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The Simultaneous Estimation of Paracetamol and Tolperisone Hydrochloride in Tablet by UV Spectrophotometric Methods

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ABSTRACT:

Two methods for simultaneous estimation of Paracetamol and Tolperisone Hydrochloride in combined tablet dosage form have been developed using Water as a solvent. The first UV spectrophotometric method was a determination using the simultaneous equation method at 242.5 nm and 260 nm. The second UV spectrophotometric method is the Q – analysis (absorption ratio) method, which involves the formation of absorbance equation at 254 nm (isoabsorptive point) and at 260 nm the maximum absorption of Tolperisone Hydrochloride. The linearity ranges for Paracetamol and Tolperisone Hydrochloride were 4-12 µg/ml and 2-18 µg/ml respectively. The accuracy of the methods was assessed by recovery studies was found to be 102.03 ± 3.79and 98.93 ± 0.90 for simultaneous equation method and 100.4 ± 1.80 and 99.40 ± 1.25 for Q analysis (absorption ratio) method for Paracetamol and Tolperisone Hydrochloride respectively. These methods are simple, accurate and rapid; those require no preliminary separation and can therefore be used for routine analysis of both drugs in quality control laboratories.

Key Words: Paracetamol , Tolperisone Hydrochloride, Q–analysis spectrophotometric method.

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Introduction:

Paracetamol (PCM) is chemically 4-hydroxyacetanilide [1], is an Analgesic and antipyretic, used for the relief of fever as well as aches and pains associated with many conditions [2]. It is official in Indian Pharmacopoeia (IP), British Pharmacopoeia (BP) and United States Pharmacopoeia (USP). IP[3] and BP[4] describe UV Spectroscopic method, while USP[5] describes Liquid Chromatographic Method for its estimation in Tablet Dosage Form. Various methods like , RP-HPLC, validated HPLC, HPTLC, Paper Chromatography, Colorimetric Methods, etc. methods[6] for estimation of Paracetamol in API & Formulations, are reported in literature for estimation of PCM in pharmaceutical dosage forms as well as in biological fluids. Tolperisone Hydrochloride is chemically 1-piperidino-2-methyl-3-(p-tolyl)-3 propanonehydrochloride [1], is a centrally acting Muscle Relaxant for the Symptometic treatment of Spasticity and Muscle Spasm [2]. Tolperisone Hydrochloride is official in Japanese pharmacopoeia JP15. JP15 [7] describes Potentiometric Titration for its estimation. Various methods like Colorimetric[8],[9], UV spectrophotometric[10] , Extractive Spectroscopic[11] , HPTLC[12] & HPLC[13],[14] methods for estimation of TOL are reported in literature for estimation of TOL in pharmaceutical dosage forms as well as in biological fluids. The combined dosage forms of PCM and TOL are available in the market for the treatment of Muscle pain or spasm. Deep literature survey reveals that, not a single analytical method is reported for the determination of these drugs in combined dosage forms. The present manuscript describes simple, accurate, precise, rapid and economic spectrophotometric methods for simultaneous estimation of PCM and TOL in tablet dosage form using distilled water as a solvent.

MATERIALS AND METHODS

Chemicals and Reagents

PCM and TOL bulk powder was kindly gifted by A.P.M.C. Pharmacy College, Himatnagar, Gujarat, India and Zydus Healthcare, Changodar, Ahmedabad, Gujarat, India Respectively. The commercial fixed dose combination Tolpidol plus was procured from the local market. All other chemicals used were of analytical grade. Distilled water and caliberated glass wares were employed throughout the work.

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. A Reptech electronic weighing analytical balance based on EMFC technology and a Toshcon ultrasonic bath (Toshniwal process instrument pvt ltd.) was used in the study.

Preparation of standard stock solutions

An accurately weighed quantity of PCM (100 mg) and TOL (100 mg) were transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with distilled water to obtain standard solution having concentration of PCM (1000 µg/ml) and TOL (1000 µg/ml). Accurately measured 10 ml of both the solutions were transferred to 100ml of volumetric flask and diluted to the mark with distilled water to obtain solution having concentration 100 µg/ml of PCM and TOL.

Method 1 :

The standard solutions of PCM (10 µg/ml) and TOL (10 µg/ml) were scanned separately in the UV range of 200-400 nm to determine λmax of both the drugs. The λmax of PCM and TOL were found to be 242.5nm and 260 nm respectively (Fig.1). Five standard solutions having concentration 4, 6, 8, 10 and 12 µg/ml for PCM and 2, 6, 10, 14 and 18 µg/ml for TOL were prepared in distilled water using the solutions having concentration 100 µg/ml. The absorbance of resulting solutions was measured at 242.5nm and 260nm and calibration curves were plotted at these wavelengths. The absorptivity coefficients of these two drugs were determined using calibration curve equations. The concentration of PCM and TOL in sample solution was determined by solving the respective simultaneous equations generated by using absorptivity coefficients and absorbance values of PCM and TOL at these wavelengths. The absorbance and absorptivities values at the particular wavelength were substituted in the following equations to obtain the concentration [15].

$$C_x = \frac{A^2 a^1{}_y - A^1 a^2{}_y}{a^2{}_x a^1{}_y - a^1{}_x a^2{}_y} \quad \ldots (1)$$

$$C_y = \frac{A^1 a^2{}_x - A^2 a^1{}_x}{a^2{}_x a^1{}_y - a^1{}_x a^2{}_y} \quad \ldots (2)$$

Where,

A^1, A^2 — absorbance of the mixture,
$a^1{}_x$, $a^2{}_x$ – denotes absorptivities of the x at 242.5nm and 260nm respectively,
$a^1{}_y$, $a^2{}_y$ — denotes absorptivities of Y at 242.5nm, 260nm respectively,
C_X = concentration of PCM.
C_Y = concentration of TOL.

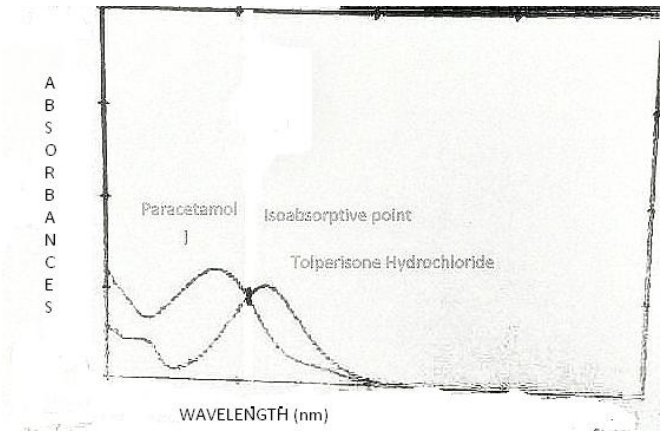

Figure 1: Overlain Spectra of Paracetamol and Tolperisone Hydrochloride

Method 2:

The standard solutions of PCM (10 µg/ml) and TOL (10 µg/ml) were scanned in the UV range of 200-400 nm to determine isoabsorptive point. The isoabsorptive point was found to be 254nm (Figure.1). Five standard solutions having concentration 4, 6, 8, 10 and 12 µg/ml for PCM and 3, 6, 9, 12 and 15 µg/ml for TOL were prepared in distilled water using the solutions having concentration 100 µg/ml. The absorbance of resulting solutions was measured at 254nm (isoabsorptive point) and 260nm (λmax of TOL) and calibration curves were plotted at these wavelengths. The absorptivity coefficients of these two drugs were determined using calibration curve equations. The concentration of PCM and TOL in sample solution was determined by solving the respective Q-analysis equations generated by using absorptivity coefficients and absorbance values of PCM and TOL at these wavelengths. The absorbance and absorptivities values at the particular wavelength were substituted in the following equations to obtain the concentration [15].

For PCM

$$C_x = \frac{Q_m - Q_y}{Q_x - Q_y} \times \frac{A_1}{AX_1} \quad \ldots (3)$$

For TOL

$$C_y = \frac{Q_m - Q_x}{Q_y - Q_x} \times \frac{A_1}{AX_1} \quad \ldots (4)$$

Where,

$$Qm = \frac{\text{Absorbance of sample at 260 nm}}{\text{Absorbance of sample at 254 nm}}$$

$$Qx = \frac{\text{Absorptivity of PCM at 260 nm}}{\text{Absorptivity of PCM at 254 nm}}$$

$$Qy = \frac{\text{Absorptivity of TOL at 260 nm}}{\text{Absorptivity of TOL at 254 nm}}$$

A1 = Absorbance of sample at isoabsorptive point, ax1 = Absorptivities of PCM at isoabsorptive point.

Validation of the proposed method:

The proposed methods were validated according to the International Conference on Harmonization (ICH) guidelines[16].

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 4-12 µg/ml and 2-18 µg/ml for PCM and TOL respectively for Simultaneous equation and 4-12 µg/ml and 3-15 µg/ml for PCM and TOL respectively for Q-analysis. Accurately measured standard solutions of PCM (4, 6, 8, 10 & 12 ml) and TOL (2, 6, 10, 14 and 18 ml) were transferred to a series of 100 ml of volumetric flasks and diluted to the mark with distilled water for simultaneous equation Method. Accurately measured standard solutions of PCM (4, 6, 8, 10 & 12 ml) and TOL (3, 6, 9, 12 and 15 ml) were transferred to a series of 100 ml of volumetric flasks and diluted to the mark with distilled water for Q-analysis Method. The absorbances of the solutions were measured at 242.5 and 260 nm against distilled water as blank for simultaneous equation Method. The absorbances of the solutions were measured at 254 and 260 nm against distilled water as blank for Q-analysis Method. The calibration curves were constructed by plotting absorbances versus concentrations and the regression equations were calculated.

Precision

The intraday and interday precision of the proposed methods was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days 3 different concentrations of standard solutions of PCM and TOL for both methods.

Accuracy (recovery study)

The accuracy of the method was determined by calculating recovery of PCM and TOL by the standard addition method. Known amounts of standard solutions of PCM and TOL were added at 80, 100 and 120 % level to prequantified sample solutions of PCM and TOL. (5000 µg/ml for PCM and 150 µg/ml for TOL) The amounts of PCM and TOL were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for five times for both methods.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

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

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

Where, σ = the standard deviation of the Intercept of Caliberation curve and S = slope of the calibration curve.

Analysis of PCM and TOL in combined Dosage Form (Tablet)

Twenty tablets were accurately weighed and average weight was calculated. The tablets were triturated to a fine powder. An accurately weighed quantity of powder equivalent to 500 mg PCM & 150mg TOL was dissolved in 10 ml methanol and sonicated for 20 min and volume was made up to 100ml. The solution was filtered through Whatman filter paper No 41 and aliquot portion of filtrate was diluted to produce solution having concentration of 10 µg/ml of PCM and 3 µg/ml of TOL. The absorbance of sample solution was measured at selected wavelengths and the concentrations of the two drugs were estimated using equations (1) and (2) for simultaneous equation method and equations (3) and (4) for absorbance ratio method. The analysis procedure was repeated six times and the results are depicted in Table 2.

RESULTS AND DISCUSSION

The overlain spectra of PCM and TOL exhibit λ max of 242.5 nm and 260 nm for PCM and TOL respectively which are quite separated from each other. Additionally one is absorptive point was observed at 254nm. This wavelength was selected for simultaneous estimation of PCM and TOL for Q value analysis and it is assumed to be sensitive wavelength. The criteria for obtaining maximum precision[15] by Simultaneous equation method were calculated and found to be out side the range 0.1-2 and for Q-analysis ratios of absorbances at 2 different Wavelengths were found to be constant. Standard calibration curves for PCM and TOL were linear with correlation coefficients (r) values in the range of 0.9995 – 0.9999 at all the selected wavelengths and the values were average of three readings with standard deviation in the range of 0.0015 – 0.0057. The calibration curves were repeated three times in a day and the average % RSD was found to be 1.06 for PCM and 1.30 for TOL; similarly the method was repeated for three different days and average % RSD was found to be 1.34 for PCM and2.10 for TOL. The accuracy of the methods was confirmed by recovery studies

TABLE-1 Regression Analysis Data and Summary of Validation Parameter of the Caliberation Curves

Parameters	Method 1				Method 2			
	PCM		TOL		PCM		TOL	
Wavelength (nm)	242.5	260	242.5	260	254	260	254	260
Beer's law limit (µg /ml)	4-12	4-12	2-18	2-18	4-12	4-12	3-15	3-15
Regression equation (y = a + bc)	y = 0.075x - 0.015	y = 0.040x + 0.002	y = 0.023x – 0.003	y = 0.035x – 0.020	y = 0.064x - 0.028	y = 0.044x - 0.009	y = 0.048x – 0.007	y = 0.056x - 0.015
Slope (b) Intercept (a)	0.075 0.015	0.040 0.002	0.023 0.003	0.035 0.020	0.064 0.028	0.044 0.009	0.048 0.007	0.056 0.015
Correlation coefficient (r^2)	0.9991	0.9996	0.9989	0.9993	0.9997	0.9997	0.9995	0.9999
LOD (µg/ml)	1.38	1.40	0.58	0.29	0.66	0.53	0.34	0.21
LOQ (µg /ml)	4.20	4.26	1.76	0.88	2.00	1.61	1.05	0.65
Precision(% RSD,n=3) Interday	2.0-6.6	2.0-8.0	2.6-7.8	2.5-7.9	1.3-2.3	1.0-6.5	0.6-6.1	0.97-4.0
Intraday	0.3-2.1	0.9-2.9	0.6-2.6	0.79-2.0	0.2-1.5	0.4-1.0	0.1-1.3	0.6-1.7

TABLE-3 Results of the Recovery Studies

Level of recovery	Amount of pure drug added (ml)		Simultaneous equation method % recovery	Q-Absorbance method %Recovery		
	PCM (100ug/ml)	TOL (100ug/ml)	PCM	TOL	PCM	TOL
80	8	2.4	105.60	100.02	102.5	100.7
100	10	3	102.46	101.07	99.5	99.33
120	12	3.6	98.05	99.69	99.25	98.19
Mean % recovery			102.03	98.93	100.4	99.40
SD*			3.79	0.9	1.80	1.25
CV**			3.76	0.91	1.80	1.26

TABLE-2 Results of Analysis of Tablet

Drugs	Simultaneous equation method % ± SD(n=5)	Q-Absorbance method %± SD(n=5)
PCM	100.3 ± 0.53	100.4 ± 0.80
TOL	96.8 ± 0.55	98.18 ± 0.58

from tablet at three different levels of standard additions and the results are depicted in Table 3. recovery in the range of 98 – 102% justifies the accuracy of both methods.

STATISTICAL COMPARISON BETWEEN METHOD1 AND METHOD2

The proposed analytical methods were compared using statistical analysis. The Student's t – test was applied and does not reveal significant difference between the experimental values obtained in the sample analysis by the two methods. The calculated t-value were found to be 0.233 and 1.152 for PCM and TOL respectively which are less than Critical t-value

($t_{crit=}$2.31) for both drugs at 5% significance level. Similarly F-test was also applied and does not reveal significant difference between the experimental values obtained in the sample analysis by the two methods. The Calculated F-value were found to be 0.438 and 0.899 for PCM and TOL respectively which are less than value in the F-table 6.39 (0.05,5).

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REFERENCES:

1) Maryadele, J.O.Neil. The Merck Index: An Encyclopedia of chemicals, drugs and biologicals. 13th Edition. Merck and Co., Inc. Whitehouse station, New Jersey; (2001): 10,1698

2) Sweetman S.C. The Martindale: The Complete Drug Reference. 33rd Edition. Pharmaceutical Press. London, UK; (2002): 2,71,1330.

3) Indian Pharmacopoiea, Vol-3, Published By the Indian pharmacopoeia commission Ghaziabad, (2007): 900,901,902.

4) British Pharmacopoeia, Vol-2, London, The British Pharmacopoeia Commission, (2001): 2294,2295,2296.

5) United State Pharmacopoeia NF, Rockville MD: United State Pharmacopoeia Convention, Inc, (2003): 16,17,18.

6) Florey Klaus, Analytical Profile of Drug Substance, 1st Indian Edn, Published By Elsevier, A Division of Reed Elsevier India Private Limited, (2005): (3), 3.

7) Japanese Pharmacopoeia JP15, 1190.

8) Jagathi V., Shaiba M., Raghavi K., Sindhura M., Spectrophotometric methods for the determination of Tolperisone, IJPI's Journal of Analytical Chemistry. 2011; 1(2): 37,38,39.

9) Sai Praveen*, P. Anupama B., Jagathi V., Devala Rao G., Spectrophotometric determination of Tolperisone using 2, 4-dinitrophenylhydrazine reagent, Indian journal of Research & Pharmaceutical Science. 2010; 1(3): 317-320.

10) Carolin Nimila, I, Balan P., Chiranjeevi N., Uma Maheswari V. , Rajasekar S., Method development and statistical validation of UV spectrophotometric method for tolperisone hydrochloride in bulk and tablet dosage form, Journal of Pharmacy Research. 2011; 4(5): 1356-1357.

11) Jagathi V, Shaiba M, Raghavi K, Sindhura M, Prashanthi R, Assay of tolperisone by extractive spectrophotometry, Research Journal of Pharmaceutical, Biological and Chemical Sciences. July – September 2010; 1(3): 654-657.

12) Liawruangrath, S., Liawruangrath, B., High performance thin layer chromatographic determination of tolperisone hydrochloride, Journal of Pharmaceutical and Biomedical Analysis**.** 1999**;** 20(1): 401-404.

13) Jung-Woo Bae, Young-Seo Park, Uy-Dong Sohn, Chang-Sun Myung, Byung-Kwon Ryu, Choon-Gon Jang and Seok-Yong Lee, HPLC Determination of tolperisone in human plasma, Archives of Pharmaceutical Research. 29(4): 339-342.

14) Murali. M and Satyanarayana. P. V. V , "Simple validated isocratic RP –HPLC method for estimation of Tolperisone in bulk and pharmaceutical dosage form" *Scholars Research Library,* Der Pharma Chemica, 2011**;** 3(5):13-19.

15) Beckett AH, Stenlake JB. Practice Pharmaceutical Chemistry. 4th Edition, Part II. New Delhi: CBS Publisher;(1997): 285-288.

16) ICH, Q2 (R1) Validation of Analytical Procedure: Text and Methodology, International Conference on Harmonization, Geneva, Switzerland; 2005.