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First Derivative Spectrophotometric Method for the Simultaneous Estimation of Tolperisone and Paracetamol in their Combined Dosage Form

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ABSTRACT:

The present manuscript describe simple, sensitive, rapid, accurate, precise and cost effective First derivative spectrophotometric zero crossing method for the simultaneous determination of Tolperisone and Paracetamol in combined dosage form. The utility of first derivative data processing program is its ability to calculate unknown concentration of components of interest in a mixture containing an interfering component. The first order derivative absorption at 217.60 nm (zero cross point for Paracetamol) was used for Tolperisone and 223.60 nm (zero cross point for Tolperisone) was used for Paracetamol. The linearity was obtained in the concentration range of 2-20 µg/ml for Tolperisone and 2-12 µg/ml for Paracetamol. The method was successfully applied to synthetic mixture because no interference from excipients was found. The suitability of these methods for the quantitative determination of Tolperisone and Paracetamol was proved by validation. The proposed methods were found to be simple and sensitive for the routine quality control application of Tolperisone and Paracetamol in pharmaceutical dosage form. The results of analysis have been validated statistically and by recovery studies.

KEYWORDS: Tolperisone, Derivative spectrophotometric method, Paracetamol, Drug analysis, Validation, Recovery.

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INTRODUCTION:

Tolperisone(TOL) is chemically 2-methyl-1-(4-methylphenyl)-3-(1-piperidyl)propan-1-one(figure 1)is a well known antispasmodic drug[1]. It is official in JP describe Potentiometery [2]. Literature survey reveals HPLC [3], GC [4] and Visible Spectrophotometry [5] methods for determination of TOL with other drugs in combination. Paracetamol is chemically N-(4-hydroxyphenyl) acetamide (Figure 2) is an analgesic, antipyretic, anti-inflammatory, antiplatelet [6]. Paracetamol (PCM) is official in IP [7] and BP [8] describes liquid chromatography method for its estimation. Literature survey reveals HPLC [9] [10] [11] [12] [13] [14] UV Spectrophotometry [15][16] and HPTLC [17] [10] method for the determination of PCM 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 TOL and PCM in their combined dosage forms. Literature survey does not reveal any simple spectrophotometric method for simultaneous estimation of TOL and PCM in combined dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise and cost effective spectrophotometric method based on First derivative spectrophotometric method for simultaneous estimation of both drugs in their combined dosage form

MATERIALS & METHODS

Materials

A Shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with

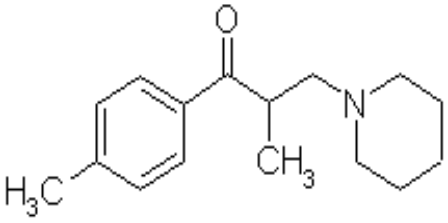

Figure 1: Structure of Tolperisone

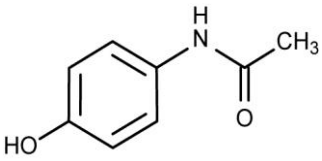

Figure 2: Structure of Paracetamol

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 system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study. TOL and PCM bulk powder was kindly gifted by Torrent Pharmaceuticals, Ghandhinagar, and Gujarat, India. Methanol (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India) were used in the study.

Methods

Preparation of Standard Solutions

A 10 mg of standard TOL and PCM were weighed and transferred to 100 ml separate volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution containing 100µg/ml each of TOL and PCM.

Methodology

The working standard solutions of TOL and PCM were prepared separately in methanol having concentration of 10µg/ml. They were scanned in the wavelength range of 200-800 nm against solvent methanol as blank. The absorption spectra thus obtained were derivatised from first to fourth order. First order derivative spectrum was selected for the analysis of both the drugs. From the overlain spectra of both the drugs (figure 3) wavelengths selected for quantitation were 217.60 nm (zero cross point for PCM) for TOL and 223.60 nm (zero cross point for TOL) for PCM.

Validation of the Proposed Method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [3].

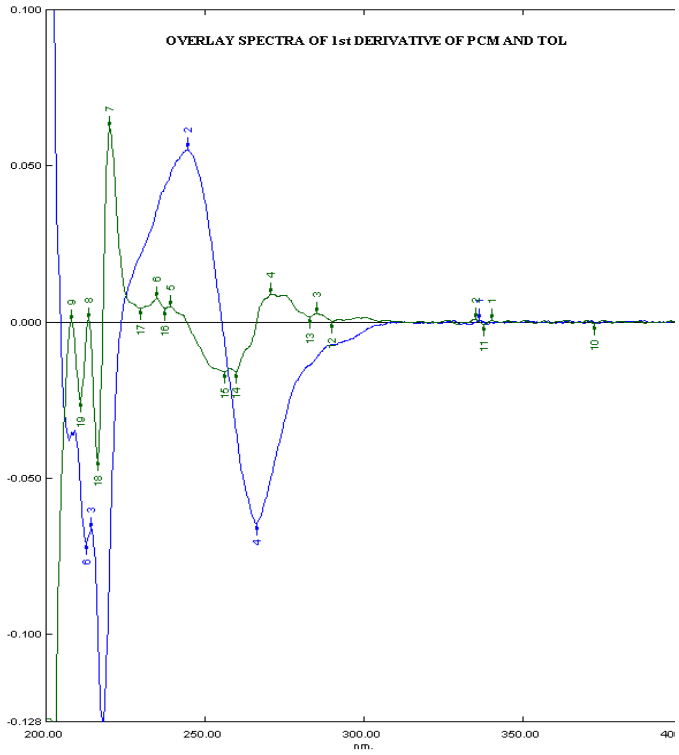

Figure 3: Overlain First order Derivative absorption spectra of TOL and PCM in methanol

Linearity (Calibration Curve)

Appropriate aliquots from the standard stock solutions of TOL and PCM were used to prepare two different sets of dilutions: Series A, and B as follows. Series A consisted of different concentration of TOL (2-16 µg/ml). Aliquot from the stock solution of TOL (100 µg/ml) was pipette out in to a series of 10 ml volumetric flask and diluted with methanol to get final concentration in range of 2-20 µg/ml (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6ml). Series B consisted of varying concentrations of PCM (2-12 µg/ml). Appropriate volume of the stock solution of PCM (100 µg/ml) was transferred into a series of 10 ml volumetric flask and the volume was adjusted to the mark with methanol to get final concentration in range of 2-12 µg/ml (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml). The calibration curve were constructed by plotting drug concentration versus the absorbance values of first derivative spectrum 217.60 nm for TOL and 223.60 nm for PCM. Statistical data for calibration curves is depicated in Table 1. The concentration of individual drugs present in the mixture was determined from the calibration curves in quantitation mode.

Method Precision (Repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 6) for TOL and PCM (8 µg/ml for TOL and 6 µg/ml for PCM drugs) without changing the parameter of the proposed spectrophotometry method.

Table 1: Regression Analysis Data and Summary of Validation Parameters for TOL and PCM by First Derivative Spectrophotometric Method

Parameters	TOL	PCM
Wavelength (nm)	217.60	223.60
Beer's law limit (µg /ml)	2-16	2-12
Regression equation (y = a + bc)	y = 0.003x-0.001	y = 0.007x + 0.005
Slope (b)	0.03	0.007
Intercept (a)	-0.001	0.005
Correlation coefficient (r^2)	0.995	0.993
LOD[a] (µg/ml)	0.55	0.42
LOQ[b] (µg /ml)	1.66	1.28
Repeatability (% RSD[c], n =6)	1.92	1.63
Precision (%RSD, n = 3)		
Interday	074-1.85	0.65-1.74
Intraday	0.62-1.95	0.42-1.63
Accuracy ± S.D[d]. (%Recovery, n= 5)	100.44 ± 1.32	100.66 ± 1.24

[a]LOD = Limit of detection, [b]LOQ = Limit of quantification, [c]RSD = Relative standard deviation. [d]S. D. = Standard deviation

Intermediate Precision (Reproducibility)

The intraday and interday 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 TOL and PCM (4, 6 and 8µg/ml for TOL and PCM). The result was reported in terms of relative standard deviation (% RSD).

Accuracy (Recovery Study)

The accuracy of the method was determined by calculating the recoveries of TOL and PCM by the standard addition method. Known amounts of standard solutions of TOL and PCM were at added at 50, 100 and 150 % level to prequantified sample solutions of TOL and PCM (4µg/ml for TOL and 6µg/ml for PCM). The amounts of TOL and PCM were estimated by applying obtained values to the respective regression line equations.

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, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$$LOD = 3.3 \times \sigma/S \quad (3)$$

$$LOQ = 10 \times \sigma/S \quad (4)$$

Where, σ = the standard deviation of the response and

S = slope of the calibration curve.

Analysis of Synthetic Sample Mixture

Tolperisone and Paracetamol were taken and then were mixed with Mcc, Starch, Lactose, Magnesium stearate and Talc. Dilute it up to mark with Methanol to get the solution containing 3µg/ml of TOL and 6µg/ml of PCM. The absorbances of final solutions were recorded at selected wavelengths for determination of TOL and PCM. The analysis procedure was repeated three times different sample.

Table 2: Recovery Data of TOL and PCM by Spectrophotometric Method

Drug	Amount taken (µg/ml)	Amount added (%)	%Recovery ± S. D. (n=5)
TOL	4	50	99.78 ± 0.17
	4	100	99.91 ± 0.54
	4	150	99.83 ± 0.38
PCM	6	50	100.11 ± 0.44
	6	100	100.80 ± 0.69
	6	150	99.88 ± 0.26

RESULTS AND DISCUSSION

The standard solutions of TOL and PCM were scanned separately in the UV range and First-order spectra for TOL and PCM were recorded. The first order derivative absorption at 217.60 nm (zero cross point for PCM) was used for Tolperisone and 223.60 nm (zero cross point for TOL) was used for Paracetamol. These two wavelengths can be employed for the determination of TOL and PCM without any interference from the other drug in their combined synthetic mixture.

Linear correlation was obtained between absorbances and concentrations of TOL and PCM in the concentration ranges of 2-16 µg/ml and 2-12 µg/ml, with R^2 value 0.995for TOL and 0.993 for PCM at both the wavelength respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression. The RSD values

Table 3: Analysis of TOL and PCM by Spectrophotometric Method

Synthetic mixture	Label Claim (mg)		Amount Found (mg)		% Label Claim ± S.D. (n=6)	
	TOL	PCM	TOL	PCM	TOL	PCM
I	150	300	150.04	300.15	101.66 ± 1.82	102.49 ± 0.91

of TOL were found to be 1.92 % at 217.60 nm. The RSD value of PCM was found to be 1.63 % at 223.60 nm. Relative standard deviation was less than 2 %, which indicates that proposed method is repeatable. The low RSD values of interday (0.74-1.85% for TOL at 217.60 nm and 0.65-1.74% for PCM at 223.60 nm, respectively) and intraday (0.62-1.95% for TOL at 217.60 nm and 0.42-1.63% for PCM at 223.60 nm, respectively) variation for TOL and PCM, reveal that the proposed method is precise. LOD and LOQ values for TOL were found to be 0.55 and 1.66 µg/ml at 217.60 nm, respectively. LOD and LOQ values for PCM were found to be 0.42 and 1.28 µg/ml at 217.60 nm, respectively. These data show that method is sensitive for the determination of TOL and PCM. The regression analysis data and summary of validation parameters for the proposed method is summarized in Table 1.

The recovery experiment was performed by the standard addition method. The recoveries of TOL and PCM were found to 100.44 ± 1.32and 100.6 ± 1.24 %for Tolperisone and Paracetamol, respectively. The results of recovery studies indicate that the proposed method is highly accurate [Table 2]. The validation parameters are summarized in [Table 1]. The proposed validated spectroscopic method was successfully applied to combined dosage form. The results obtained for TOL and PCM were comparable with the corresponding label claim percentage [Table 3]. No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of TOL and PCM in pharmaceutical dosage forms.

CONCLUSION

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of TOL and PCM in synthetic mixture. The method utilizes easily available and cheap solvent for analysis of TOL and PCM hence the method was also economic for estimation of TOL and PCM from dosage form. The common excipients and other additives are usually present in the capsule dosage form do not interfere in the analysis of TOL and PCM in method, hence it can be conveniently adopted for routine quality control analysis of the drugs in combined pharmaceutical formulation.

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