Research Article **CODEN: AJPCFF** **ISSN: 2321 – 0915**

Asian Journal of Phytomedicine and Clinical Research

Journal home page: www.ajpcrjournal.com

https://doi.org/10.36673/AJPCR.2022.v10.i02.A07

A RESEARCH ARTICLE ON ANALYTICAL METHOD VALIDATION OF TAPENTADOL

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ABSTRACT

Objective: To develop a simple, sensitive, specific, and inexpensive HPLC method for measuring of tapentadol hydrochloride in its tablet dosage form. **Method:** The chromatographic separation was achieved by using Waters Spherisorb C18R 150mm x 4.6mm (5µm) and Shimadzu C18 R 150mm x 4.6mm (5µm) column as stationary phase using phosphate buffer pH 2.5 and methanol at 80:20 ratio as mobile phase at a flow rate of 1ml/min. in isocratic mode. The detection of the selected wavelength after scanning the standard solution was found to be 215nm. **Result:** The tapentadol calibration curve was linear from 80% to 120% solution. The value of the correlation coefficient (r^2) was found to be 0.9938. The % recovery of Tapentadol was found in a range of 99.86 – 101.44. Intermediate precision was performed and %RSD was found to be 0.96. The limit of detection of Tapentadol was found to be 0.027346µg/ml and the limit of quantitation was found to be0.4453µg/ml.

KEYWORDS

Tapentadol, HPLC and RSD.

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INTRODUCTION

Tapentadol Hydrochloride [3-[(2*R*, 3*R*)-1-(dimethylamino)-2-methylpentan-3-yl] phenol; Hydrochloride] is a novel opioid analgesic having two mechanisms of action. The first mechanism of action is agonist activity at µ opioid receptor and the second one is norepinephrine reuptake inhibitor. The physical appearance of Tapentadol Hydrochloride is light brown solid. The molecular formula of Tapentadol Hydrochloride is $C_{14}H_{24}ClNO$ having a molecular weight of 257.8, a melting point of 209 – 210 □, and a boiling point of 323.493 □ at 760mmHg.

Tapentadol HCl is used to treat moderate to severe short-term pain. It belongs to a class of drugs known as opioid analgesics. It works in the brain to change how the body feels and responds to pain.

According to the severity of the pain and previous treatment experience, the dosing regimen should be individualized.A single dose of 50mg Tapentadol tablets at the interval of every 4 to 6 hours should be given to patients. Initial higher starting doses might require depending on the severity of pain and also it depends on the patient's previous history of analgesic requirements. On the initiation of dosing, an additional dose can be given as soon as one hour after the initial dose, if the pain continues. The dose should then be standardized individually to an extent that provides sufficient analgesia and minimizes unwanted effects under the close direction of the prescribing physician.Total daily doses higher than 700mg of Tapentadol on the first day of treatment and daily maintenance doses of more than 600mg of Tapentadol have not been studied and are therefore it is not recommended.

Pharmacokinetic Properties

After oral administration Tapentadol is quickly absorbed. The mean absolute bioavailability after single-dose administration in an empty stomach is approximately 32% due to extensive first-pass metabolism. After 1.25 hours of administration of Tapentadol tablets, maximum serum concentrations are observed.

Tapentadol is widely distributed all over the body. After IV administration, the volume of distribution for Tapentadol is 540 ± 98L. The serum protein binding is about 20% which is low protein binding.

It is highly metabolized and about 97% of the core compound is metabolized. The metabolism of Tapentadol takes place by conjugation with glucuronic acid to produce glucuronides.

99% of Tapentadol and its metabolites are excreted through the kidneys.

Side effects

Hives
Chest pain
Fast heartbeats
Difficult breathing
Swelling face, lips, tongue, or throat.

Tapentadol tends to slow or stop breathing, and death may occur. Naloxone can be used or emergency medical attention should be provided if the patient has slow breathing with long pauses, blue-colored lips, or if you are hard to wake up.

Drug–Drug Interactions

Duloxetine, Venlafaxine, Amitriptyline, Desvenlafaxine, and Escitalopram

Tapentadol with these drugs increase the risk of a rare but serious condition called serotonin syndrome (such as: confusion, hallucination, seizure, extreme change in blood pressure, increase heart rate, fever, excessive sweating, shivering or shaking, blurred vision, muscle spasm or stiffness, tremor, incoordination, stomach cramp, nausea, vomiting, and diarrhea).

Pregabalin, Orphenadrine, Oxycodone, Quetiapine, Naloxone and Diazepam

Tapentadol with these drugs causes CNS depression and leads to serious side effects including respiratory distress, coma, and even death.

Topiramate

Tapentadol with Topiramate may increase side effects such as dizziness, drowsiness, confusion, and difficulty concentrating. Elderly people may experience impairment in thinking, judgment, and motor coordination.

Albuterol

Both Tapentadol and Albuterol can increase blood pressure and heart rate and combining them may enhance these effects.

MATERIAL AND METHODS

Chemicals and reagents

Paindol – 50 tablets containing Tapentadol 50mg were manufactured by the FR and D department of Accord Pharmaceuticals Pvt. Ltd. having batch No.R21112. Working standards were developed against a reference standard. HPLC grade Methanol was purchased which was manufactured by Merck, HPLC grade Water which was manufactured by Fischer, and SQ grade Potassium di-hydrogen phosphate was manufactured by HI-MEDIA.

Instrumentation

Two HPLC of the different brands were used for analysis purposes. The first HPLC was of Waters, USA having model E2695 PDAand the second, was of Shimadzu, Japan having model 2030i Prominence.

Chromatographic system

The chromatographic system was isocratic having mobile phase Phosphate buffer pH 2.5 and Methanol in the ratio 80:20. Flow rate was 1ml/min. The column used was C18 R 150mm x 4.6mm (5µm). The detector wavelength was set at 215 nm for monitoring separation. The sample injection volume was 20µL. The peak area was determined using Empower software.

Preparation of reference solution

50mg of Tapentadol hydrochloride working standard was weighed and dissolved in a 100ml volumetric flask using mobile phase and mixed well. Further dilution was done by taking 5ml to 25ml with the mobile phase and filtered using 0.2µm filter paper.

Preparation of test solution

Powdered sample equivalent to 50mg of Tapentadol Hydrochloride was weighed and dissolved in 70ml of mobile phase and was diluted to 100ml with same, mixed well, and filtered with Whatman No.1. Further dilution was done by taking 5ml to 25ml with mobile phase and filtered using 0.2µm filter paper.

Assay

Powder of 20 tablets was prepared by grinding tablets to a fine, uniform particle using a mortar and pestle. The average weight of the tablet was calculated and a sample equivalent to 50mg was weighed and transferred into a 100ml volumetric flask. The mobile phase was used for dissolving and the volume make-up of the solution. The solution was sonicated for 10 minutes and filtered using Whatman No.1 filter paper. A portion of the filtered sample (5ml) was diluted into a 25 ml volumetric flask with mobile phase and mixed well.

Method validation

As per ICH guidelines, the developed method was validated.

Accuracy

The accuracy of an experimental procedure conveys the closeness of positioning linking the value, which is gained either as a usual true value or an accepted equivalent value and the value found, i.e. experimental result. The accuracy of an analytical method is specified by the recovery of the analytical result.

It is performed by either spiking or taking linear concentrations of samples over the range of 80% to 120% of the target concentration with triplicate samples in each concentration.

Precision

Precision is the closeness among analytical outcomes of an analytical method. It expresses within laboratories variations i.e. different analysts on different days and carried out under the same conditions of measurement. It is denoted by relative standard deviation, RSD.

Precision was determined by injecting a series of standards or analyzing a series of samples from multiple samplings from a homogeneous lot. Three analysts performed precision on different days and the result obtained.

Specificity

Specificity was performed to check the interference of the sample component expected to be present in the sample matrix. Initially, a placebo sample was injected and observed for the response. It showed the response of area was 0 and also retention time was 0. After that, 100% standard solution was injected and the response of area was 6902377, and retention time was 4.741 minutes. Similarly, the mixture of placebo with standard using 80% to 120% of placebo solution was injected and all samples showed response as well as retention time similar to the standard solution.

Limit of detection

The Limit of Detection (LOD) is defined as the least concentration of an analyte in a sample solution that can be detected, but not necessarily quantified. Based on the signal-to-noise ratio of 3, the LOD of Tapentadol was determined and was found to be 0.027346µg/ml.

Limit of Quantitation (LOQ)

Quantitation limits based on visual inspection are determined by initiating the minimum level of analyte which can be measured with acceptable accuracy and precision. Quantitation limits based on signal-to-noise ratio can only be applied to procedures that express baseline noise. Based on the signal-to-noise ratio of 10, the LOQ of Tapentadol was determined and was found to be 0.4453µg/ml.

Linearity

The linearity of an analytical procedure is its ability to obtain test results directly proportional to the concentration of analyte in the sample. For performing linearity, a minimum of 5 concentrations were taken. The graph of concentration of analyte vs. response was plotted. The concentration was plotted against the x-axis and the response was plotted against the y-axis.

Linearity for assay of Tapentadol tablet was studied from 80% to 120% of the test concentration and R^2 was found to be 0.9938.

Range

The scope of the analytical method is the interval between the upper and lower limits of the concentration of the object to be analyzed in the sample, where the analytical method has been proven to have a reasonable degree of accuracy and linearity.

Robustness

In the analytical method, robustness is the size range that is unaffected by small but intentional differences in method parameters and provides a signal of reliability in normal use.

The robustness of Tapentadol tablets was performed by changing the flow rate of the mobile phase, changing column temperature, and using different HPLC columns.

At flow rate, 0.5ml/min assay obtained was 100.01%, at flow rate 1ml/min assay obtained was 100.4%, and at the flow rate, 1.5ml/min assay obtained was 99.6%.

At temperature 35□ assay obtained was 99.6%, at temperature 40□ assay obtained was 100.4%, and at temperature 45□ assay obtained was 100.31%

By changing the column, the assay obtained by using the Waters column was 100.28% whereas by using the Shimadzu column assay obtained was 101.1%.

System suitability test

A system suitability test was performed on HPLC systems to determine the accuracy and precision of the system by injecting 6 replicate injections of the solution containing 100% of the analyte.

The theoretical plate count was 26501.7 which is greater than 2000. A tailing factor was reckoned to be 1.56 which is lesser than 2 and % RSD of response was found to be 0.22 which is lesser than 2. Hence, system suitability was found comprehensible provided in the guidelines.

Table No.1: Result of accuracy (recovery)

S.No	Spike level	Sample weight taken (mg)	Recovery	Recovery %
1	80%	80	79.89	99.86
2	90%	90	90.95	101.06
3	100%	100	98.69	98.69
4	110%	110	111.76	101.60
5	120%	120	121.73	101.44

Table No.2: Result of precision standard run

S.No	Chromatogram	Analyst-I (day-1)		Analyst-II (day-2)		Analyst-III (day-3)	
		Area	RT	Area	RT	Area	RT
1	Chromatogram 1	6632960	4.723	6558483	4.719	6870462	4.707
2	Chromatogram 2	6626090	4.725	6559451	4.719	6869201	4.707
3	Chromatogram 3	6639685	4.725	6555715	4.716	6870486	4.703
4	Chromatogram 4	6636800	4.722	6543803	4.713	6874750	4.694
5	Chromatogram 5	6636052	4.721	6562052	4.712	6834623	4.691
6	Chromatogram 6	6631706	4.723	6552064	4.713	6859147	4.688

S.No	Description	Area	RT	Area	RT	Area	RT	Limit
1	Average	6633882	4.723	6555261	4.715	6863111.5	4.698	NA
2	SD	4758.124	0.001602	6569.1362	0.003141	14887.99	0.008445	NA
3	% RSD	0.072	0.034	0.100	0.067	0.217	0.180	NMT 2%
4	Avg. RSD (%)	Area	0.130		Retention Time		0.093	

Table No.3: Result obtained for specificity of tapentadol tablets

S.No	Sample	Placebo (%)	Placebo		Standard	
			Area	RT	Area	RT
1	Placebo	100	-	-	NA	NA
2	Standard 100µg/ml	0	-	-	6902377	4.741
3	Placebo + Std. 100µg/ml	80	-	-	6915630	4.734
4	Placebo + Std. 100µg/ml	90	-	-	6916308	4.726
5	Placebo + Std. 100µg/ml	100	-	-	6933710	4.724
6	Placebo + Std. 100µg/ml	110	-	-	6925353	4.725
7	Placebo + Std. 100µg/ml	120	-	-	6919711	4.724
8	Average		-	-	6918848.2	4.729
9	Std. deviation		-	-	10507.52	0.006986
10	RSD (%)		-	-	0.152	0.148

Table No.4: Assay of formulation for LOD

S.No	Conc. (µg/ml)	Area				SD	%RSD
		Std1	Std2	Std3	Mean		
1	16.0	369098	371074	368137	369436.3	1497.446	0.405333
2	18.0	553611	557282	556305	555732.7	1901.245	0.342115
3	20.0	740982	742823	744053	742619.3	1545.597	0.208128
4	22.0	925279	952854	930640	936257.7	14620.66	1.561607
5	24.0	1110350	1108975	1115950	1111758	3694.619	0.332322

Table No.5: Assay of formulation for LOQ

S.No	Conc. (µg/ml)	Area				SD	%RSD
		Std1	Std2	Std3	Mean		
1	160	3212204	3219198	3218312	3216571	3808.077	0.118389
2	180	4876254	4856666	4842294	4858405	17046.63	0.350869
3	200	6509518	6509879	6501598	6506998	4680.308	0.071927
4	220	8135179	8137321	8156747	8143082	11882.31	0.145919
5	240	9782159	9776174	9782798	9780377	3653.9	0.03736

Table No.6: Range observation

S.No	Assay (%)	Range order
1	99.4	98.10
2	100.3	99.28
3	101.43	99.40
4	100.68	100.30
5	98.1	100.68
6	99.28	101.32
7	101.32	101.42
8	101.88	101.43
9	101.42	101.46
10	101.46	101.88
11	Lowest Value	98.10
12	Highest Value	101.88
13	Range	98.10µg/ml – 101.88µg/ml
14	Range Limit	80.00µg/ml – 120.00µg/ml

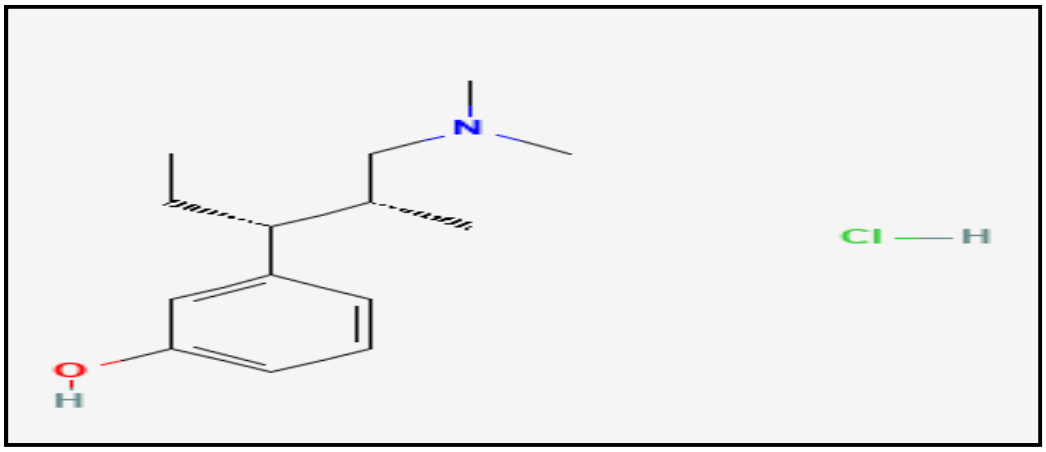

Figure No.1: Structure of Tapentadol hydrochloride

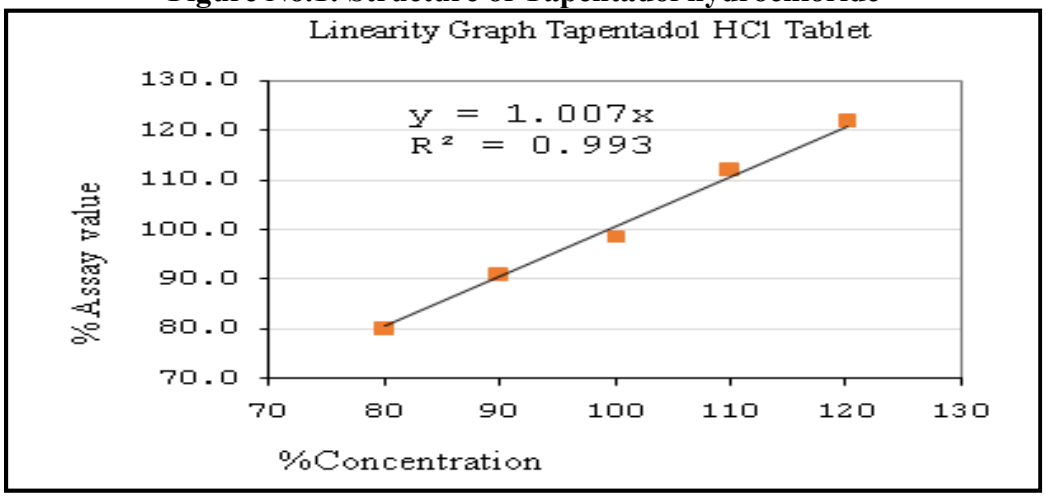

Figure No.2: Linearity graph of Tapentadol tablets

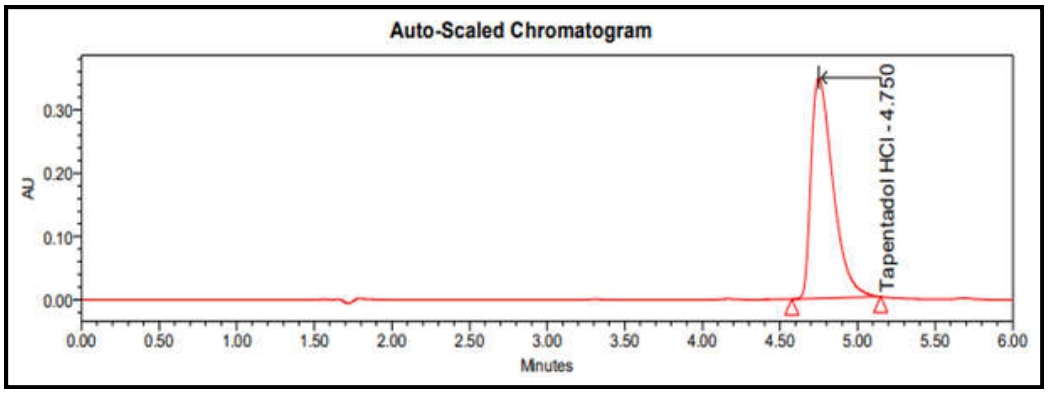

Figure No.3: Chromatogram of Tapentadol

CONCLUSION

A rapid, accurate, user-friendly, and reproducible HPLC method for the estimation of Tapentadol hydrochloride in tablet formulations was developed and validated according to ICH guidelines. LOD and LOQ measurements are also established for further application of this method. The broad linearity range was found to be less than 2 for all parameters using readily available mobile phases and RSD values. This method can be used in industry and academia to estimate Tapentadol in tablet dosage forms.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Gyani Inder Singh Institute of Professional Studies, Dehradun, Uttarakhand, India for providing necessary facilities to carry out this research article.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Please cite this article in press as: Pramod Yadav *et al*. A research article on analytical method validation of Tapentadol tablets, *Asian Journal of Phytomedicine and Clinical Research,* 10(2), 2022, 43-50.