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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS FOR THE SIMULTENEOUS ESTIMATION OF PARACETAMOL AND TRAMADOL IN COMBINE DOSAGE FORM

***Mayur S. Patil, Sachin S. Rane, Milind E. Chaudhari, Prof. (Dr.) Rajesh Y. Chaudhari, Prof. (Dr.) Vijay R. Patil**

Department of Pharmaceutical Chemistry, Hon'ble Loksevak Madhukarrao Chaudhari College of Pharmacy, Faizpur. Tal. Yawal Dist. Jalgaon Maharashtra. PIN- 425503.

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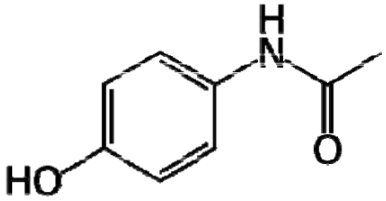
***Corresponding Author**
Mayur S. Patil
Department of Pharmaceutical Chemistry, Hon'ble Loksevak Madhukarrao ChaudhariCollege of Pharmacy, Faizpur. Tal. Yawal Dist. Jalgaon Maharashtra. PIN- 425503.

ABSTRACT

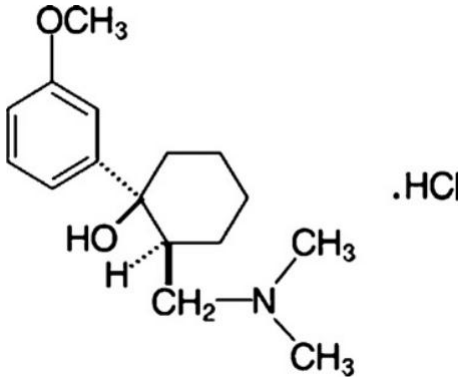
A simple, robust, precise, UV spectroscopic method has been developed for the simultaneous estimation of paracetamol and tramadol in bulk and capsule dosage forms. In this paper the estimation of those drugs was carried out by simultaneous equation method. This method is based on measurement of absorption at 243nm and 272nm i.e, λmax of paracetamol and tramadol respectively. The linearity observed for paracetamol is in the range of 10-50 µg/ml and for tramadol is in the range of 5-25 µg/ml. The accuracy of methods was assessed by recovery studies and was found to be within the range of 99%-101% for both paracetamol and tramadol. The developed methods were validated with respect to linearity, accuracy (recovery), and precision. The method can be employed for estimation of pharmaceutical formulations with no interference from any excipients and diluents. The results were validated as per ICH guidelines.

KEYWORDS: Paracetamol, Tramadol, Simultaneous estimation Method, ICH, Validation.



Paracetamol

Fig. 1: Chemical structure of Paracetamol.



Tramadol

Fig. 2: Chemical structure of Tramadol.

INTRODUCTION

Tramadol hydrochloride is a centrally acting analgesic, used for treating moderate to severe pain. Tramadol hydrochloride possesses agonist actions at the opioid receptor and effects reuptake at the noradrenergic and serotonergic systems. Tramadol is a compound with agonist activity. Chemically it is [2- (dimethylaminomethyl)-1-(3-methoxyphenyl) cyclohexanol]. It is used to treat moderate to moderately severe pain and most types of neuralgia, including trigeminal neuralgia. Paracetamol is official in Indian Pharmacopoeia and British Pharmacopoeia.[1] Paracetamol Chemically is *N*-(4-hydroxyphenyl)acetamide and I.P. & B.P. both suggest titrimetric and UV spectrophotometric assay method for paracetamol in bulk and tablet formulations is available in the form of oral drops, tablets, capsules and injections.[2,3] There are various methods available for estimation of tramadol hydrochloride like UV spectrophotometric[3], spectrofluorometry,[4] HPLC,[5] gas chromatography,[6] GC-MS and LCMS,[7] capillary electrophoresis,[8] HPTLC,[9] HPTLC-densitometry[10,11] etc. Paracetamol estimated simultaneously with other drugs by UV and RP-HPLC methods.[13,14] also there are some UV, HPLC and other methods for estimation in combination, however some of these methods are costlier and time consuming. To overcome these difficulties spectrophotometric analysis serves to be the quickest, promising and reliable method for routine analytical needs. The aim of the present study is to develop a new simple, rapid, reliable and precise UV spectrophotometric method for analysis of tramadol from tablet formulation; method is based on measurement of UV absorbance of tramadol hydrochloride in methanol diluted with distilled water.

MATERIALS AND METHODS

Instrument

The proposed work was carried out on a Shimadzu UV-visible spectrophotometer (model UV-1800 series), which possesses a double beam double detector configuration with a1 cm quartz matched cell. All weighing was done on electronic balance (Sansui-vibra DJ-150S-S). A Fast clean ultrasonicate cleaner (India) was used for degassing the mobile phase.

Chemicals and Reagents

Tramadol was procured from Aristo Laboratories Ltd. Indore M.P. and paracetamol from Ajanta Pharmaceuticals, Jalgaon M.S. The commercial pharmaceutical formulation 'Calpol T' tablets, manufactured by Glaxo Smith Kline. Ltd. Mumbai, containing 325 mg of PCM and 37.5 mg of TRD was collected from local market. Dibasic sodium phosphate, monobasic sodium phosphate and water used were of analytical grade (Qualigens Fine Chemicals, Mumbai, India). A 0.45 µm nylon filter (Pall life Sciences, Mumbai, India) was used. All other chemicals and reagents used were analytical grade unless otherwise indicated.

Selection of Solvents

On the basis of solubility study Phosphoric acid buffer pH 8.0 was selected as the solvent for dissolving PCM and TRD.

Preparation of stock solution and selection of wavelength

A) Paracetamol Stock Solution[A]

An accurately weighed quantity of PCM (10 mg) was taken in 10mL volumetric flask and dissolved in Phosphoric acid buffer pH 8.0 (10 mL) with the help of ultrasonication for about 10 min. Then the volume was made up to the mark using Phosphoric acid buffer ph8.0 to get Paracetamol standard stock solution (1 mg / mL).

B) Paracetamol Working Standard Solution [A1]

Paracetamol standard stock solution 1 mL was diluted to 10 mL using Phosphoric acid buffer pH 8.0 to get working standard solution100 µg / mL.

C) Tramadol Stock Solution [C]

An accurately weighed quantity of TRD (10 mg) was taken in 10mL volumetric flask and dissolved in Phosphoric acid buffer pH 8.0 (10 mL) with the help of ultrasonication for about

10 min. Then the volume was made up to the mark using Phosphoric acid buffer pH 8.0 to get Tramadol standard stock solution (1 mg / mL)

D) Tramadol Working Standard Solution [C1]

Tramadol standard stock solution 5 mL was diluted to 50 mL using Phosphoric acid buffer pH 8.0 to get working standard solution 100 µg / mL.

Determination of λ Max of Individual Component

An appropriate aliquot portion of PCM and TRD were transferred to two separate 10 mL volumetric flasks, the volume was made up to the mark using Phosphoric acid buffer ph8.0 to Obtain PCM (50 µg/mL) and TRD (5 µg/mL). Drug solutions were scanned separately between 200 nm to 400 nm.PCM shows **λ** max at 243 nm while TRD at 272 nm, respectively (Figure1).

Overlay Spectra of Paracetamol and Tramadol

The overlain spectrum of both drugs was recorded (Fig.1) and two wavelengths 274.0 nm (λ max of PCM) and 250.2 nm (λ max of TRD) were selected for further study.

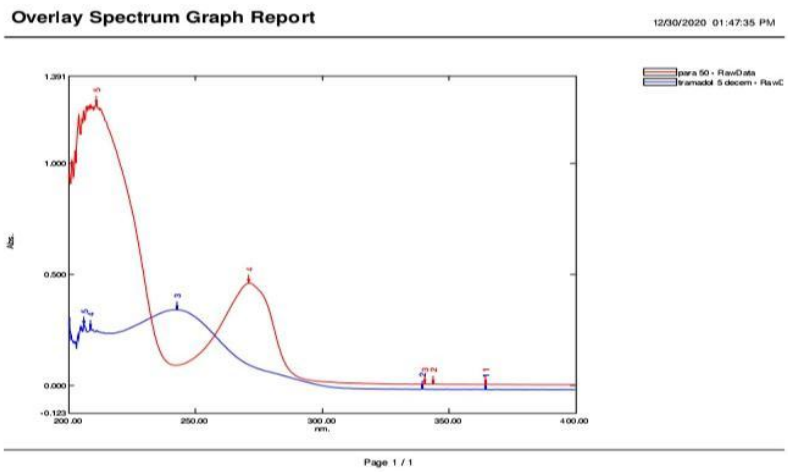


Fig. 3: Overlay Spectra of Paracetamol and Tramadol.

Linearity Study for Paracetamol

An accurately measured aliquot portion of working standard solution of PCM was transferred to five separate 10 mL volumetric flasks. The volume was made up to the mark using phosphoric acid buffer pH 8.0 to obtain concentrations (10-50 µg/mL). Absorbance of these solutions was measured at 243 nm, (Table1) Calibration curve was plotted, absorbance Vs concentration as shown in (Fig. 4).

Linearity Study for Tramadol

Accurately measured aliquot portions of working standard solution of TRD were transferred to five separate 10 mL volumetric flasks. The volume was made up to the mark using phosphoric acid buffer pH 8.0 to obtain concentrations (5-25 µg/mL). Absorbance of these solutions was measured at 272 nm, (Table 1). Calibration curve was plotted, absorbance Vs concentration as shown in (Fig. 5).

Table 1: Regression and Optical characteristics of PCM and TRD.

Parameters	Value For PCM	Value For TRD
Beer's law limit (µg/mL)	10-50	5-25
Correlation Coefficient (r)	0.995	0.997
Regression equation		
Slope	0.005	0.026
Intercept	0.011	0.057

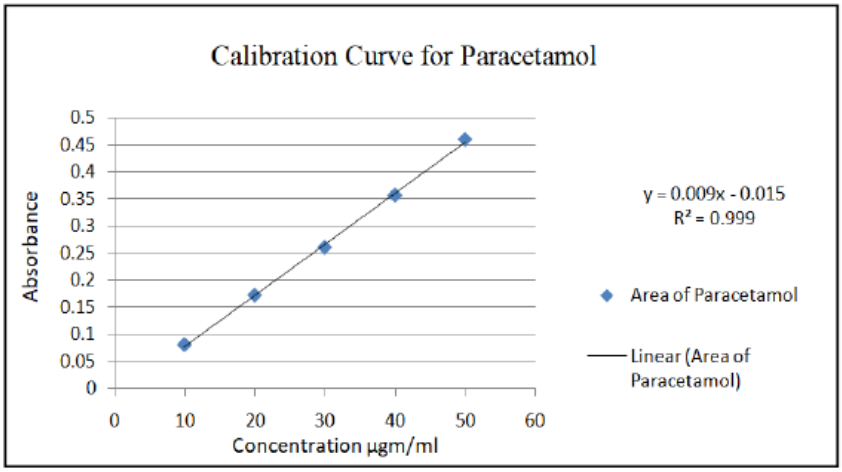


Fig. 4: Calibration Curve of Paracetamol at 243 nm Wavelength.

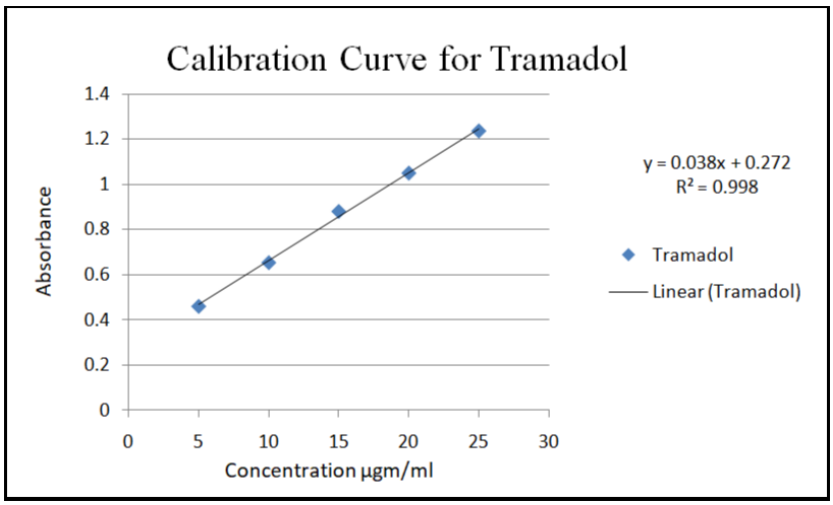


Fig. 5: Calibration Curve of Tramadol at 272 nm Wavelength Estimation of Laboratory Mixture by Proposed Method.[15]

In order to see the feasibility of proposed method for simultaneous estimation of PCM and TRD in marketed pharmaceutical formulations, the method was first tried for estimation of drugs in standard laboratory mixture. Accurately weighed PCM (500 mg) and TRD (50 mg) were taken in 100 mL volumetric flask, dissolved in Phosphoric acid buffer ph8.0 (60 mL) with the help of ultrasonication for about 10 min and the volume was made up to mark using the same. Appropriate aliquot portion (1 mL) was transferred to 10 mL volumetric flask and further diluted using Phosphoric acid buffer ph8.0 to get PCM (5 g/ mL) and TRD (50 g/ mL). The absorbance was recorded at 243 nm and 272 nm against solvent as blank.

Amount of each drug was estimated using following equations,

$$C_x = \frac{A_2 \times ay_1 - A_1 \times ay_2}{ax_2\, ay_1 - ax_1\, ay_2}$$

$$Cy = \frac{A_1 \times ax_2 - A2 \times ax_1}{ax_2\, ay_1 - ax_1\, ay_2}$$

Where;

A1 and A2 are the absorbance of diluted mixture at λ1 and λ2

Cx and Cy are the concentration of X and Y respectively

ax1 and ax2 are absorptivities of X at λ1 and λ2 respectively

ay1 and ay2 are absorptivities of Y at λ1 and λ2 respectively

The results are reported in (Table 2).

Table 2: Results of Estimation of Paracetamol and Tramadol Standard Laboratory Mixture.

Analyte	%Concentration estimated (Mean ±S.D.)	%R.S.D.
PCM	99.63 ± 0.15270	0.153264
TRD	99.84 ± 0.39820	0.398815

*Average of five determinations; R.S.D. = Relative Standard Deviation

Application of the Proposed Method for Estimation of Drugs in Tablets

Twenty 'Calpol T' Tablets powder containing PCM (325 mg) and TRD (37.5 mg) was weighed and ground to fine powder. A quantity of sample equivalent to PCM (50 mg) and TRD (5 mg) was transferred into 100 mL volumetric flask containing Phosphoric acid buffer pH 8.0 (60 mL), sonicated for 15 min and the volume was made up to the mark and filtered

through Whatman filter paper (No. 45). This solution was (1 mL) transferred to 10 mL volumetric flaks, dissolved and volume was adjusted to the mark. The absorbance of the solutions was measured at 243 nm and 272 nm against blank. The concentrations of two drugs in sample were determined by using simultaneous equations.

The results are reported in the (Table 3).

Table 3: Results of Estimation of Paracetamol and Tramadol in Tablets.

Analyte	Label claim (mg/tab)	% Label claim estimated (Mean ± S.D.)		%R.S.D.
PCM	500	99.72 ±	0.64	0.3294038
TRD	50	10.90 ±	0.84	0.15961778

*Average of five determinations; S.D. =Standard Deviation

Validation of Proposed Method

The Proposed method was validated as per the ICH guidelines.[16,17]

Accuracy [Recovery Study]

Accuracy of proposed method was ascertained on the basis of recovery study performed by standard addition method. A known amount of standard drug solutions were added to the tablet powder to make final concentrations in the range of 80%, 100% and 120% and re-analyzed it by the proposed method. The absorbance recorded and the % recoveries were calculated using formula.

$$\% \text{ Recovery} = [A - B/ C] X 100$$

Where,

A = Total amount of drug estimated.

B = Amount of drug found on preanalysed basis.

C = Amount of Pure drug added.

The results are reported in (Table 4).

Table 4: Recovery Study.

Drug in mixture solution (µg/mL)		%Recovery ± S.D.	
PCM	**TRD**	**PCM**	**TRD**
30	5	99.58±0.29	99.58±0.59
40	10	99.67±0.64	99.72±0.64

15 99.52±0.84 99.60±0.84 S.D. =Standard Deviation

Precision

Precision was determined as intra-day and inter-day variations. Intra-day precision was determined by analyzing PCM (30, 40, and 50 µg/mL) and TRD (5, 10, and 15 µg/mL) for three times on the same day. Inter-day precision was determined by analyzing the same concentration of solutions for three different days over a period of week. The results are reported in (Table 5).

Table 5: Precision Study.

Precision	Paracetamol	%R.S.D.	Tramadol	%R.S.D.
Interday, n = 3	99.25 ±0.2316	±0.298	99.97 ± 0.2643	±0.2356
Intraday, n = 3	99.01 ±0.1520	±0.1591	99.20 ± 0.3516	±0.3271

RSD = Relative standard deviation

Ruggedness

Ruggedness of the proposed method was determined by analysis of aliquots from homogenous slot by two different analyst using same operational and environmental conditions. The results are reported in (Table 6).

Table 6: Ruggedness Study.

	PCM50 µg/mL Amount Found in µg/mL Mean S.D. (n = 3)	% R.S.D.	TRD 5 µg/mL Amount Found in µg/mL Mean S.D.(n = 3)	% R.S.D.
Analyst-I	49.17 0.7549	0.7879	4.85 0.2645	0.2104
Analyst-II	49.98 0.1892	0.1732	4.90 0.6658	0.6296
Day-I	49.91 0.3294	0.3824	4.75 0.1953	0.1559
Day-II	49.96 0.9470	0.9369	4.88 0.8911	0.8140

LOD: Limit of detection of Paracetamol and Tramadol were found to be 1.9233289 µg/mL and 1.39119137 µg/mL respectively.

LOQ: Limit of Quantitation of Paracetamol and Tramadol were found to be 5.82826941 µg/mL and 4.21573143 µg/mL respectively.

RESULTS AND DISCUSSION

A simultaneous UV Spectrophotometric Estimation method was developed for PCM and TRD. The method employs 243 nm as λ1 and 272 nm as λ2 for formation of equations. PCM and TRD obeys Beer's law in the concentration range 10-50 µg/ml (R^2=0.9961) and 5-25 µg/ml (R^2=0.9996) respectively. The mean recovery for PCM and TRD was found to be99.59

% and 99.63 % respectively. The developed method were validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values.

CONCLUSION

The proposed simultaneous UV Spectrophotometric Estimation method presented in this paper has advantages of simplicity, accuracy, precision and convenience for quantitation of PCM and TRD. The proposed method can be used for the quality control of PCM and TRD in typical laboratories.

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