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Validated Spectrophotometric Method for the Determination of Paracetamol and Tamadol Hydrochloride in Tablet dosage form

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Abstract:

A simple, precise and accurate method was developed for the estimation of Paracetamol (PCM) and Tramadol Hydrochloride (TRM) in tablet dosage form using second order derivative spectrophotometry. Wavelengths selected for quantitation were 264 nm for Paracetamol (zero crossing point of TRM) and 224.06 nm for Tramadol Hydrochloride (zero crossing point of PCM). The method was validated with respect to linearity, accuracy, precision, limit of detection and limit of quantitation in accordance with the international conference on harmonisation (ICH) guidelines. Linearity was observed in concentration range of 2-14 µg/ml for each Paracetamol and Tramadol Hydrochloride. The limit of detection and limit of quantitation were found to be 0.396 µg/ml and 1.2 µg/ml for Paracetamol while 0.429 µg/ml and 1.3 µg/ml for Tramadol Hydrochloride, respectively. The percentage recovery of Paracetamol and Tramadol Hydrochloride was found to be 99.29 ± 0.57 and 99.61 ± 0.29, respectively. The % R.S.D. values for intra-day and inter-day precision study were <2.0%, confirming that the method was precise. The method can be successfully employed for the simultaneous estimation of Paracetamol and Tramadol Hydrochloride in tablet dosage form.

Keywords: Paracetamol, Tramadol Hydrochloride, Recovery, Second derivative, Validation.

Introduction

Paracetamol (PCM) is chemically N-(4-hydroxyphenyl) acetamide (Figure 1) also known as acetaminophen [1], is a popular analgesic and antipyretic drug widely used for management of pain and fever [2]. It is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP), United States Pharmacopeia (USP) and Japanese Pharmacopoeia (JP). IP [3] and JP [4] describe UV method for its estimation, IP and USP [5] describes liquid chromatography method for its estimation, while EP [6], BP [7] describes titration method for its estimation. Literature survey reveals UV [8-9], HPLC[10], GC [11], LC-MS [12] methods for estimation of PCM alone.

Literature survey also reveals UV [13-16], HPLC [17-22] HPTLC [23] methods for determination of PCM with other drugs in combination. Tramadol hydrochloride (TRM) is a well known anti-inflammatory analgesic drug[24]. Chemically it is (1RS, 2RS)-2-[(Dimethylamino) methyl]-1-(3-methoxyphenyl) cyclohexanol hydrochloride [25] (Figure 2). It is official in Indian Pharmacopoeia (IP), British pharmacopoeia (BP) and European pharmacopoeia (EP). IP [26], BP [27] & EP [28] describes potentiometric titration and HPLC methods for its estimation. Literature survey reveals UV spectroscopy [29], HPLC [30], Chemiluminance [31] methods for determination of TRM.

Literature survey also reveals UV spectroscopy [32-34], HPLC [35-36], methods for the determination of TRM with other drugs combination. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of PCM and TRM in their combined synthetic mixture or dosage forms. Literature survey reveals only simultaneous equation method [37], area under curve [38] and RP-HPLC [39] method for PCM and TRM in combined dosage forms. The present manuscript describes simple, sensitive, rapid, accurate, precise and cost effective second order derivative spectrophotometric method for simultaneous estimation of both drugs in tablet dosage form.

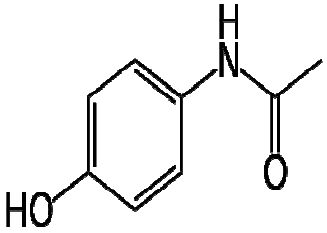

Figure 1- Chemical structure of Paracetamol

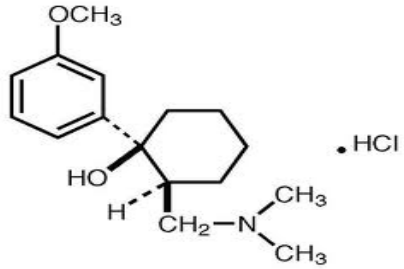

Figure 2- Chemical structure of Tramadol HCL

MATERIALS AND METHODS

APPARATUS

A Shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe 2.0 system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

REAGENTS AND MATETIALS

Paracetamol (PCM) and Tramadol Hydrochloride (TRAMA) were kindly supplied as a gift samples from Anvi Lifescience Pvt. Ltd., Mehsana, Gujarat, Laboratory prepared Distilled Water (Millipore, Catalogue no TANKPE030, Made in France) as a solvent and Whatman filter paper no. 41 (Millipore, USA) were used in the study.

PREPARATION OF STANDARD STOCK SOLUTION

An accurately weighed PCM and TRM powder (10 mg) were weighed and transferred to 100 ml separate volumetric flasks and dissolved in distilled water. The flasks were shaken and volumes were made up to mark with distilled water to give a solution having concentration 100 µg/ml for both of drugs.

DETERMINATION OF WAVELENGTH FOR MEASUREMENT

The standard solutions of PCM (10 µg/ml) and TRM (10 µg/ml) were scanned separately in the range of 200-400 nm. The zero order spectra thus obtained was then processed to obtain second derivative spectrum. The zero crossing points were found to be 224.06 nm and 264 nm for PCM and TRM, respectively (Figure 2). Wavelengths selected for quantification were 264 nm for PCM (zero crossing point for TRM) and 224.06 nm for TRM (zero crossing point for PCM).

CALIBRATION CURVE (LINEARITY)

The calibration curves were plotted over a concentration range of 2-14 µg/ml for both the drug. Accurately measured mix standard solutions of PCM and TRM (0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with distilled water. The absorbance of the derivatised spectra was measured at 264 nm and 224.06 nm for PCM and TRM, respectively with distilled water as blank. Six replicate analysis were carried out. $d^2A/d\lambda^2$ Vs concentration were plotted to obtain the calibration curve. Both drugs obey the Beer's law between 2-14 µg/ml with R^2 value of 0.9990 and 0.9998 for PCM and TRM, respectively.

METHOD PRECISION (REPEATABILITY)

The precision of the instrument was checked by repeated scanning and measuring the absorbance of solution of (n = 6) PCM and TRM (10 µg/ml) without changing the parameters of proposed method.

INTERMEDIATE PRECISION(REPRODUCIBILITY)

The intra-day and inter-day precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of PCM and TRM (6, 10 and 12 µg/mL for both PCM and TRM). The result was reported in terms of relative standard deviation (% RSD).

LIMIT OF DETECTION (LOD) & LIMIT OF QUANTIFICATION (LOQ)

LOD and the LOQ of the drug were calculated using the following equations as per ICH guidelines.

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma /S$$

Where σ = the standard deviation of the response

S = Slope of calibration curve.

ACCURACY (% RECOVERY STUDY)

The accuracy of the method was determined by calculating recovery of PCM and TRM by the standard addition method. PCM (2.8, 3.5 and 4.2 µg/ml) and TRM (8, 10, 12 µg/ml) were added to prequantified sample solutions of PCM (3.5 µg/ml) and TRM (10 µg/ml). The amount of PCM and TRM were determined by applying obtained values to the regression equation of the calibration curve. The accuracy was repeated for three times at each level.

ANALYSIS OF PCM AND TRAMA IN TABLET

The response of the sample solution was measured at 264 nm and 224.06 nm for quantitation of PCM and TRM, respectively. The amounts of the PCM and TRM present in the sample solution were calculated by fitting the responses into the regression equation for PCM and TRM in the proposed method.

RESULTS AND DISCUSSION

Zero-order absorption spectra of 10 µg/ml of each of PCM and TRM showed overlapping peaks in Figure 3. Distilled water was used as the solvent. Since both the drugs exhibit good solubility in it and no interference due to excipients of the tablet formulation were observed. Derivative spectroscopy, based on a mathematical transformation of the spectra zero-order curve into the derivative spectra, allows a fast, sensitive and precise resolution of a multicomponent mixture and overcomes the problem of overlapping of a multicomponent system. Derivative spectroscopy on the basis of zero-crossing measurements involves measurement of the absolute value of the total derivative spectrum at an abscissa value corresponding to the zero-crossing wavelength of the derivative spectra of individual components, which should be only a function of the concentration of other component. The spectroscopic parameters including derivative order, wavelength and $\Delta\lambda$ values should be optimized to obtain maximum resolution, sensitivity and reproducibility. In this study second derivative technique (D2) traced with $\Delta\lambda$= 8 nm was used to resolve the spectral overlapping. The optimums D2 values without interference for PCM and TRM were 264 and 224.06 nm, , respectively (Figure 4).

The linearity of the method was established from second derivative spectra by measurement of the absorbance of standard solutions. The calibration curves were constructed by plotting the D2 value against PCM and TRM at 264 nm and 224.06 nm, respectively. Linear correlation was obtained between second derivative absorbance versus concentrations of PCM and TRM in the range of 2-14 µg/ml for both the drugs. The linear equations for the calibration plots were y = 0.0001x and y = 0.0001x with regression (r2) being 0.9990 and 0.9998 for PCM and TRM, respectively. The precision of the method was expressed as relative standard deviation (RSD %). The % R.S.D. values for intraday precision study and inter-day study listed in (Table 3) were <2.0%, confirming that the method was sufficiently precise.

The % RSD for absorbance values of PCM and TRM were found to be 1.03 and 1.41, respectively, as given in Table 1. The recovery was found to be 99.29 % for PCM and 99.61 % for TRM (Table 2). The LOD and LOQ values were 0.396 µg/ml and 1.3 µg/ml for PCM while 0.429 µg/ml and 1.4 µg/ml for TRM, respectively. When the synthetic mixture was analyzed, the % purity was found to be 99.88 % for PCM and 99.51 % for TRM (Table 3).

CONCLUSION

In this proposed method the linearity is observed in the concentration range of 2-14 µg/ml with co-efficient of correlation, (r^2) = 0.9990 for PCM at 264 nm and (r^2) = 0.9998 for TRM at 224.06 nm. The result of the analysis of tablet by the proposed method is highly reproducible and reliable and it is in good agreement with the taken amount of the drug. The additives present in tablet did not interfere with determination of PCM and TRM. The method can be used for the routine analysis of the PCM and TRM in combined dosage form without any interference of excipients.

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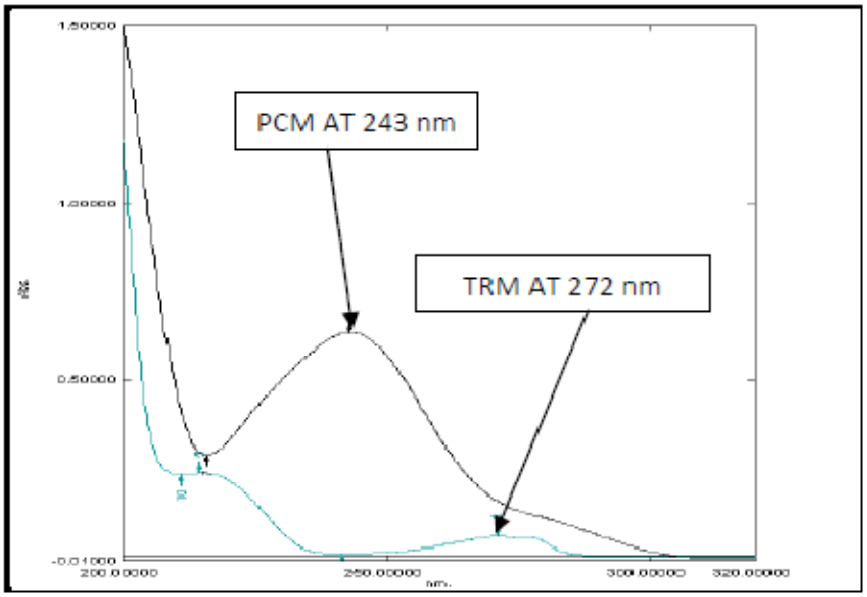

Figure 3: Zero order overlain spectra of PCM (10 µg/ml) and TRM (10 µg/ml)

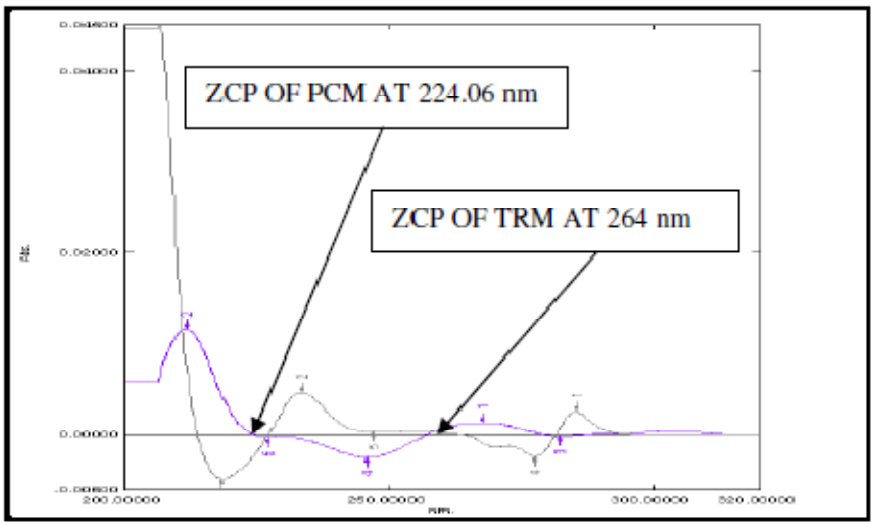

Figure 4: Overlain second order derivative spectra of PCM and TRM

Table 1 Regression Analysis Data and Summary of Validation Parameter for the Proposed Method

Validation parameters		PCM	TRAMA
Analytical Wavelength (nm)		264	224.06
Linearity		2-14 µg/ml	2-14 µg/ml
Regression equation y=mx+c		y = 0.0001x	y = 0.0001x
Correlation coefficient (r2)		0.9990	0.9998
Repeatability (n=6) % RSD		1.03280	1.41772
Intermediate Precision	Intra-day (n=3)	0.52-1.16	0.58-1.21
	Inter-day (n=3)	0.77-1.49	0.88-1.54
%Recovery (n=3) ± SD	Level 1	99.28% ± 1.05	98.625% ± 0.28
	Level 2	100.57% ± 0.21	99.1% ± 0.20
	Level 3	98.08% ± 0.45	101.16% ± 0.38
Limit of Detection (µg/ml)		0.396	0.429
Limit of Quantification (µg/ml)		1.2	1.3
% Assay ± SD (n= 6)		99.88 ± 0.80	99.51 ± 0.45

RSD = Relative standard deviation.

LOD = Limit of detection.

LOQ = Limit of quantification.

S.D. = standard deviation

Table 2: Recovery data of proposed method

DRUG	LEVEL	Amount of Sample Taken (µg/ml)	Amount of Standard Spiked (µg/ml)	Total Amount Recovered (µg/ml)	% Mean Recovery ± SD (n=3)
PCM	80%	3.5	2.8	2.78	99.28% ± 1.05
	100%	3.5	3.5	3.52	100.5% ± 0.21
	120%	3.5	4.2	4.15	98.08% ± 0.45
TRAMA	80%	10	8	7.89	98.62% ± 0.28
	100%	10	10	9.91	99.10% ± 0.20
	120%	10	12	12.14	101.1% ± 0.38

S. D. is Standard deviation and n is number of replicate

Table 3 Analysis of Sample by proposed method (n = 6)

Sample No.	Label claim (mg)		Amount found (mg)		% Label claim (n = 6)	
	PCM	TRM	PCM	TRM	PCM	TRM
1	325	37.5	326.4	37.27	100.44	99.41
2	325	37.5	322.1	37.32	99.12	99.52
3	325	37.5	320.7	37.20	98.66	99.22
4	325	37.5	324.1	37.13	99.72	99.02
5	325	37.5	322.7	36.87	99.31	98.32
6	325	37.5	327.4	37.19	100.74	99.18
MEAN			323.89	37.16	99.66	99.11
S.D.					0.80	0.45

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