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Development And Validation Of Spectrophotometric Methods For Simultaneous Estimation Of Paracetamol And Tapentadol In Combined Pharmaceutical Dosage Form

Khokhar Vishal G.* and Agola Ashwin

Department of Quality Assurance, N. R. Vekaria Institute of Pharmacy, C. L. College Campus, Bilkha Road, Junagadh-362001, Gujarat, India.

***Corres.author: vishalkhokhar@gmail.com**
Phone Number: 07600986375

Abstract: Three simple, rapid, precise and accurate spectrophotometric methods have been developed for simultaneous analysis of Paracetamol (PCM) and Tapentadol (TAP) in combined pharmaceutical dosage form. Method A, Simultaneous Equation Method (Vierodt's method) applies measurement of absorptivities at two wavelengths, 257.00 nm (λmax of Paracetamol) and 289.00 nm, (λmax of Tapentadol) in zero order spectra. Method B, Absorbance Correction Method. It involves measurment at 309.00 nm (Corrected wavelenth) and 289.00 nm (λmax of Tapentadol) in zero order spectra. Method C, Dual Wavelength Method, involves measurement of difference in absorbance at 248.5 nm & 265.5 nm for estimation of Tapentadol and 282.00 nm & 296.5.00 nm for estimation of Paracetamol. Developed methods were validated according to ICH guidelines. The calibration graph follows Beer's law in the range of 6.0 to 30.0 µg/mL for both drug with R square value greater than 0.999. Accuracy of all methods was determined by recovery studies and showed % recovery between 98 to 102%. Intraday and inter day precision was checked for all methods and mean %RSD was found to be less than 2 for all the methods. The methods were successfully applied for estimation of Paracetamol and Tapentadol in marketed formulation.

Keywords: - Simultaneous Equation Method (Vierodt's method), Absorption Correction Method, Dual Wavelength Method, Paracetamol, Tapentadol.

Introduction:

Tapentadol (TAP) chemically is 3 - [(1R,2R) -3 -(dimethylamino) –1 - ethyl-2 -methylpropyl] phenol Mono hydrochloride (Fig. 1), is an agonist at the µ-opioid receptor and as a norepinephrine reuptake inhibitor [1].This dual mode of action provides analgesia at similar levels of more potent narcotic analgesics such as hydrocodone, oxycodone and morphine, but with a more tolerable side effect profile. Tapentadol is not official in Pharmacopoeia. Paracetamol (PCM) Chemically is 4-hydroxyacetanilide (Fig. 2), used as antipyretic and analgesic [2]. Paracetamol is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP) and United States Pharmacopoeia (USP) [2,3,4].

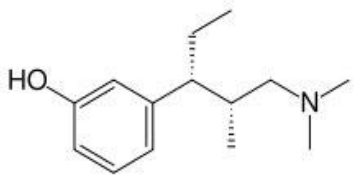

Fig. 1 Tapentadol

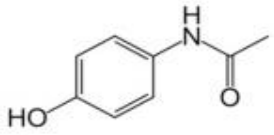

Fig. 2 Paracetamol

Objective of Study:

Survey of literature revealed that numbers of method have been reported in literature for the individual analysis of Tapentadol and Paracetamol by UV spectrophotometric and RP-HPLC method. UV spectrophotometric method available in literature for simultaneous determination of Paracetamol with other drugs like Aceclofenac, Lornoxicam, Etodolac, Nimesulide [5,6,7,8]. RP-LC method available in literature for simultaneous determination of Paracetamol with Tapentadol [9]. RP-HPLC and UV Spectrophotometric method available in literature for determination of Tapentadol [10,11,12,13]. However, to our knowledge, there is no reported uv-spectrophotometric method available for simultaneous estimation of Tapentadol and Paracetamol.

The aim of the present work was to develop easy, economic, accurate, specific and precise spectrophotometric methods for simultaneous estimation of Paracetamol and Tapentadol in bulk drugs and combined pharmaceutical formulations and validation of newly developed analytical methods.

Materials And Methods:

Apparatus and Software: Shimadzu UV-1800 double beam spectrophotometer connected to a computer loaded with Shimadzu UV Probe 2.34 software was used for all the spectrophotometric measurements. The absorbance spectra of the reference and test solutions were carried out in 1cm quartz cells over the range of 200-400 nm. Contech analytical balance with minimum weighing capacity of 1mg was used in the study.

Reagents and Chemicals:

Solvent: NaOH analytical reagent grade (Poly-Chem Limited, Mumbai, India). Water, single distilled water.

Preparation of Stock Solution: Accurately weighed PCM and TAP (in quantities of 25.0 mg) were transferred to two separate 25 ml volumetric flasks, dissolved with the use of 0.1N NaOH and volume was made up to the mark with same 0.1N NaOH to obtain stock solution of PCM (1000 µg/mL) and TAP (1000 µg/mL)

Preparation of Working Standard Solution: From the above solution, standard stocks solutions of PCM (100 µg/mL) and TAP (100 µg/mL) were prepared by transferring 1 mL aliquots to 100 mL volumetric flasks and making up the volume with 0.1N NaOH.

Preparation of Calibration Curve of Standard PCM and TAP: From working std. solution of PCM (100 µg/ml) 0.6, 1.2, 1.8, 2.4 and 3.0 mL were transferred to 10 ml volumetric flasks and volume were made up to the mark with 0.1N NaOH. This gives 6.0 to 30.0 µg/mL of PCM. From working std. solution of TAP (100 µg/mL) 0.6, 1.2, 1.8, 2.4 and 3 mL were transferred to 10 mL volumetric flasks and volume were made up to the mark with 0.1N NaOH. This gives 6.0 to 30.0 µg/mL of TAP.

<u>Method A- Simultaneous Equation Method (Vierodt's Method):</u>

If a sample containing two absorbing drug (X and Y) each of which absorbs at λmax of other. It may possible to determine both drugs by the technique of simultaneous equations (Vierodt's method) provided that certain criteria apply. The information required is the aborptivities of X at and λ1 and λ2 ax1 and ax2 respectively (a) The aborptivities of Y at and λ1 and λ2 ay1 and ay2 respectively (b) The absorbances of the diluted sample at λ1 and λ2 , A1 and A2 respectively. Let Cx and Cy be the concentrations of X and Y respectively in the diluted sample. Two equations are constructed based upon the fact that at λ1 and λ2 the absorbance of the mixture is the sum of the individual absorbance of X and Y. From the stock solutions, working standard solutions of PCM (100 µg/ml) and TAP (100µg/ml) were prepared. By appropriate dilutions, the solutions with concentrations 6.0-30.0 µg/ml (for both) were prepared and scanned between 200 to 400 nm (Fig. -3). calibration curve of

absorbance versus concentration were prepared. The calibration curves were found to be linear in the concentration range under study (Fig. 3.1). For PCM and TAP, analytical wavelengths of 257.00 nm and 289.00 nm were selected respectively. Absorptivity of PCM and TAP were calculated at both the wavelengths. The concentrations of PCM and TAP can be calculated from following equations [14] :

Cx (PCM) = (A2 ay1 – A1 ay2) / (ax2 ay1 – ax1 ay2)

Cy (TAP) = (A1 ax2 – A2 ax1) / (ax2 ay1 – ax1 ay2)

Where; Cx & Cy are concentrations of PCM and TAP respectively in gm/100 ml in the sample solution. A1 & A2 are the absorbances of the mixture at 257.00 nm & 289.00 nm respectively; aX1 and aX2 = Absorptivity of PCM at 257.00 nm and 289.00 nm; aY1 and aY2 = Absorptivity of TAP at 257.00 nm and 289.00 nm.

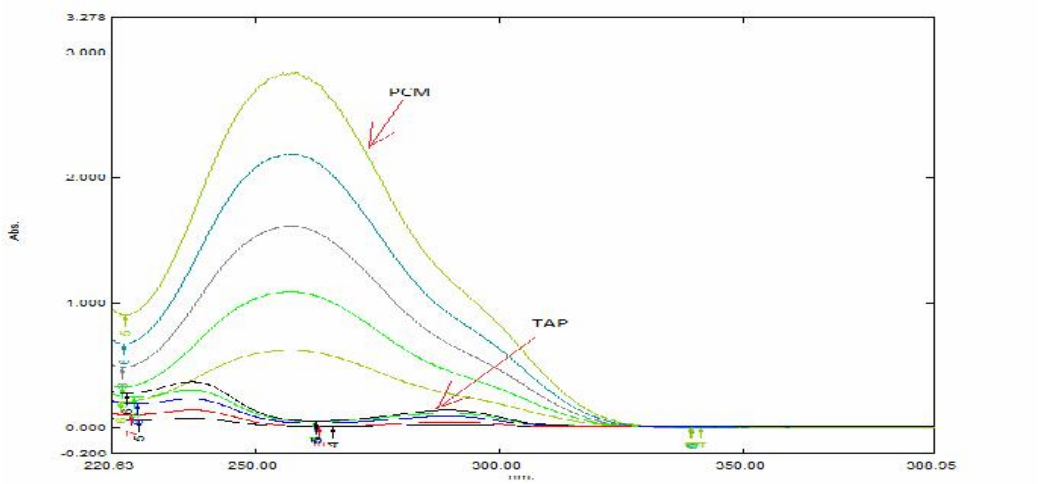

Fig -3, Zero order overlain spectra (PCM and TAP)

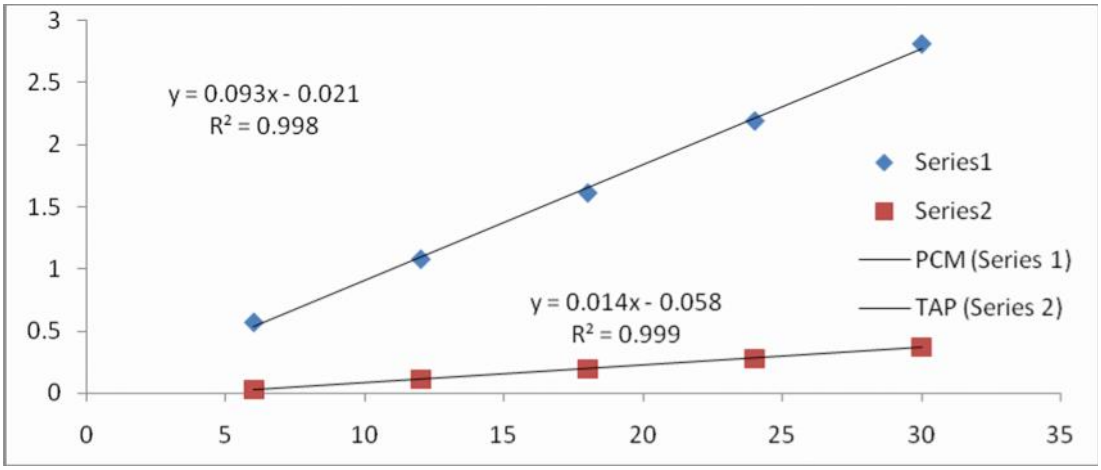

Fig. – 3.1 Calibration graph of PCM (257 nm) and TAP (289)

Method B- Absorbance Correction Method:

This method involves measurement of absorbance at 289 nm and 309 nm. At 309 nm, TAP shows no absorbance and PCM can be estimated directly without any interference of TAP. TAP shows maximum absorbance at 289 nm where PCM is having considerable interference. So, absorbance of TAP at 289 nm is corrected from total absorbance and then it is related to concentration of TAP. Calibration graphs are prepared at 289 nm and 309 nm for TAP and PCM respectively (Figure 4).

- $C_Y = \frac{A_{309\ nm}}{A\ (1\%,\ 1cm)_{309\ nm\ of}}$
- $Ay_{289\ nm} = Cy * A\ (1\%,\ 1cm)_{289\ nm\ of\ PCM}$
- $CAx_{289\ nm} = A_{289\ nm} - Ay_{289\ nm}$
- $C_x = \frac{CAx_{289\ nm}}{A\ (1\%,\ 1cm)_{289\ nm\ of\ TAP}}$

Where,

- Cx = Conc. Of TAP in gm/100ml
- Cy = Conc. Of PCM in gm/100ml
- $A_{289\ nm}$ = Absorbance of mixture at 289 nm
- $A_{309\ nm}$ = Absorbance of mixture at 309 nm
- $CAx_{289\ nm}$ = Corrected absorbance of TAP at 289 nm
- $Ay_{289\ nm}$ = Absorbance of PCM at 289 nm

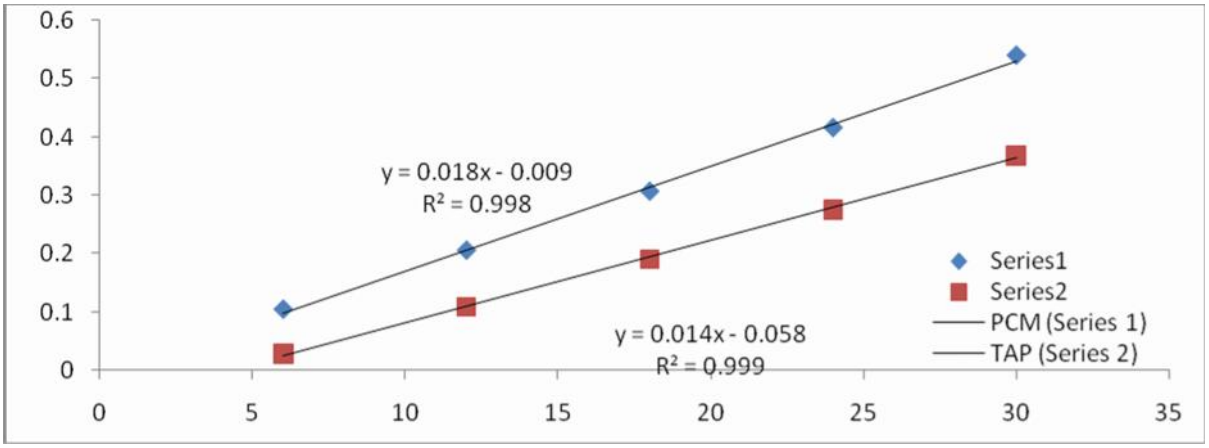

Fig. – 4 Calibration graph of PCM (309 nm) and TAP (289 nm)

Methd C- Dual Wavelength Method:

The solutions of standard PCM and TAP were prepared in the range of 6.0 to 30.0 µg/mL for both. The absorption spectra of the solutions of PCM and TAP were recorded in the range of 200 nm to 400 nm (fig - 3). For estimation of each drug, difference in the absorbance at two wavelengths was measured in zero order spectra as the difference for other drug at this two wavelength is zero. For TAP, the difference in absorbance of 282.00 nm and 296.5 nm as the difference is zero for PCM were plotted against the concentration of TAP. Similarly, for the estimation of PCM, the difference in absorbance of 248.5 nm and 265.5 nm (difference is zero for PCM) were plotted against the concentration of PCM. Calibration graph for PCM and TAP are shown below (Fig 5).

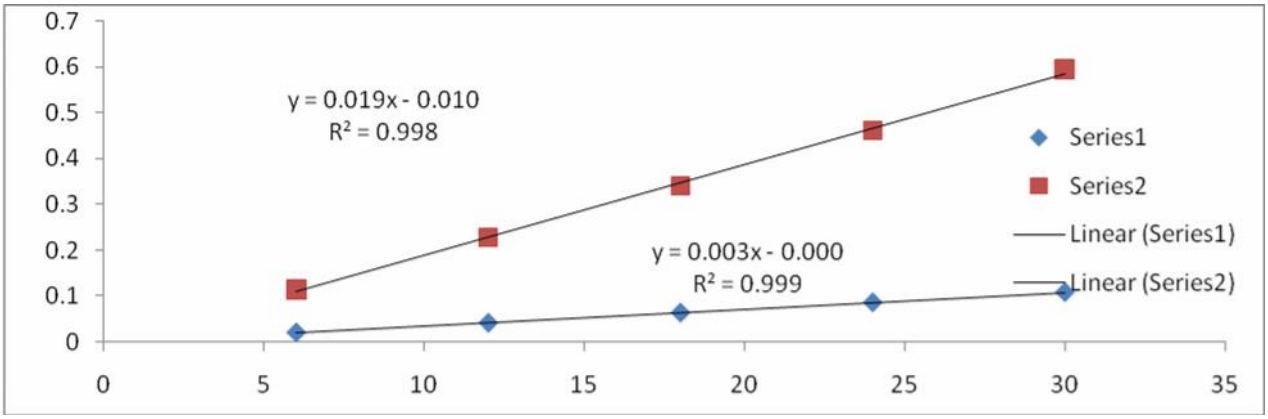

Fig. 5, Calibration graph of PCM (282.0-296.5 nm) and TAP (248.5-265.5 nm).

Assay of Commercial Formulation by Method A and B:

20 tablets were powdered and an amount equivalent to 10 mg TAP and 65 mg PCM was weighed and dissolved in 0.1N NaOH. Solutions were filtered using whatmann filter paper grade 1. Appropriate dilutions were prepared in 0.1N NaOH taking suitable aliquots of the clear filtrates and subjected to analysis using all methods described above. The result of analysis is reported **(Table 1).**

Table 1: Results of Simultaneous Estimation of Marketed Formulation for Method A and B:

Labelled Claim :- PCM : TAP (325mg : 50mg)

Method	TAP *±SD	PCM *±SD
A	99.41 ±0.0537	100.12 ±0.1201
B	100.12 ± 0.0231	100.15 ±0.0212
C	99.75±0.0989	100.34±0.0221

Results And Discussion:

Developed spectrophotometric methods for the simultaneous estimation of DIC and TOL were validated according to ICH guidelines and data complying with the standards were obtained [11]. The results of validation parameters for all the two developed methods are reported (Table 2 and 3).

Table 2: Summary of Validation Parameters by Developed Methods:

Parameters	Method A		Method B		Method C	
	TAP	PCM	TAP	PCM	TAP	PCM
Analytical wavelength (nm)	289.00	257.00	289.00	309.0	248.5 -265.5, 282.00-296.5.00	
Beer's range (µg/mL)	6-30	6-30	6-30	6-30	6-30	6-30
Slope	0.0141	0.093	0.0141	0.018	0.0036	0.0199
Intercept	0.0588	0.0213	0.0588	0.0092	0.0009	0.0109
Correlation coefficient	0.9991	0.9983	0.9991	0.998	0.9998	0.9986
Intraday precision (%RSD)	0.987	0.921	0.298	0.367	0.872	0.529
Interday precision (%RSD)	1.012	0.873	0.932	0.768	1.213	0.825
LOD (µg/mL)	1.084	0.114	1.084	0.391	0.861	0.5467
LOQ (µg/mL)	3.21	0.3463	3.219	1.185	2.611	1.9597

Table 3: Results of Recovery Study of TAP and PCM by developed methods

Method	% Spiking	% Recovery ± S.D.	
		TAP	PCM
A	50	99.54±0.0289	101.05±0.1173
	100	99.43±0.0245	99.98±0.0173
	150	99.50±0.0393	101.02±0.1224
B	50	100.34±0.0222	100.21±0.0765
	100	100.22±0.0763	100.42±0.0547
	150	101.15±0.0876	101.25±0.0231
C	50	99.54±0.0231	100.52±0.0872
	100	99.22±0.0478	100.89±0.0472
	150	99.59±0.0462	101.65±0.0382

Conclusion:

Three Spectrophotometric methods (Simultaneous equation method, Absorbance correction method and Dual wavelength method) were developed for simultaneous estimation of PCM and TAP in their combined formulation without prior separation. Methods were found to be precise and accurate as can be reflected from validation data. Developed methods were successfully applied for estimation of PCM and TAP in marketed formulation.

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