Method development and validation for simultaneous estimation of tapentadol and paracetamol by UV spectrophotometry

Abstract

A simple, rapid, accurate and precise UV Spectrophotometric method was developed for the simultaneous estimation of tapentadol (TAP) and paracetamol (PCM) in the pharmaceutical dosage forms. This method utilizes 0.1N HCl as solvent. λmax of tapentadol and paracetamol was found to be 272.5 nm and 244.0 nm respectively, but we selected 257 nm for paracetamol, because at this wavelength tapentadol is showing linear absorbance values. Linearity was observed in the concentration range of 4-28 µg/ml for both the drugs. The R^2 value was found to be 0.9991 and 0.9986 for tapentadol and paracetamol respectively. The method was validated statistically and by recovery studies. The mean percentage recovery was found to be between 95%-105% for tapentadol and paracetamol.

Keywords: 0.1N HCl, Tapentadol, Paracetamol, UV spectrophotometry, Simultaneous Equation.

1. Introduction

Tapentadol is chemically 3-[(1R, 2R)-3-(dimethyl amino)-1-ethyl-2-methyl propyl phenol monohydrochloride. It is a centrally acting analgesic with a dual mode of action as an agonist of µ-opioid receptor and as a norepinephrine reuptake inhibitor. Tapentadol is indicated for the treatment of moderate to severe pain for both acute and chronic musculoskeletal pain. It is specifically indicated for controlling the pain for diabetic neuropathy when around the clock opioid medication is required.

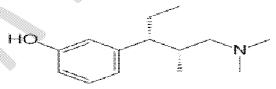

Figure 1: Chemical structure of tapentadol.

Paracetamol is chemically 4-hydroxy acetanilide. Paracetamol is a good and promptly acting analgesic and antipyretic. The main mechanism of action of paracetamol is inhibition of COX (cyclooxygenase) enzyme, and recent findings suggest that it is highly selective for COX-2. Paracetamol is mainly indicated for treatment of pain and fever.

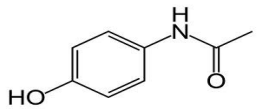

Figure 2: Chemical structure of paracetamol.

Literature survey reveals that different analytical techniques including Colorimetric, UV Spectrophotometric (Chandani, 2013; Samil *et al.*, 2013; Khokhar and Aswin, 2013; Vishal Khokhar and Shah, 2013) and RP-HPLC (Deepti and

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Pawan,2013; Dharmishtha, 2013;Ramanaiah*et al.*, 2012) methods were reported on tapentadol and paracetamol combination.

2. Materials and Methods

2.1Apparatus: Double beam UV-Visible Spectrophotometer, SL 244(model), ELICO (brand); Electronic balance, capacity (220gm), readability (0.001 gm), SHIMADZU (brand); Ultra Sonicator, Citizen (brand). The curves of the UV Visible spectra of standard and sample solutions were recorded in 1 cm quartz cells at scan range of 200 - 400nm.

2.2 Materials and reagents: Tapentadol reference standard was obtained as gift sample from MSN laboratories, Hyderabad. Paracetamol reference standard was obtained as gift sample from HETERO Drugs Ltd, Hyderabad. Marketed formulation (ZYNTAP) of tapentadol and paracetamol was obtained from the local medical shop. Each film coated tablet contains 50 mg of tapentadol and 325 mg of paracetamol. All the chemicals used were of analytical grade purchased from SD fine chemicals, Mumbai.

2.3 Method development

2.3.1 Preparation of stock and standard solutions: Standard stock solutions of tapentadol and paracetamol were prepared by dissolving 100 mg of each drug in 100 ml of 0.1N HCl individually to get the concentration of 1000 µg/ml. 10 ml of each drug solution was taken from the stock solution and diluted to 100 ml with 0.1N HCl in 100 ml volumetric flask to get the concentration of 100µg/ml individually.

2.3.2 Selection of the λmax: The working standard dilutions of each drug were scanned from 200 – 400nm to determine the λmax individually, and it was found to be 244 nm and 272.5nm for paracetamol and tapentadol, respectively. But we selected 257nm for paracetamol, because at this wavelength tapentadol is showing linear absorbance values. Spectrums of λmax of tapentadol and paracetamol were shown in Figures 3 and 4, respectively.

2.3.3 Assay of tapentadol and paracetamol in tablets: Twenty tablets of ZYNTAP tablets were carefully weighed and ground to finely divided powder. Accurate weight equivalent to 100mg of paracetamol and 15.5 mg of tapentadol was weighed and dissolved in 0.1N HCl.This solution was kept overnight to dissolve drugs completely in the solvent.

Solutions were filtered using whatmann filter paper grade 1. The filtrate was diluted with 0.1N HCl to obtain the appropriate dilutions of 26:4 µg/ml, 13:2 µg/ml, 6.5:1 µg/ml (paracetamol: tapentadol).The concentration of the drug in the marketed formulation was calculated by using the following simultaneous equations

$$C_P = A_2ay_1 - A_1ay_2 \,/\, ax_2ay_1 - ax_1ay_2$$
$$C_T = A_1ax_2 - A_2ax_1 \,/\, ax_2ay_1 - ax_1ay_2$$

Where C_P = Concentration of paracetamol

C_T = Concentration of tapentadol
A_1, A2 = Absorbances of paracetamol and tapentadol at 257nm and 272.5nm
ax_1, ax_2 = Absorptivities of paracetamol at 257nm and 272.5nm
ay_1, ay_2= Absorptivities of tapentadol at 257nm and 272.5nm

3. Results

3.1. Determination of λmax of standard drugs

The λmax oftapentadol and paracetamol determined was shown in the following Figures 3 and 4, respectively.

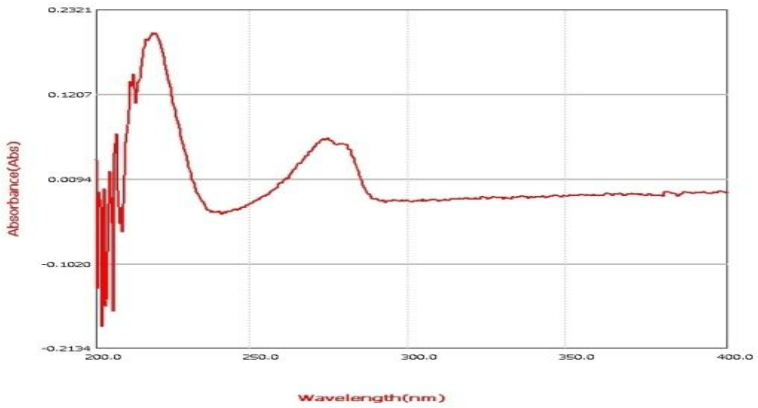

Figure 3: UV Spectrum (λmax) of tapentadol.

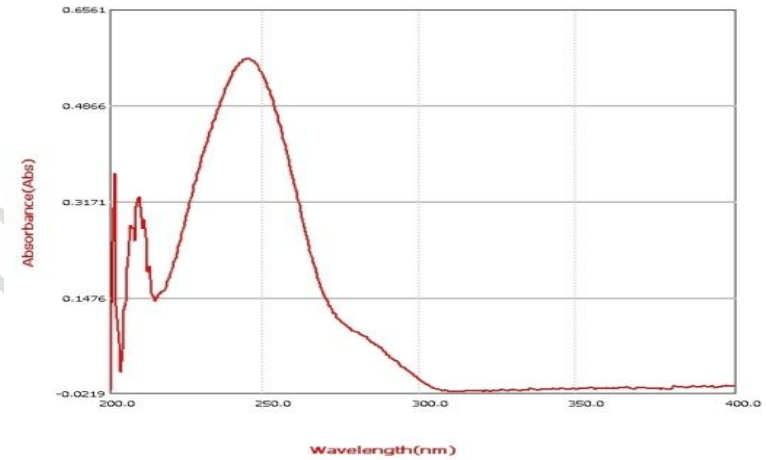

Figure 4: UV Spectrum (λmax) of paracetamol.

3.3. Validation of the developed method

3.1. Linearity

In the UV method,the working solutions were scanned at 200 – 400nm against a similarly prepared blank. The linearity range of both the drugs was found to be

between 4-28 µg/ml. The linearity spectrums of tapentadol and paracetamol standard drugs were shown in Figures5 and 6, respectively. The Calibration curve for tapentadol at 272.5 nm and 257 nm were shown inFigures 7, 8, respectively. Figures 9, 10 are calibration curve of paracetamol at 257 nm and 272.5 nm,respectively.

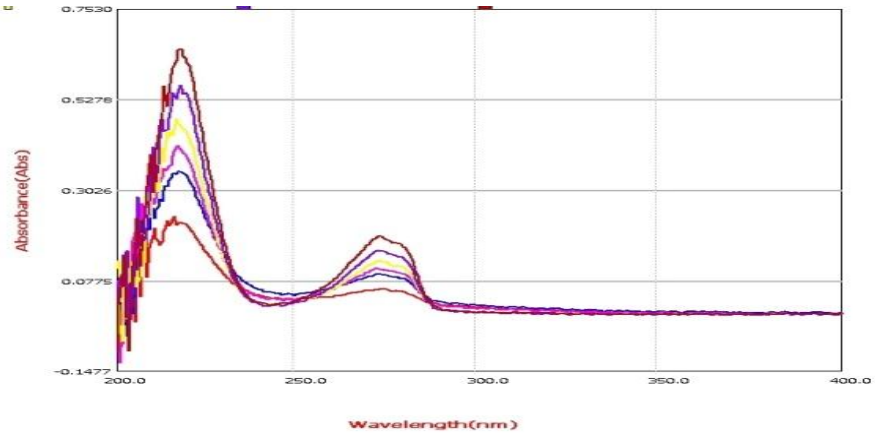

Figure 5: Linearity spectrum of tapentadol at 272.5nm.

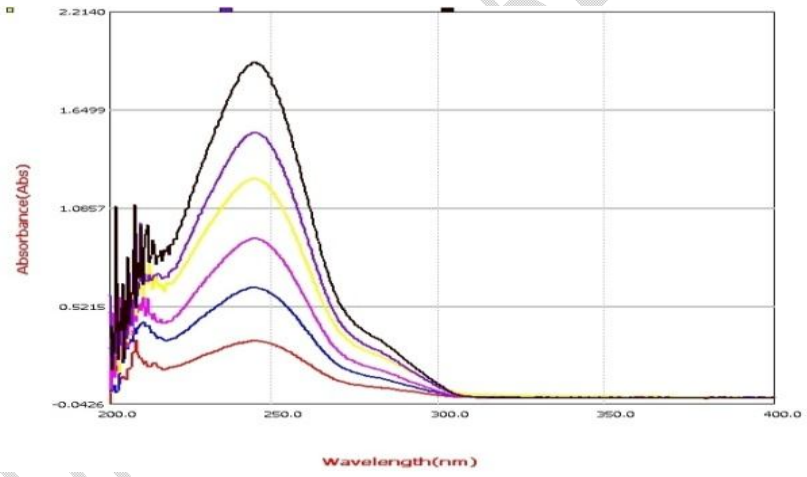

Figure 6: Linearity spectrum of paracetamol at 257nm.

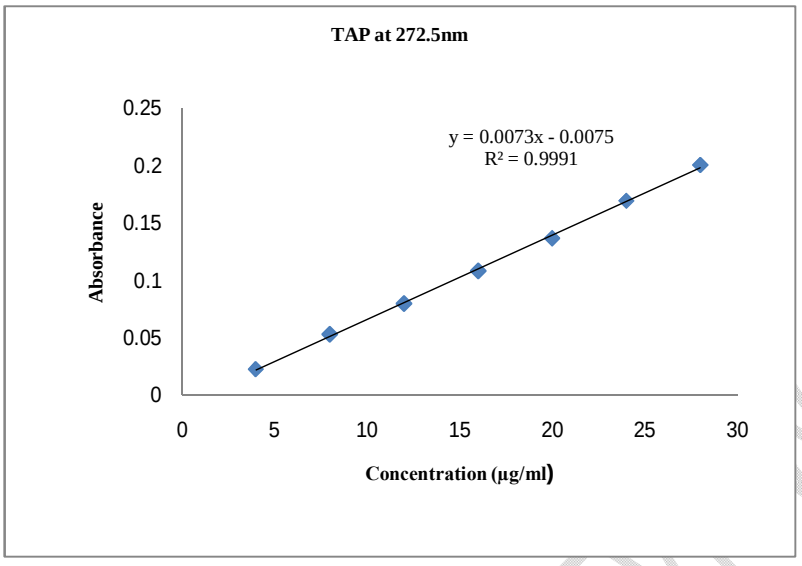

Figure 7: Calibration curves of tapentadol at 272.5nm.

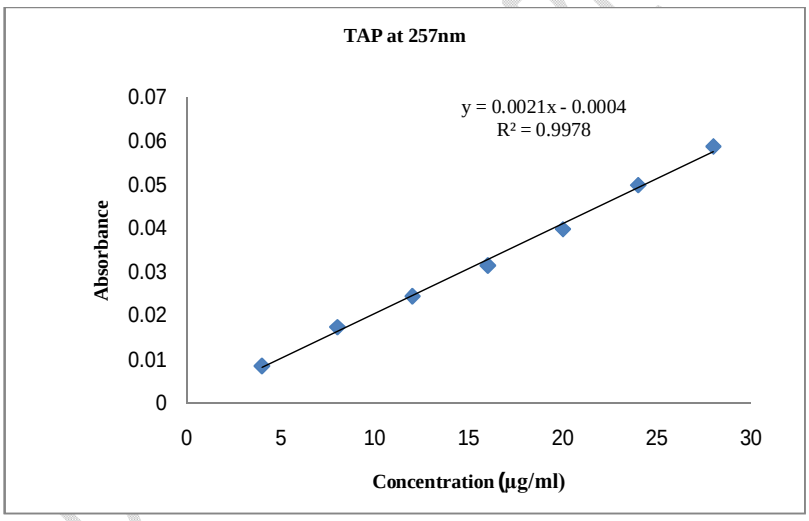

Figure 8: Calibration curves of tapentadol 257nm.

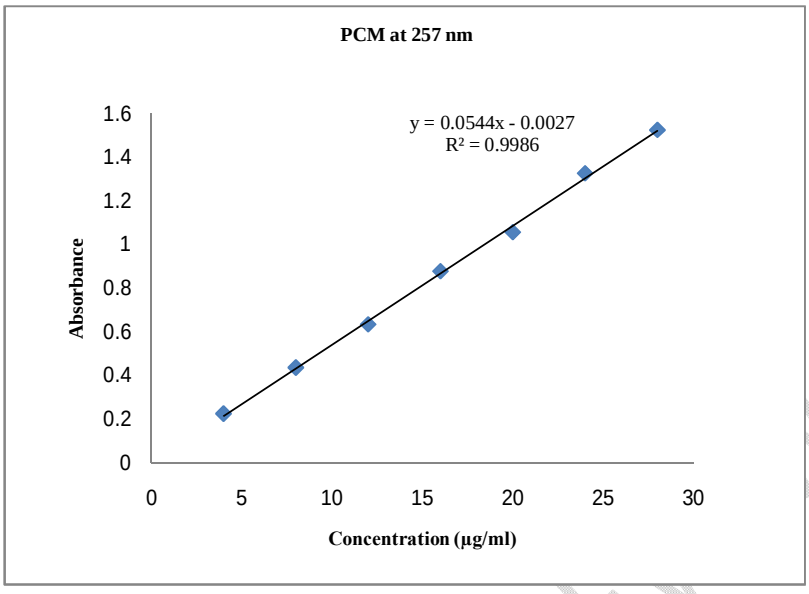

Figure 9: Calibration curves of paracetamol at 257nm.

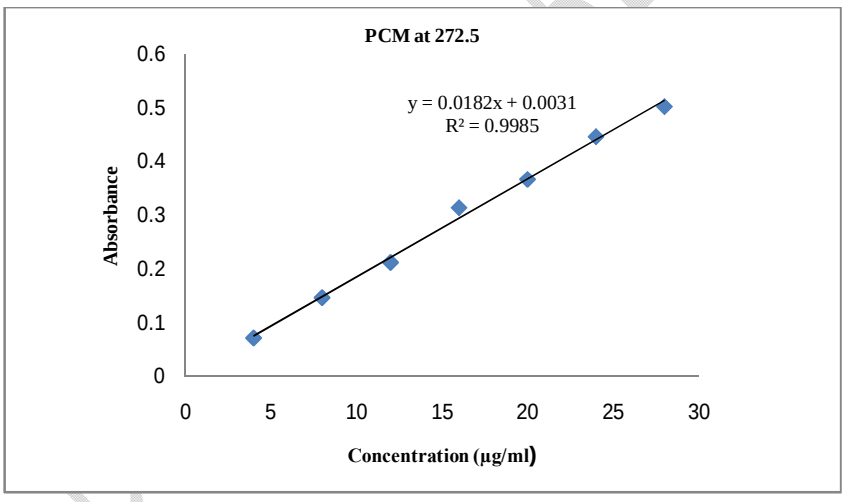

Figure 10: Calibration curves of paracetamol at 272.5nm.

3.2. Sensitivity

LOD and LOQ values represent the sensitivity of the method. LOD was calculated by using the formula 3.3×standard deviation of intercepts/mean of slopes of linearity graphs. LOQ was calculated by using the formula 10×standard deviation of intercepts/mean of slopes of linearity graphs. The results found were shown in the following Table 1.

Table 1: LOD and LOQ values of tapentadol and paracetamol

S.No	Parameter (µg/ml)	Tapentadol at		Paracetamol at	
		257nm	272.5nm	257nm	272.5nm
1.	LOD	0.1625	0.1039	0.1588	0.5576
2.	LOQ	0.4926	0.3149	0.4814	1.6898

3.3. Precision studies

3.3.1. System Precision: System precision was performed with mixture of standard drugs (n=5) and the results observed were shown in the following Tables 2 and 3.

Table 2: Results of system precision

Concentration (µg/ml) (TAP:PCM)	Absorbance of TAP and PCM at	
	257 nm	272.5 nm
12:12	0.6740	0.3047
12:12	0.6732	0.3047
12:12	0.6741	0.3046
12:12	0.6727	0.3033
12:12	0.6724	0.3047

Table 3: Statistical report of system precision

Parameter	For TAP and PCM at	
	257 nm	272.5 nm
Mean	0.67328	0.3044
Standard deviation	0.00076	0.000616
% RSD	0.112822	0.20251

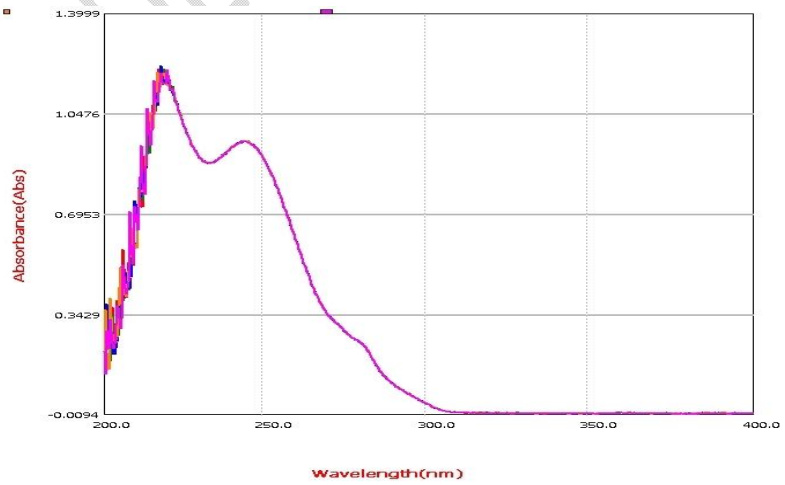

Figure 11: System precision spectrum of tapentadol and paracetamol.

3.3.2. Method Precision

Method precision was performed with marketed formulation (n=6) and the results observed were shown in the following Tables 4 and 5.

Table 4: Results of method precision

Concentration (PCM:TAP) (µg/ml)	Absorbance at 257 nm	Absorbance at 272.5 nm
26:4	0.8061	0.3068
26:4	0.8059	0.3055
26:4	0.8054	0.3083
26:4	0.8048	0.3075
26:4	0.8064	0.3055
26:4	0.8044	0.3062

Table 5: Statistical report of method precision

Parameter	At 257 nm	At 272.5 nm
Mean	0.8055	0.306633
Standard deviation	0.00078	0.001124
% RSD	0.09802	0.366459

3.3.3. Repeatability

Repeatability was performed with mixture of standard drugs(n=5) and the results observed were shown in the following Tables 6 and 7. The percentage RSD was found to be less than 2%.

Table 6: Results of repeatability

Concentration (µg/ml)	Absorbance at 257 nm			Absorbance at 272.5 nm		
	S1	S2	S3	S1	S2	S3
4:4	0.2366	0.2373	0.2372	0.0998	0.1005	0.0998
8:8	0.4374	0.4383	0.4395	0.1957	0.1957	0.1965
12:12	0.6715	0.6742	0.6754	0.3053	0.3041	0.3040

Table 7: Statistical report of repeatability

Mean		Standard deviation		% RSD	
257 nm	272.5 nm	257 nm	272.5 nm	257 nm	272.5 nm
0.237033	0.100033	0.000379	0.000404	0.159722	0.404011
0.4395	0.195967	0.001054	0.000462	0.240321	0.235693
0.6754	0.304467	0.001997	0.000723	0.296497	0.237602

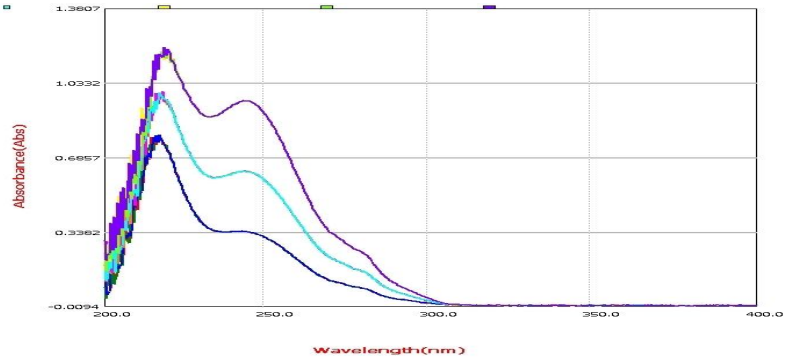

Figure 12: Repeatability spectrum of tapentadol and paracetamol.

3.3.4. Intra-day precision: Intraday precision was performed with mixture of standard drugs (n=5) and the results observed were shown in the following Tables 8 - 10. The percentage RSD was found to be less than 2%.

Table 8: Results ofintra-day precision at 257nm

Concentration (µg/ml)	Absorbance at 257 nm				
	S1	S2	S3	S4	S5
4:4	0.2150	0.2147	0.2143	0.2138	0.2134
8:8	0.3921	0.3916	0.3912	0.3909	0.3902
12:12	0.6481	0.6477	0.6470	0.6467	0.6462

Table 9: Results of intra-day precision at 272.5nm

Concentration (µg/ml)	Absorbance at 272.5 nm				
	S1	S2	S3	S4	S5
4:4	0.0983	0.0983	0.0986	0.0986	0.099
8:8	0.1724	0.1727	0.1732	0.1726	0.1724
12:12	0.3029	0.3033	0.3044	0.3033	0.3032

Table 10: Statistical report of intra-day precision

Mean		Standard deviation		% RSD	
257 nm	272.5 nm	257 nm	272.5 nm	257 nm	272.5 nm
0.21424	0.09856	0.00065	0.000288	0.303578	0.292306
0.3912	0.17266	0.000718	0.000329	0.183445	0.190336
0.6462	0.30342	0.000764	0.000572	0.117988	0.188465

3.3.5. Inter-day precision: Inter day precision was performed with mixture of standard drugs (n=5) for 5 days, once in each day and the results observed wereshown in the following Tables 11-13. The % RSD was found to be less than 2%.

Table 11: Results of inter-day precision at 257nm

Concentration (µg/ml)	Abs. at 257 nm				
	S1	S2	S3	S4	S5
4:4	0.2805	0.2736	0.2758	0.2737	0.2682
8:8	0.4880	0.4760	0.4719	0.4688	0.4664
12:12	0.8148	0.8017	0.7986	0.7882	0.7766

Table 12: Results of inter-day precision at 272.5nm

Concentration (µg/ml)	Abs. at 272.5 nm				
	S1	S2	S3	S4	S5
4:4	0.0327	0.0324	0.0321	0.0316	0.0312
8:8	0.0687	0.0684	0.0679	0.0673	0.0666
12:12	0.1255	0.1247	0.1239	0.1227	0.1216

Table 13: Statistical report of inter-day precision

Mean		Standard deviation		% RSD	
257 nm	272.5 nm	257 nm	272.5 nm	257 nm	272.5 nm
0.27436	0.0320	0.004437	0.000604	1.617055	1.887976
0.47422	0.06778	0.0085	0.000847	1.792318	1.249275
0.79598	0.12368	0.014404	0.001556	1.809579	1.25831

3.4. Recovery studies

Recovery studies were performed with both the standard drug and the marketed formulation. To a fixed concentration of marketed formulation 80%, 100% and 120% of the standard drug was added and the resulting solutions absorbance was determined. From the absorbance values amount of the drug present was calculated and then percentage recovery was calculated by using the following formula.

Percentage recovery = Amount found – Amount added / Actual amount

The percentage recovery was found to be between 95-105% and the results were shown in the following Table 14.

Table 14: Results of recovery studies for tapentadol and paracetamol

% Spiking	% Recovery ± SD	
	Tapentadol	Paracetamol
80	98.62±0.20	100.21±0.26
100	97.94±0.05	100.32±0.23
120	96.64±0.78	100.47±0.33

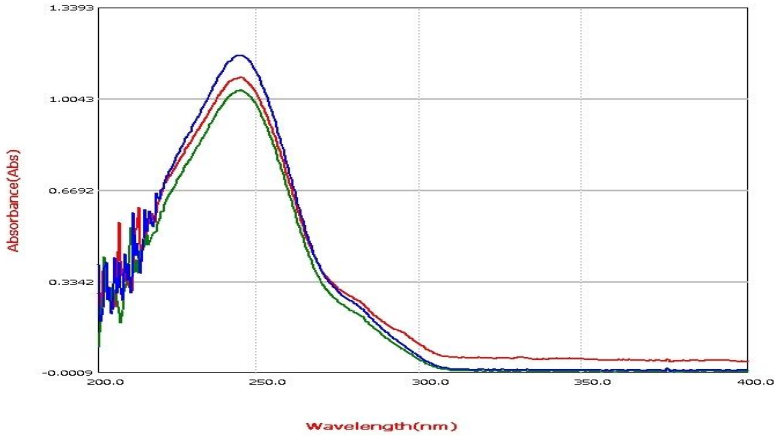

Figure 13: Accuracy spectrum of Tapentadol and Paracetamol.

3.2. Assay

The results of assay were found to be between 95-105% and were shown in the following Table 15.

Table 15: Results of assay of the marketed formulation

Dosage form	Labeled claim	Concentration (µg/ml) TAP:PCM	%Purity of TAP ±SD	%Purity of PCM ±SD
Tablet	Paracetamol 325 mg	4:26	102.4±0.99	100±0.96
		2:13	101.3±0.45	100.3±0.39
	Tapentadol 50 mg	1:6.5	99.9±0.61	100.7±0.68

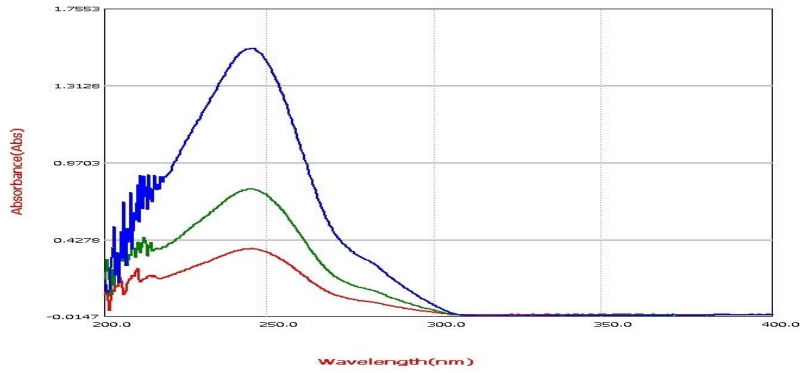

Figure 14: Assay spectrum of tapentadol and paracetamol.

4.Discussion

ZYNTAP is one of the analgesics combination drugs. The proposed method is useful so much because it is a method easy to apply. For this, UV Spectrophotometric method that enables us to quantify both the drugs without derivatization in commercial dosage forms was developed and validated.

Tapentadol and paracetamol have UV-absorbing molecules from the specific chromophores in the structure that absorb at a particular wavelength and this fact was successfully applied for their quantitative determinations using the UV spectrophotometric method.

The UV spectrum of tapentadol and paracetamol was shown in Figure 1. The standard calibration curves of tapentadol and paracetamol in 0.1N HCl were shown in Figures 7-10 at both the wavelengths.

Good linearity was observed for boththe standard drugs in the concentration range of 4-28µg/ml. The R^2 value was found to be 0.9991 and 0.9986 for tapentadol and paracetamol respectively. The LOD and LOQ values found to be 0.5576 and 1.6898 for standard tapentadol and 0.1625 and 0.4926 for standard paracetamol at 272.5nm and 257nm respectively. In the precision studies the percent relative standard deviation was found to be less than 2. In the assay the percentage purity was found to be between 95-105%. In the recovery studies the percentage recovery was found to be between 95-105%. All the parameters were validated according to the ICH guidelines.

5. Conclusion

A spectrophotometric method was developed for simultaneous estimation of tapentadol and paracetamol in their combined formulation by simultaneous equation method without prior separation. Method was found to be precise, accurate and sensitive as can be reflected from validation data. Developed method was successfully applied for estimation of tapentadol and paracetamol in marketed formulation.Our developed method was found to be accurate, precise and sensitive.

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