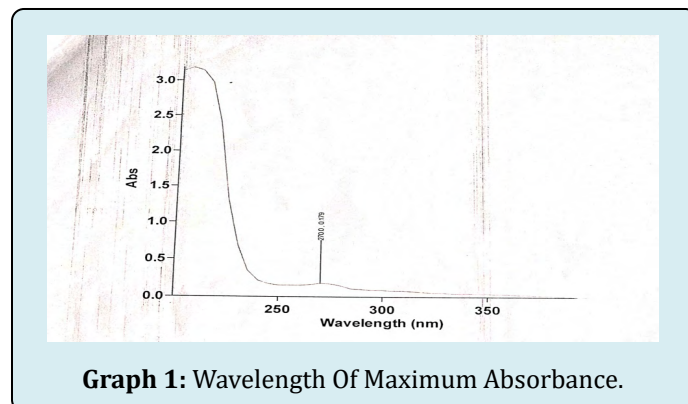
The Ultra Violet-Spectroscopy was conceded out with a Cary 60 Single Beam UV spectrometer manufacturer by Agilent Tech, Digital Weight Balance: TX323L, Shimadzu was used.

### Preparation of Standard Stock Solution of Tramadol

Accurately weigh about 50mg of the drug and transferred to 50ml of volumetric flask and dissolved it in 50ml of 0.1N HCl. Then volume was made up to the mark with 0.1N HCl. The 10ml of previously set solution was diluted with 50ml of HCl. This standard solution contained 100 $\mu$ g of drug per ml.

### Determination of wavelength of maximum absorbance ( $\lambda$ max)

1ml of standard stock solution was pipette out and transferred to a 10ml of volumetric flask. The volume was made up to the mark with 0.1N HCl. The solution contained 100 $\mu$ g/ml of the drug. Then 1ml of the solution is taken in a 10ml volumetric flask was added to it then volume was made up to the mark with 0.1N HCl. This solution contains 10 $\mu$ g/ml of the drug. The absorbance of this solution was scanned in the range of 200-400nm against 0.1N HCl as a blank.



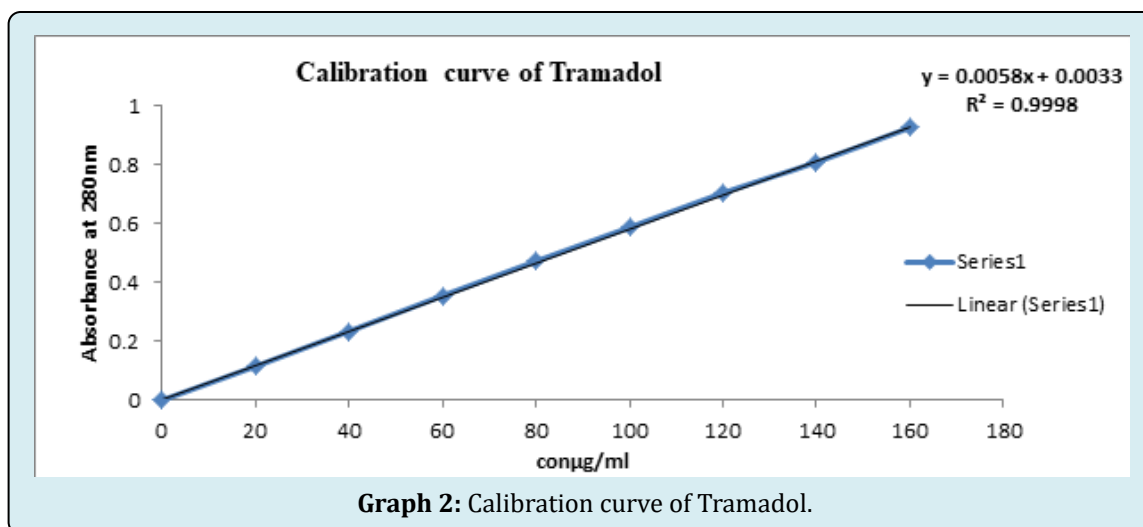
### Preparation of calibration curve for Tramadol at 270nm

1,2,3,4,5,6,7 and 8ml standard stock solution (200 $\mu$ g/ml) was pipette out into a series of 10ml volumetric flask. Then the volumes were made up to the mark with 0.1N HCl and mixed to obtain the solutions in the concentration range of 20, 40, 60, 80, 100, 120, 140, 160 $\mu$ g/ml of drug.

The absorbance of these resultant solutions were measured at 270nm against 0.1N HCl as a blank and graph was plotted between absorbance obtained and the concentration of the solution. (Table1).

Concentration ( $\mu$ g/ml)	Absorption			Mean	E1%	Absorptivity	Molar Absorptivity
	A1	A2	A3				
0	0	0	0	0			
20	0.1152	0.1150	0.1151	0.1151	57.55	5.755	1515.867
40	0.2340	0.2328	0.2362	0.2340	58.5	5.85	1540.89
60	0.3545	0.3545	0.3548	0.3546	59.1	5.91	1556.694
80	0.4718	0.4721	0.4716	0.4718	58.97	5.897	1553.2698
100	0.5862	0.5857	0.5863	0.5861	58.61	5.861	1543.7874
120	0.7036	0.7038	0.7028	0.7032	58.6	5.86	1543.524
140	0.8081	0.8074	0.8076	0.8077	57.69	5.769	1519.5546
160	0.9241	0.9246	0.9254	0.9247	57.79	5.779	1522.1886

**Table1:** Linearity, Range, E 1% 1CM, Absorptivity (L gm<sup>-1</sup>cm<sup>-1</sup>), and Molar Absorptivity (L mol<sup>-1</sup>cm<sup>-1</sup>).



### Repeatability

Pipetted out 1ml of standard solution shifted into a series of nine 10ml analytical flask and diluted with 0.1N HCl

to get the concentration of 20µg/ml. Optical density of the resultant solutions was dignified at 270nm 0.1N HCl used as a blank. The results were obtained and concise in the (Table 2).

Repeatability					
Nominal Con µg/ml	Absorbance	Observed Con (µg/ml)	Mean Con µg/ml	SD	% RSD
20	0.1111	17.2	17.2	0.00288	0.01676
20	0.1156	18.0			
20	0.1097	17.0			
20	0.1113	17.3			
20	0.1067	16.4			
20	0.1112	17.2			

**Table 2:** Study of Repeatability.

### Intra-Day Precision

Pipette outs 2.5, 5, 7.5ml working solutions were transferred into separate 10ml analytical flasks and diluted with 0.1N HCl to get the concentration 50,100,150µg/ml.

Absorbance of the subsequent solutions was measured at 270nm 0.1N HCl used as a blank. Such six repetitions were performed with in a day 0, 3 and 6 hrs interval. The result was summarized in the (Table3). The % RSD was less than 2%.

Intra-Day Precision									
Nominal Con µg/ml	Absorbance			Observed Conc. (µg/ml)			Mean Conc. (µg/ml)	SD	%RSD
	0 hr	3hr	6hr	0hr	3hr	6hr			
50	0.2710	0.2693	0.2642	45.5	45.2	44.3	45.0	0.624500	1.387777
100	0.5324	0.5431	0.5403	91.8	93.7	93.2	92.9	0.984886	1.060157
150	0.8124	0.8135	0.812	141.3	141.5	141.3	141.4	0.115470	0.081681
							<b>Mean</b>		<b>0.843205</b>

**Table 3:** Study of Intra-Day Precision.

### Inter- Day Precision

Pipette out 2.5, 5, 7.5ml working solutions were transferred into separate 10ml analytical flasks and diluted with 0.1N HCl to get the concentration 50,100,150µg/ml. Absorbance of the subsequent solutions was measured

at 270nm 0.1N HCl used as a blank. Such six studies were performed for day one two day three intervals. The result was summarized in the (Table4).The % RSD was less than 2%.

Inter-Day Precision									
Nominal Conc µg/ml	Absorbance			Observed Conc. (µg/ml)			Mean Conc. (µg/ml)	SD	%RSD
	0 hr	24hr	48hr	0hr	24hr	48hr			
50	0.2898	0.2843	0.2735	48.5	48.3	47.7	48.2	0.416333	0.86436
100	0.5693	0.5745	0.5533	99.6	99.2	98.8	99.2	0.400000	0.403226
150	0.8560	0.8572	0.8463	149.6	149.3	148.9	149.3	0.351188	0.235276
								<b>Mean</b>	<b>0.500954</b>

**Table 4:** Study of Inter-Day Precision.

### Accuracy

The accuracy was assessed by the standard addition method of three replicate determinations of three different solutions containing 80,100,120µg/ml of Tramadol Hydrochloride. The average % recoveries for three different

concentrations was found to be 99.79 using proposed UV spectrophotometric method. The higher values indicated that the proposed UV spectrophotometric method was accurate for the determination of Tramadol Hydrochloride in pharmaceutical dosage form. Results of recovery studies are summarized in (Table 5).

Accuracy				
Recovery	Nominal Conc. (µg/ml)	Absorbance	Observed conc. (µg/ml)	% Recovery
80%	90=50+40	0.5043	89.8	99.78
80%	90=50+40	0.5203	89.9	99.89
80%	90=50+40	0.5248	90.0	100.00
100%	100=50+50	0.6096	99.7	99.70
100%	100=50+50	0.5866	99.9	99.90
100%	100=50+50	0.5868	99.9	99.90
120%	110=50+60	0.6450	109.2	99.27
120%	110=50+60	0.6523	109.7	99.73
120%	110=50+60	0.6449	109.9	99.91
		<b>Mean</b>		<b>99.79</b>

**Table 5:** Accuracy.

### Specificity

Specificity study was carried out by observing any interference in absorbance of drug in the existence of conjoint excipients like Starch, Talc, Lactose, Magnesium

Stearate etc. Absorbance of 100µg/ml drug solution with and without excipients was measured at 270nm. The results obtained were summarized in the (Table 6).

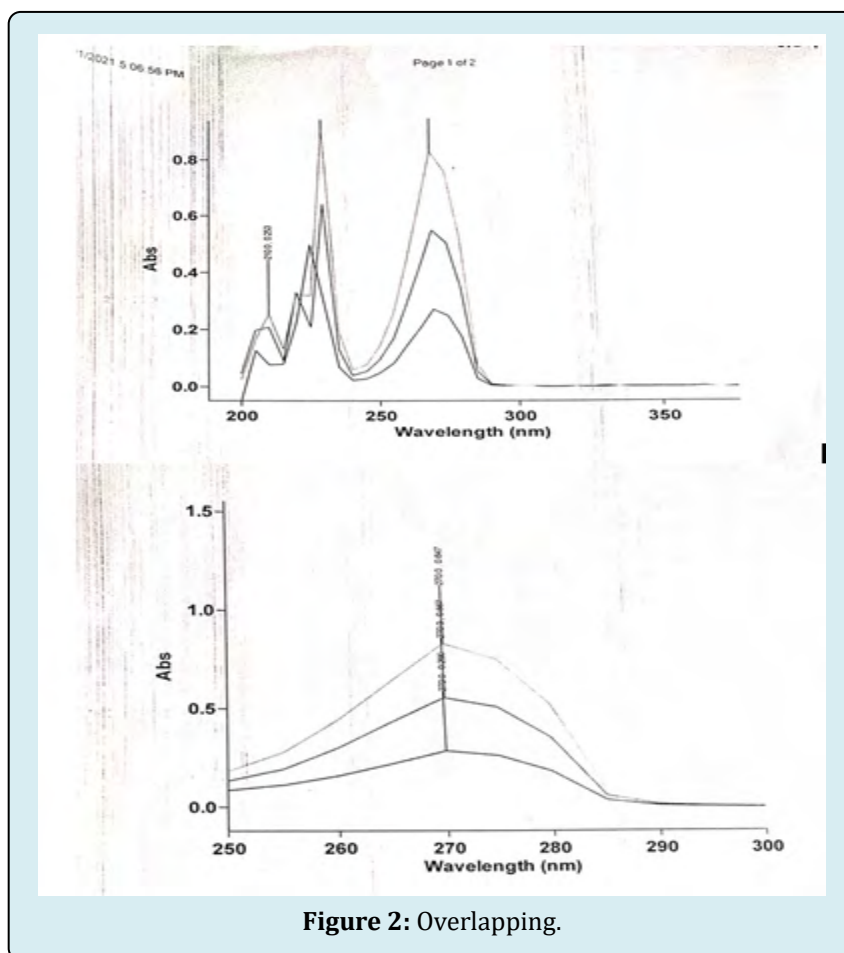
Specificity					
Nominal con( $\mu\text{g/ml}$ )	Without Excipients		With Excipients		% Interference
	Absorbance	Observed Conc. ( $\mu\text{g/ml}$ )	Absorbance	Observed Conc. ( $\mu\text{g/ml}$ )	
100	0.5855	101.2	0.5568	96.1	0.95
100	0.5822	100.6	0.5796	100.1	1.00
100	0.5815	100.5	0.5721	98.8	0.98
100	0.5600	96.7	0.5562	96.0	0.99
100	0.5612	96.9	0.5717	98.7	1.02
				Mean	0.987811

**Table 6:** Study of Specificity.

### % Assay of Tramadol tablets two different brands (TRD-contin 100mg, Tramacon-™ SR-100mg)

Taken 5 tablets and weighed accurately for calculated the average weight. The tablets were powdered and amount of powdered containing 100 mg of Tramadol was transferred into 100ml of volumetric analytical flasks and dissolve

in 50ml of 0.1N HCl and then sonicate the solution after 20minutes make up the volume up to the mark with 0.1N HCl and then filter the solution. Then take 10ml of filtered solution in 100ml of volumetric analytical flasks and make up the volume up to the mark with 0.1N HCl. The absorbance of this resultant solution was estimated at 270nm (Table 7 and Table 8).



**Figure 2:** Overlapping.

Sr. No	Absorbance	Conc.(µg/ml)	Dil. Factor	Content (mg)	Label claim(mg)	%Assay
1	0.5669	97.9	1000	97.9	100	97.9
2	0.5672	97.9	1000	97.7	100	97.7
3	0.5661	97.9	1000	97.6	100	97.6
					Mean	97.73

**Table 7:** Brand A (TRD-Contin 100mg).

Sr. No	Absorbance	Conc.(µg/ml)	Dil. Factor	Content (mg)	Label claim(mg)	%Assay
1	0.6346	102.6	1000	102.60	100	102.6
2	0.6233	101.6	1000	101.90	100	101.9
3	0.6239	101.7	1000	101.70	100	101.7
					Mean	102.06

**Table 8:** Brand B (Tramacon-™ SR-100mg).

## Results and Discussion

The present study describes development and validation of simple and economic UV spectrophotometric method for the estimation of Tramadol Hydrochloride in bulk and tablet dosage form using absorbance maxima method. Solubility studies indicated that a Tramadol Hydrochloride shows better solubility in proposed diluents i.e 0.1N HCl solution the  $\lambda_{max}$  of Tramadol Hydrochloride was found to be 270nm. Because of cost effective and minimal maintenance, the present UV spectrophotometric methods can be preferred at small scale industries as compared to other reported methods.

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