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Research Article

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RP-HPLC DETERMINATION OF RELATED SUBSTANCES OF TAPENTADOL IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A sensitive, specific, precise and linear reverse phase HPLC method was developed for the analysis of related substances in Tapentadol in bulk and pharmaceutical dosage form. The known related substances are methoxy impurity [(2R, 3R)-3-(3-methoxyphenyl)-N,N,2-tri methyl pentanamine] and alcohol impurity [(2S)-1-dimethylamino)-3-(3-methoxyphenyl)-2-methylpentan-3-ol hydrochloride]. The method was carried out on a Zodiac C_{18} column (250 mm x 4.6 mm; 5µ) using a mobile phase mixture of phosphate buffer pH 7.0, acetonitrile and methanol in a gradient elution at a flow rate of 1.0ml/min at wavelength of 220 nm. The retention time of tapentadol was found to be 14±0.1 min, methoxy impurity was found to be 39.75±0.1 min and alcohol impurity was found to be 30.9±0.1min. The method can be used for the detection and quantitative estimation of known and unknown impurities in drug and pharmaceutical dosage form.

KEYWORDS

Tapentadol, RP-HPLC, phosphate buffer, Zodiac C18 Column, Tablets, Related substances.

INTRODUCTION

Tapentadol is 3-[(1R, 2R)-3-(dimethylamino)-1-ethyl-2-methyl propyl] phenol hydrochloride, it is a centrally acting analgesic with a dual mode of action as an agonist of the µ-opioid receptor and a norepinephrine reuptake inhibitor[1]. It approved in United States for treatment for moderate to serve acute pain. Tapentadol is not official in any pharmacopoeia. A few reports are in literature study on tapentadol drug-drug interaction potential in human liver[2]. N-desmethyltapentadol in urine specimens[3]. RP-HPLC[4]. Since this drug is being marketed in domestic and international market the present investigation by the author describes a rapid, accurate and precise RP – HPLC method for the determination of related substances from bulk sample and pharmaceutical dosage form. The detector responses were linear in the concentration range of 5.06 – 40.46 µg/ml of drug and its related substances. The method was validated as per ICH guidelines.

EXPERIMENTAL

Chromatographic Conditions

Agilent 1200 series with high pressure liquid chromatographic instrument provided with Auto sampler, and VWD & photo diode array detector, thermostatted column compartment connected with EZ Chrom software connected with a Zodiac C_{18} column (250 mm x 4.6 mm ; 5 μ). HPLC grade methanol, acetonitrile , water were purchased from E. Merck Co; Mumbai, India, and potassium dihydrogen phosphate, potassium hydroxide AR grade were purchased from E. Merck Co; Mumbai. India were used in the study.

Drug Samples

The reference sample and impurities supplied by Bio-Leo Analytical Labs India (P) Ltd, Prasanthinagar, Hyderabad. Branded formulation of Tapentadol was purchased from local market.

Mobile phase

Accurately weigh 6.8g of potassium dihydrogen phosphate was weighed out and dissolved in 1000ml of HPLC grade water and adjust pH 7.0±0.05 with 10% KOH solution. The buffer was used as mobile phase preparation A, a mixture of buffer, acetonitrile and methanol in the ratio of 30:20:50 v/v used as mobile preparation B, a mixture of methanol and acetonitrile in the ratio of 50:50 v/v used as diluent, the solutions were filtered through 0.45µ membrane filter and was degassed and Tapentadol and its impurities were eluted in a gradient program given in **Table 1**. The mobile phase was sonicated by using Biotechnics India Sonicator, Mumbai; the flow rate of the mobile phase was maintained at 1.0ml/min. The column temperature was maintained at 45ºC and the detection of the drug was carried out at 220nm.

Table 1: Gradient Programme

Time(in min)	Mobile phase - A	Mobile phase - B
0.01	35	65
15	35	65
35	10	90
46	10	90
46.5	35	65
55	35	65

Standard Préparation

Weigh accurately about 29.0 mg of Tapentadol hcl (Equivalent to 25 mg of Tapentadol) working standard and Transfer into 25 ml volumetric flask, add 15 ml Of diluent and sonicate for 5 minutes to dissolve and make up to the volume with diluent. Further dilute 2 ml of this solution to 100 ml with diluent.

Sample Preparation

Weigh and powder 10 tablets. Transfer the tablets powder equivalent to 100 mg of Tapentadol and transfer into 50 mL volumetric flask, add 30 mL of diluent and sonicate for 15 min and dilute to volume with diluent. Further filter the solution with 0.45 micron filter, discard first 5 mL of the filtrate.

Placebo Preparation:

Weigh and transfer the placebo powder equivalent to 100 mg into 50 mL volumetric flask, add 30 mL of diluent and sonicate for 15 min and dilute to volume with diluent. Further filter the solution with 0.45 micron filter, discard first 5 mL of the filtrate.

VALIDATION PARAMETERS

Linearity of the detector response was determined by taking measurement at Six concentration prints (6 replicates at each point) working dilution of Tapentadol, Methoxy impurity and alcohol impurity in the range of 5.06-40.46µg/ml.

The specificity of the method was performed by injected samples of related substances on placebo equivalent to the amount present in test preparation, and spiked known impurities with blend mixture of Tapentadol 100mg tablets.

The precision was performed by prepared six sample preparations representing a single batch and the % of impurities was determined, the intermediate precision also performed by prepared six preparations of a single batch by different analysts, different columns, different day and different instruments.

The accuracy of the test method was performed by prepared known quantities of impurities at the level of LOQ, 50%, 100%, 150% and 200% of target concentration. The percentage recovery of the amount added was estimated at each level.

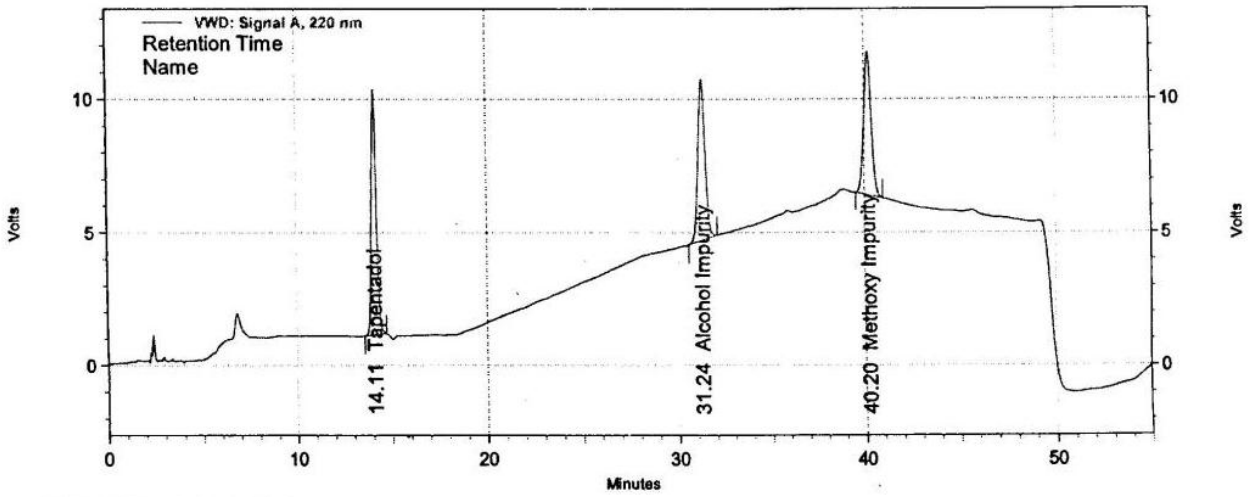
The robustness of the test method was performed as such condition and each of altered conditions such as column temperature, buffer, pH, extraction and flow variation, and also bench top stability initial and 24hours and 48 hours was performed, and filter paper variation was studied.

The LOD & LOQ of were determined by injected solutions of Tapentadol and impurities by S/N ratio method.

RESULTS AND DISCUSSION

The present study was aimed at developing a sensitive precise and accurate HPLC method for the separation of Tapentadol and impurities in bulk drug and in pharmaceutical dosage. A system suitability solution of low concentrations of Tapentadol with impurities given in **Fig.1** and the impurities with Tapentadol in Tablets preparation given in **Fig.2**.

Fig 1 : Chromatogram of Tapentadol and impurities



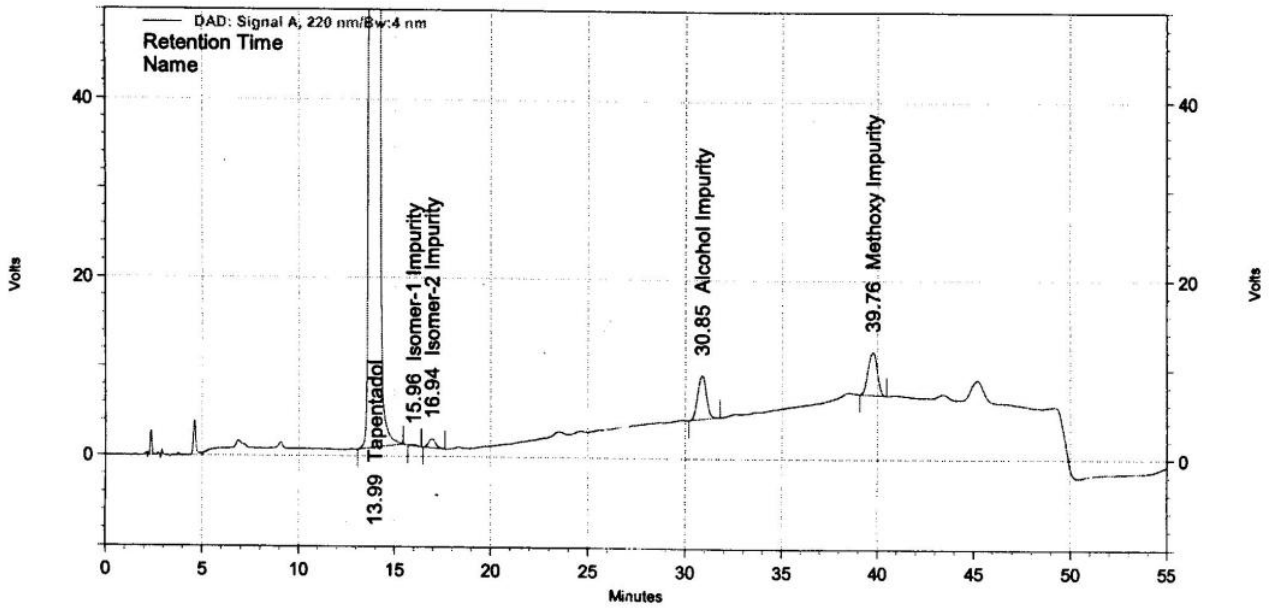
VWD: Signal A, 220 nm
Results

Name	Retention Time	Area	Area Percent
Tapentadol	14.11	2745499	34.82
Alcohol Impurity	31.24	2629493	33.35
Methoxy Impurity	40.20	2509931	31.83
Totals		7884923	100.00

The peak areas of Tapentadol were reproducible as indicated by low coefficient of variation. A good linear relationship (r^2 = 1.0) was observed for Tapentadol, r^2 = 0.9998 was observed for methoxy impurity and r^2 = 0.9998 was observed for alcohol impurity the regression characteristics are given in **Table 2**.

The specificity of the proposed method was observed that there was no interference of blank and placebo at the retention time of analyte and impurity peaks. Peak purity of analyte and individual impurities not be less than 0.99 indicates the method is specific. The results of specificity data for degradation study are given in **Table 3**.

Fig 2. Chromatogram of Tapentadol with impurities in tablet preparation



DAD: Signal A, 220 nm/Bw:4 nm Results

Name	Retention Time	Area	Area Percent	Peak purity
Tapentadol	13.99	30394139	97.73	1.00000
Isomer-1 Impurity	15.96	5919	0.02	0.99290
Isomer-2 Impurity	16.94	51734	0.17	1.00000
Alcohol Impurity	30.85	316037	1.02	1.00000
Methoxy Impurity	39.76	333403	1.07	1.00000
Totals		31101232	100.00	

The precision was established by six replicate injections at LOQ level of the test preparation containing impurities of interest. The values of relative standard deviation were found to be within the acceptance limit, indicating the injection repeatability of the method. The results are presented in **Table 4**. The intermediate precision (ruggedness) of the method was by carried out was found to be within the acceptance limit, which shows that the method is rugged. The results are presented in **Table 5**.

High recovery values obtained from the different dosage form by the proposed method indicates the method is accurate. The impurity content in tablets was quantified using the proposed analytical method are given in **Table 6**.

The percentage of individual and total impurities observed were deliberate changes in the method proves that the method is robust. The robustness study results are presented in **Table 7**. The difference between initial and bench top stability sample for % of individual impurities and total impurities were found within the acceptance criteria which indicates the solution were stable up to 48 hours. The results are presented in **Table 8**. The lowest value of LOD and LOQ as obtained by the proposed method indicates the sensitivity of the method. The results are presented in **Table 9**.

The filter paper variation of the method was carried out by injected filtered through different 0.45 µ membrane filters, the difference between

% of individual and total impurities were found within the acceptance limit. The results are presented in **Table 10**. Hence it can be concluded that the proposed HPLC method is sensitive, specific and reproducible for the determination of known and unknown related substances in Tapentadol and in pharmaceutical dosage form.

Table 2: Linearity of Tapentadol and impurities

S. No	Linearity Level	Concentration (ppm)	Average area of Tapentadol	Average area of Methoxy impurity	Average area of Alcohol impurity
1	25.0%	5.06	677567	537163	547837
2	50.0%	10.12	1431271	1200327	1254668
3	100.0%	20.23	2771905	2522604	2632847
4	150.0%	30.35	4165940	3778187	3880785
5	200.0%	40.46	5575700	4969776	5196770

	Tapentadol	Methoxy impurity	Alcohol impurity
Correlation coefficient	1.0000	0.9998	0.9998
Slope	137485	125667	129897
% of Y-Intercept	4.9599	4.9816	4.9337
Residual sum square	0.9999	0.9995	0.9996
Residual standard deviation	23251	47583	46133

Table 3: Specificity study

S. No.	Name of the Impurity/Analyte	Peak Purity	RT (Individual)	RT (Spiked sample)
1	Tapentadol	1.00000	13.99	13.99
2	Methoxy impurity	1.00000	39.75	39.76
3	Alcohol impurity	1.00000	30.89	30.85
4	Isomer-1 impurity	0.99290	16.05	15.96
5	Isomer-2 impurity	1.00000	16.98	16.94

Table 4: Precision study

S. No	Amine amide impurity	Lactam impurity
1	439383	235863
2	429742	241116
3	430631	240618
4	437916	235429
5	447665	228964
6	432343	230521
Avg:	436280	235419
SD:	6812.9	5006.8
% RSD:	1.56	2.13

Table 5: Intermediate Precision (ruggedness) study

S. No	Methoxy impurity(%w/w)	Alcohol impurity(%w/w)
1	1.00	1.01
2	1.00	1.00
3	0.99	1.00
4	1.00	1.00
5	0.99	1.00
6	1.00	1.00
7	1.00	1.00
8	1.00	1.00
9	1.00	1.00
10	1.00	1.00
11	1.00	1.00
12	1.00	1.00
Avg:	1.00	1.00
SD:	0.00	0.00
% RSD:	0.39	0.29

Note: For Analyst – 1, Column-1 & System -1 results refer Precision

Table 6 : Recovery of Methoxy impurity

Spike level	Amount Added (ppm)	Amount Recovered(ppm)	% Recovery	% Mean Recovery
LOQ level	0.441	0.433	98.25	98.84
LOQ level	0.441	0.439	99.56	
LOQ level	0.441	0.435	98.71	
50%	10.03	10.40	103.76	104.32
50%	10.03	10.51	104.88	
50%	10.03	10.42	103.96	
100%	20.05	20.66	103.04	102.88
100%	20.05	20.60	102.72	
100%	20.05	20.52	102.35	
150%	30.08	31.12	103.47	103.05
150%	30.08	30.86	102.62	
150%	30.08	31.18	103.67	
200%	40.10	39.98	99.71	99.42
200%	40.10	39.76	99.14	
200%	40.10	39.70	99.01	

Recovery of Alcohol impurity

Spike level	Amount Added (ppm)	Amount Recovered (ppm)	% Recovery	% Mean Recovery
LOQ level	0.40	0.402	99.78	99.38
LOQ level	0.40	0.402	99.64	
LOQ level	0.40	0.398	98.71	
50%	10.08	10.35	102.77	102.68
50%	10.08	10.39	103.08	
50%	10.08	10.30	102.19	
100%	20.15	20.25	100.49	100.17
100%	20.15	20.26	100.53	
100%	20.15	20.04	99.47	
150%	30.23	31.26	103.42	103.05
150%	30.23	31.36	103.74	
150%	30.23	30.82	101.97	
200%	40.30	41.02	101.78	101.01
200%	40.30	40.40	100.25	
200%	40.30	41.12	102.03	

Table 7: Robustness study

Condition		% Total Impurities	% Difference	Theoretical plates	Tailing factor
Normal Condition (i.e as such condition)		0.50	NA	17421	1.07
Flow changed to 0.9ml/min		0.45	0.05	18321	1.05
Flow changed to 1.1ml/min		0.46	0.04	17227	1.04
Column Temperature changed to 40°C		0.46	0.04	16827	1.04
Column Temperature changed to 50°C		0.48	0.02	19184	1.04
Buffer pH changed to 6.8		0.47	0.03	16942	1.08
Buffer pH changed to 7.2		0.47	0.03	18016	1.04
Extraction time	10 min	0.46	0.04	17421	1.07
	15 min	0.48	0.02		
	20 min	0.48	0.02		

Table 8: Solution stability

Time (hours)	% of Total impurities	% of difference
Initial	0.46	NA
After 24 hours	0.46	Nil
After 48 hours	0.47	0.01

Table 9: LOD Study

S. No	Name of the Component	S/N Ratio	% level of component w.r.t to sample concentration	Value (µg/ml)
1	Tapentadol	2.91	0.003	3.0
2	Methoxy impurity	3.32	0.007	7.0
3	Alcohol impurity	3.42	0.006	6.0

LOQ Study

S. No	Name of the Component	S/N Ratio	% level of component w.r.t to sample concentration	Value (µg/ml)
1	Tapentadol	10.10	0.01	10.0
2	Methoxy impurity	10.38	0.02	20.0
3	Alcohol impurity	10.20	0.02	20.0

Table 10: Filter Variation Study

	Centrifuged	Nylon Filter	PVDF Filter
% of Isomer-1 impurity	0.02	0.02	0.02
% Difference	NA	Nil	Nil
% of Isomer-2impurity	0.13	0.13	0.12
% Difference	NA	Nil	0.01
% of Total impurities	0.48	0.50	0.46
% Difference	NA	0.02	0.02

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