Research Article

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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC AND UV-SPECTROSCOPY METHODS FOR QUANTITATIVE DETERMINATION OF TAPENTADOL IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

The aim of the study was to develop UV spectroscopy and RP-HPLC method for the analysis of Tapentadol in marketed tablets. Chromatographic separation was achieved on a Inertsil ODS 3v (250×4.6, 5µm) with mobile phase consisting of Sodium dihydrogen phosphate (pH 7.0) buffer: Acetonitrile in the ratio of 80:20 v/v. The effluent was monitored at 290nm. A sharp peak was observed at 3.2min. UV Spectrophotometric method was performed at 290 nm using methanol as the solvent. R^2=1.000 for HPLC method and R^2=0.999 for UV Spectrophotometric method. Validation as per ICH guidelines and statistical analysis showed that both the methods were precise, accurate, sensitive, and can be used for the routine analysis of Tapentadol in pharmaceutical dosage forms.

Keywords: Tapentadol, RP-HPLC, UV-Spectrophotometric, ICH guidelines.

INTRODUCTION:

Tapentadol is a centrally acting analgesic with a dual mode of action as an agonist of the µ-opioid receptor and as a nor epinephrine reuptake inhibitor. It is also an agonist of the σ_2 receptor, though the function of this orphan receptor remains controversial. While its analgesic actions have been compared to tramadol and oxycodone, its general potency is somewhere between tramadol and morphine in effectiveness. It has opioid and nonopioid activity in a single compound.

In the US, Tapentadol is FDA approved for the treatment of moderate to severe acute pain. Due to the dual mechanism of action as an opioid agonist and norepinephrine reuptake inhibitor, there is potential for off-label use in chronic pain. IUPAC name of the Tapentadol is 3-[(1*R*, 2*R*)-3-(dimethylamino)-1-ethyl-2-methylpropyl] phenol hydrochloride, Molecular formula is C14H23NO and Molecular weight of Tapentadol is 221.339 g/mol.

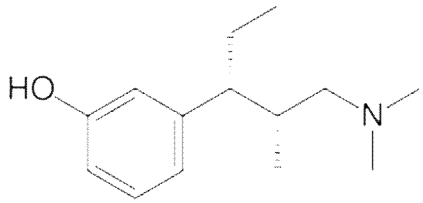

Fig. a. Structure of Tapentadol

Tapentadol is estimated by various methods either by HPLC or with the combination of HPLC, with UV Spectrophotometer or with HPTLC, methods as per literature.

MATERIALS AND METHODS:

Tapentadol were obtained as a memento sample from Dr.Reddys, Hyderabad. Acetonitrile, methanol HPLC grade rankem New delhi, Methanol hong yang chemical corp china, milli-Q water it was purified by milli

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pore corporation's system mfg barnstead. The analysis was carried out on HPLC Waters 2695 connected with PDA detector 2998 and Empower2 soft ware. The analysis was carried out on UV Lab India UV visible spectrometer (model-UV-VIS SPECTROPHOTOMETER 3000^{+})

Chromatographic Conditions
Column: Inertsil ODS 3V
Flow rate: 1ml/min
Injection volume: 10µl
Column Temperature: Ambient
Mobile phase A: Sodium dihydrogen phosphate: Acetonitrile (80:20)
Detection: 290nm.
Diluent: Methanol

Preparation of standard solution:
For HPLC method, an accurately weighed quantity, 100mg of Tapentadol was transferred into 100ml of volumetric flask and add 20ml of diluent and sonicate for 30 mins make up the volume with methanol. Transfer above solution 5ml into 50ml volumetric flask and dilute the volume with Diluent. Typical chromatogram for Tapentadol is shown in Fig. 1.

Preparation of the sample solutions:
Ten tablets were weighed, powdered and a 270mg of sample was transferred to a 100 ml volumetric flask containing 20ml of diluent. The mixture was then sonicate for 30 min to dissolve the material completely. An aliquot of supernatant solution 5 ml was transferred to50 ml volumetric flask, dilute the volume with diluent.

Method Validation:
Both the methods were validated by following ICH recommendations for validation of analytical Procedures.

Linearity and range:
For the HPLC method, stock solution of Tapentadol was suitably diluted with the Acetonitrile to get concentrations in the linear range of 25-150% were injected into the HPLC system. The calibration curve for Tapentadol was constructed by plotting the ratio of the peak area of Tapentadol (Y) against concentration (X) and linearity was evaluated by linear regression equation. The slope, intercept and correlation coefficient values were recorded. For the UV Spectrophotometric method, calibration graph was prepared 25-150% Tapentadol and absorbance was recorded at 290 nm. Each experiment was performed in six replicates Fig. 2 & 3.

Accuracy:
Accuracy was determined by standard addition method. To a pre-analyzed sample formulation a known quantity of standard was added at three levels (50, 100 and 150% of the assay concentration). Absorbance was measured directly for the UV Spectrophotometric method. The experiment was performed in six replicates. %RSD and %recovery were calculated for all the concentrations.

Precision:
The precision of the method was studied by repeatability (within-day) and intermediate precision (inter-day).The intra-day precision studies were carried out by estimating the response six times on the same day using three different concentrations. For UV Spectrophotometric method of Tapentadol and inter-day precision studies were done by repeating the above procedure on three different days. The results of precision studies were expressed as %RSD.

Specificity:
Purity of Tapentadol in HPLC method by comparing the individual spectrum at three regions i.e. peak start, peak apex and peak end. The specificity of the method was further assessed by comparing the chromatograms obtained from standards and from placebo solutions prepared using the excipients most commonly present in pharmaceutical formulations, Specificity of UV Spectrophotometric method was determined from the absorption spectra of Tapentadol reference standard and that of formulation.

Robustness:
Robustness of HPLC method was studied to evaluate the effect of small but deliberate variations of the chromatographic conditions on the method parameters. Robustness was determined by changing individually the flow

rate (1±0.1ml/min), temperature (25±5°C), pH and mobile phase evaluating their effects on peak parameters.
In case of UV robustness was studied on sample temperature and wave length max.

System suitability tests:
The test was carried out by making six replicate injections of a standard solution of Tapentadol. The peak area of the sample, number of theoretical plates (N), tailing factor (T),and capacity factor (k') were analyzed.

FORCED DEGRADATION STUDIES:
Control Sample:
Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 280 mg of Tapentadol into a 100 ml volumetric flask add about 70 ml of methanol, and sonicate for 30 minutes with shaking at controlled temperature and dilute to volume with methanol and mix. Filter the solution through 0.45 µm membrane filter. Transfer 5.0ml of the above solution into a 50 ml volumetric flask and dilute to volume with diluent.

Acid Degradation Sample:
Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 280 mg of Tapentadol into a 100 mL volumetric flask add about 70 mL of methanol, and sonicate for 30 minutes with intermittent shaking at controlled temperature. Then add 10 mL of 5 N acid, refluxed for 30 min at 60˚C, then cooled to room temperature, neutralize with 5 N NaOH and di-lute to volume with methanol and mix. Filter the solution through 0.45 µm membrane Filter. Transfer 5.0 mL of the above solution into a 50 mL volumetric flask and dilute to volume with diluent.(Fig.4)

Base Degradation Sample:
Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 282 mg of Tapentadol into a 100 mL volumetric flask add about 70 mL of methanol, and sonicate for 30 minutes with intermittent shaking at controlled temperature. Then add 10 mL of 5 N Base (NaOH), refluxed for 30 min at 60˚C, then cooled to room temperature, neutralize with 5 N Acid (HCl) and dilute to volume with methanol and mix.

Filter the solution through 0.45 µm membrane Filter. Transfer 5.0 mL of the above solution into a 50 mL volumetric flask and dilute to volume with diluent. (Fig.5)

Peroxide Degradation Sample:
Weigh and finely powder not fewer than 20 Tablets. Accurately weigh and transfer equivalent to 281 mg of Tapentadol into a 100 mL volumetric flask add about 70 mL of methanol, and sonicate for 30 minutes with shaking at controlled temperature. Then add 2 ml of 30% Peroxide, refluxed for 30 min at 60°C, then cooled to room temperature and dilute to volume with methanol and mix. Filter the solution through 0.45 µm membrane filter. Transfer 5.0ml of the above solution into a 50ml volumetric flask and dilute to volume with diluent. (Fig.6)

Thermal Degradation Sample:
Powders collected from 20 tablets are exposed to heat at 105oC for about 5 days. Then Weigh and finely powder not fewer than 20 tablets. Accurately weigh and transfer equivalent to 280 mg of Tapentadol into a 100 ml volumetric flask add about 70 ml of methanol, and sonicate for 30 minutes with shaking at controlled temperature and dilute to volume with methanol and mix. Filter the solution through 0.45 µm membrane filter. Transfer 5ml of the above solution into a 50 ml volumetric flask and dilute to volume with diluent. Similarly Humidity, water hydrolysis stress samples are prepared and checked for their purity by proposed method. From the above data of degradation profile it can be concluded that there is no interference found for of Tapentadol peak. (Fig 7)

RESULTS AND DISCUSION:
For HPLC method:
System suitability results were given by table 1A and system suitability parameters are retention time, tailing and plate count were shown uniformity and %RSD was less than 1. Linear correlation was found r2=0.99 given in table1B. And for UV r^2=0.999.The method accuracy was evaluated by recovery studies

those values are given by table 2. Tapentadol recovery was founded 101% as per ICH 97%-103% and also percentage RSD was very low so method is accurate. And plot the graph three different concentrations versus areas to construct the linear regression equation and to calculate the value of correlation co efficient. Precision results were shown by table 3. Method robustness results was given by table 4, they were not observed marked changes of those trails compared to other trails so it proves method was robust. Stability studies results are given in table 5.

For UV method:

The absorption spectrum of Tapentadol solution in methanol was recorded between 200-400nm. Therefore, 290 nm was used as analytical wavelength (λ_{max}). Figure 9 represents the absorption spectra of Tapentadol in methanol. Linear correlation were obtained between the absorbance and concentration of Tapentadol in the range of 50-150µg/mL Correlation coefficient(fig10), the calibration data are summarized in Table 6, precision results are given the table 7 and accuracy results are given in table 8, and the results of degradation was summarized in table 9.

Table 1A: System Suitability Test Parameters for the proposed method.

Parameters	Tapentadol
Retention Time (min)	3.28mins
Theoretical plates	4579
Tailing factor	1.6

Table 1B: LENIARITY OF TAPENTADOL BY HPLC

LIN 25%	555890
LIN 50%	1116635
LIN 75%	1673640
LIN 100%	2228914
LIN 125%	2782306
LIN150%	3335847

Table 2: Accuracy results of Tapentadol by HPLC

Spiked level	%recovery	Mean
50%	100	100
50%	100	
50%	100	
100%	100	
100%	100	
100%	100	
150%	100	100
150%	100	
150%	100	

Table 3: precision results of Tapentadol by HPLC

S. No	Sample weight	%Assay
1	270	99
2	270	99
3	270	99
4	270	99
5	270	99
6	270	99
Mean		99
Std.dev		0.17
%RSD		0.17

Table 4: Robustness of Tapentadol

S. No	Sample name	Name	RT	Tailing	Plate count
1	Flow1	Tapentadol	3.892	1.8	3947
2	Flow2	Tapentadol	2.589	1.8	3586
3	Temp1	Tapentadol	1.232	1.9	4013
4	Temp2	Tapentadol	3.115	1.8	4077
5	MP1	Tapentadol	3.868	1.8	4047
6	MP2	Tapentadol	2.602	1.8	3450
7	pH1	Tapentadol	3.863	1.7	4009
8	pH2	Tapentadol	2.614	1.8	3822

Table 5: Degradation Studies

	Sample weight	% Assay	purity angle	Purity Threshold
Acid	270.00	91	0.68	0.91
Base	270.00	91	0.89	1.12
Peroxide	270.00	92	1.15	1.32
Water	270.00	91	0.81	1.02
Heat	270.00	89	0.96	1.21

Table 6: leniarity of tapentadol by uv

LIN 25%	0.1638
LIN 50%	0.3277
LIN 75%	0.437
LIN 100%	0.6564
LIN 125%	0.758
LIN150%	0.9832

Table 7: precision For UV

METHOD PRECISION (INTRA DAY)

S. No	Sample Weight	Absorbance	% Assay
1	270	0.6545	100.0
2	270	0.6539	100.0
3	270	0.6548	99.9
4	270	0.6550	99.9
5	270	0.6553	99.8
6	270	0.6556	99.97
Avarage Assay:			99.9
STD			0.10
%RSD			0.10

B) Interlay precision

S. No	Sample Weight	Absorbance	% Assay
1	270	0.6507	100
2	270	0.6514	100
3	270	0.6502	99.9
4	270	0.6521	99.9
5	270	0.6518	99.8
6	270	0.6513	99.8
Average Assay:			99.9
STD			0.09
%RSD			0.09

Table 8: accuracy results for UV

Spiked Level	Sample Weight	Sample Absorbance	µg/ml added	µg/ml found	% Recovery	% Mean
50%	140.00	0.3270	53.846	55.01	102.161	100.6698
50%	140.00	0.3274	53.846	54.42	101.066	
50%	140.00	0.3276	53.846	53.19	98.781	
100%	290.00	0.6562	111.538	110.52	99.087	98.7824
100%	290.00	0.6561	111.538	110.96	99.481	
100%	290.00	0.6558	111.538	109.06	97.778	
150%	435.00	0.9837	167.308	167.22	99.947	100.0430
150%	435.00	0.9808	167.308	167.60	100.174	
150%	435.00	0.9817	167.308	167.10	99.223	

Table 9: Stress testing

Type	Sample Weight	Sample Absorbance	% Assay	%DEG	observation
Acid	280.00	0.5980	91.2	-9	degraded
Base	279.00	0.6011	91.2	-8	degraded
Peroxide	276.00	0.5990	91.4	-9	degraded
Water	278.00	0.6422	98.0	-2	degraded
Humidity	278.00	0.6401	97.7	-2	degraded
Heat	276.00	0.5846	89.2	-11	degraded

Fig.1: A typical standard chromatogram of Tapentadol

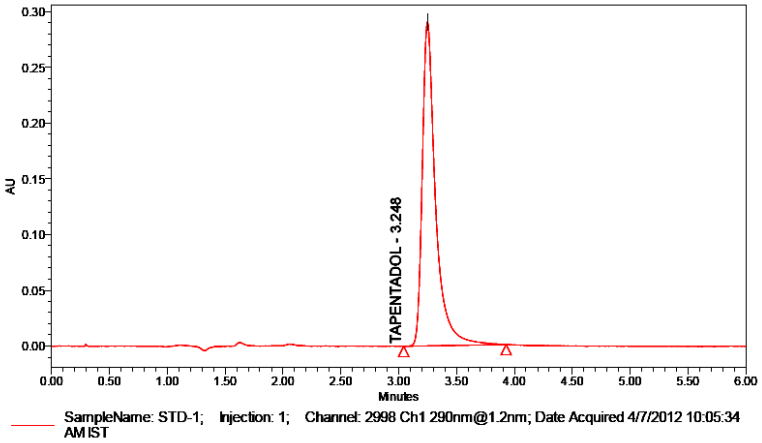


Fig. 2: Calibration curve of Tapentadol by HPLC

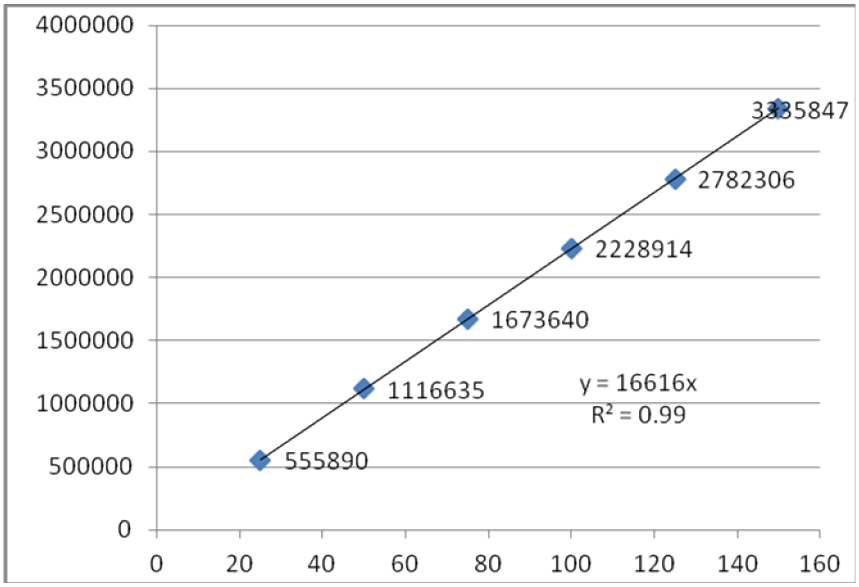


Fig 3: Calibration curve of Tapentadol by UV

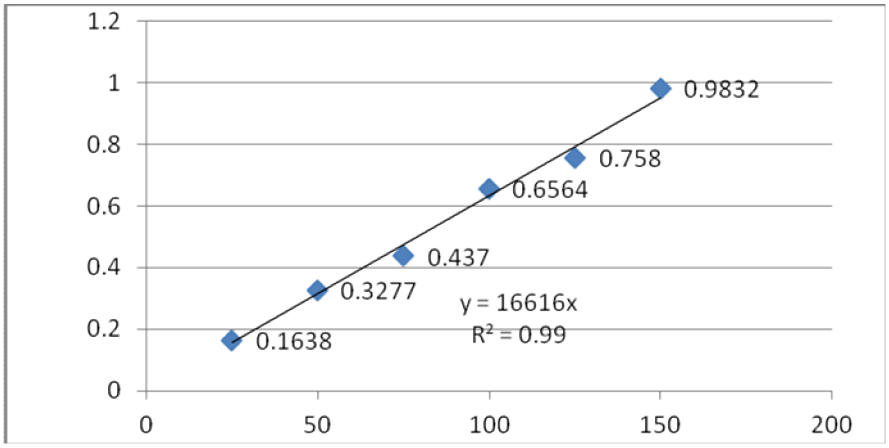


Fig. 4: Chromatogram for acid

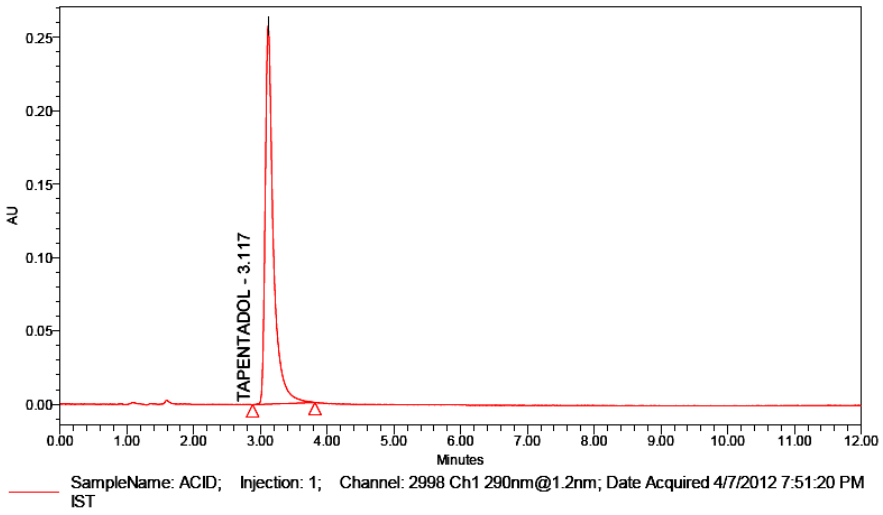


Fig 5: Chromatogram for base

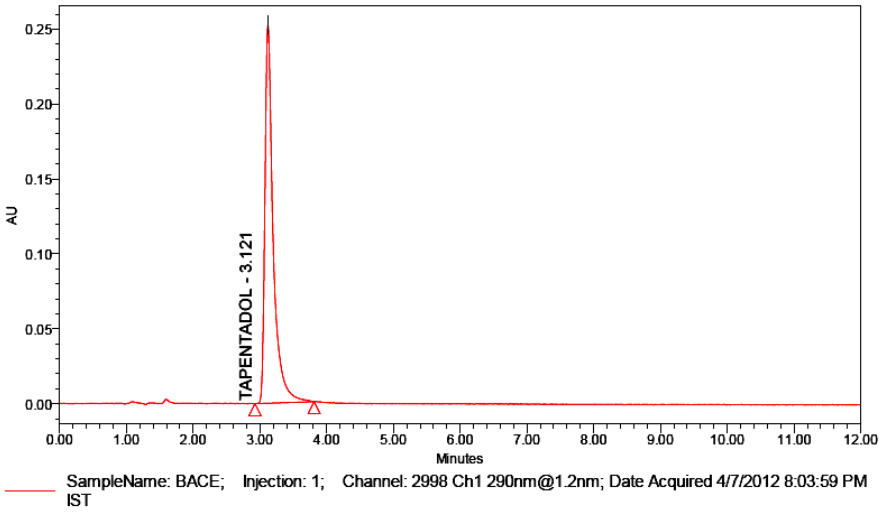


Fig. 6: Chromatogram for peroxide

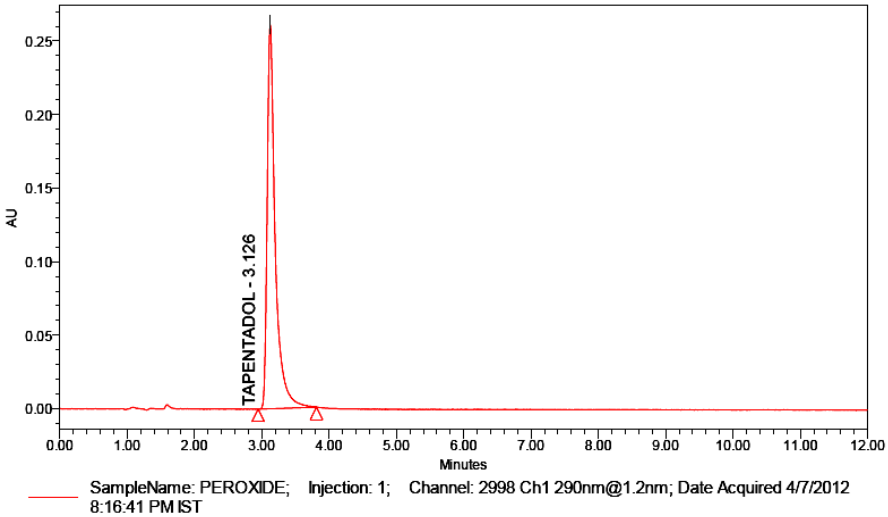


Fig 7: Chromatogram for heat

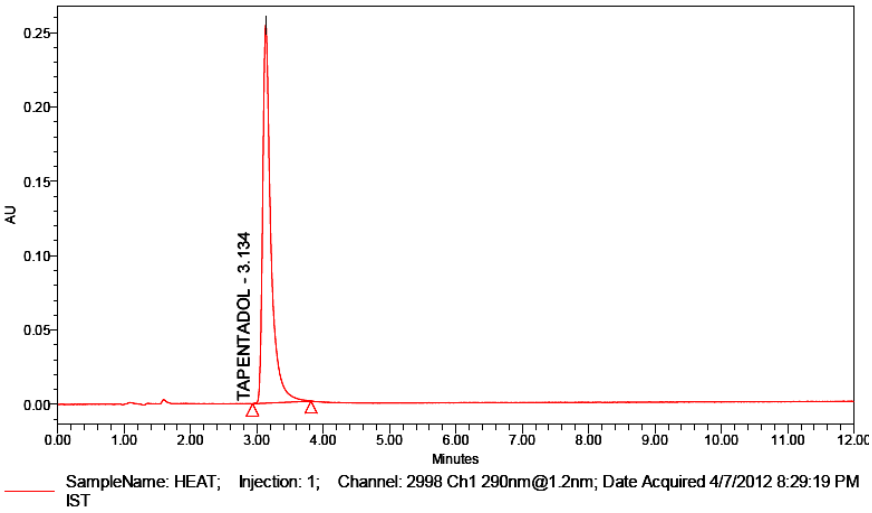


Fig 8: Chromatogram for humidity

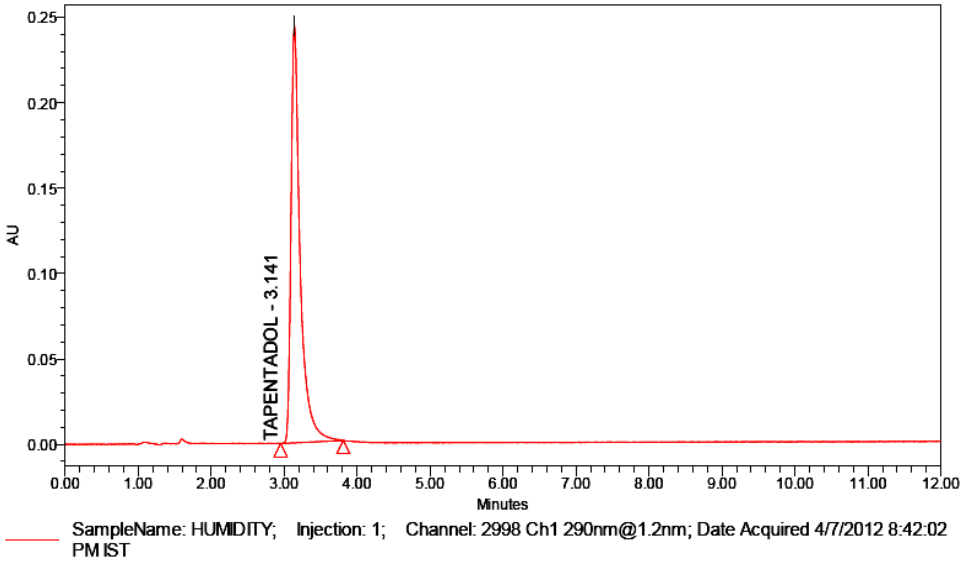


Fig. 9: UV Spectrum of Tapentadol

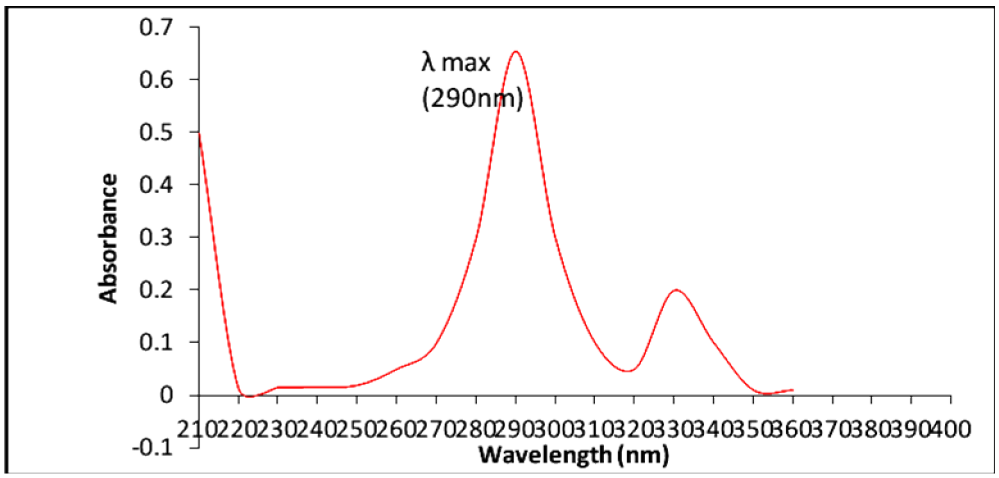
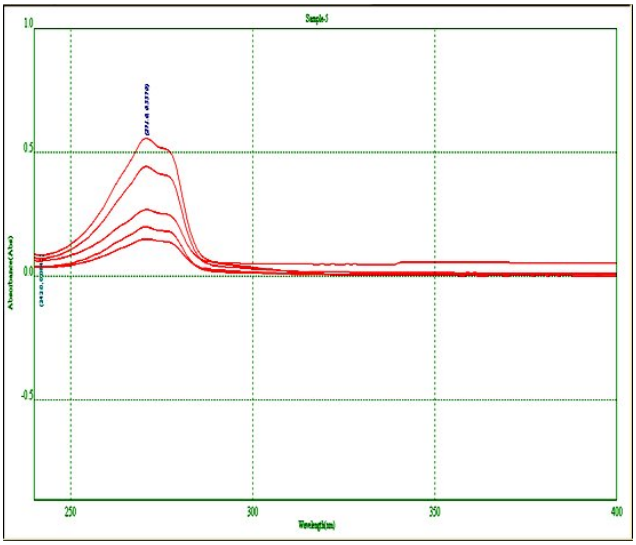


Fig. 9: Overlay spectrum of Tapentadol



CONCLUSION:

The validated HPLC and UV methods were found to be accurate precise and reliable. UV spectroscopic method was simpler and sensitive than HPLC method and the same may be used as an alternative method and advanced instrument like HPLC are not available for routine quantification purpose. In degradation studies It was observed that purity angle is less than the threshold value and hence the peak is said to be pure and under these conditions tapendol can be estimate without any interference. Both the methods can be employed for the routine quality control of Tapentadol in tablet dosage form.

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