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Simultaneous spectrophotometric estimation of Paracetamol and Dexketoprofen Trometamol in pharmaceutical dosage form

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ABSTRACT

NSAIDs are often used for their anti-inflammatory, analgesic, and/or antipyretic effects. The aim of combining two analgesic agents is to get synergistic analgesic effects of the individual drugs. Two simple spectrophotometric methods have been developed for simultaneous estimation of Paracetamol and Dexketoprofen Trometamol from pharmaceutical dosage form. Method-I involved simultaneous equation method and Method-II is the Q-absorbance method. For simultaneous equation method the absorbances of the standard solutions were taken at two wavelengths 243.0 nm (working wavelength of paracetamol) instead of 248 .0 nm (λmax of paracetamol) and 253.0 nm (λmax of dexketoprofen trometamol). For Q-absorbance method the absorbances of the standard solutions were taken at two wavelengths 248.0 nm (λmax of paracetamol) and 266.0 nm (Isobestic point), in methanol and linearity range selected was 8-28 µg/ml for paracetamol and 0.4-1.4 µg/ml for dexketoprofen trometamol for both methods based on the ratio of the two drugs in combined dosage form. The accuracy and precision of the methods were determined and validated statistically. All the methods showed good reproducibility and recovery with RSD less than 2. The methods were found to be rapid, specific, precise and accurate and can be successfully applied for the routine analysis of paracetamol and dexketoprofen trometamol in bulk and in pharmaceutical dosage form.

Key words: Paracetamol, Dexketoprofen Trometamol,Simultaneous equation method,Q-absorbance method.

INTRODUCTION

NSAIDs are often used for their anti-inflammatory, analgesic, and/or antipyretic effects. Synergistic drug combinations improve effective treatment of pain, minimizing drug specific adverse effects. Formulations with propionic acid derivative, particularly their enantiomeric

S(+)-form and paracetamol are prescribed for the treatment of acute musculoskeletal pain. [1] Paracetamol(PCM) is 4-hydroxy acetanilide and is official in I.P.[2] It is a centrally and peripherally acting analgesic and antipyretic agent. Dexketoprofen trometamol (DKP.TRIS) chemically, 2-amino-2-(hydroxymethyl) propane-1,3-diol; 2-(3-benzoylphenyl propanoic acid is a water-soluble salt of the dextrorotatory enantiomer or (*S*)-(+)-enantiomer of the nonsteroidal anti-inflammatory drug (NSAID) ketoprofen. The enantiomer is a relatively new oral NSAID with analgesic, anti-inflammatory and anti-pyretic properties and is one of the most potent in vitro inhibitors of prostaglandin synthesis.[3]. The combination of 500mg of paracetamol and 25mg of dexketoprofen trometamol is available in tablet dosage form.

Various methods such as HPLC [4-7], HPTLC [8], UV [9] spectrophotometry methods have been reported for paracetamol but few methods have been reported for dexketoprofen trometamol in formulation. An attempt has been made to develop simple, rapid and accurate spectrophotometric method for simultaneous estimation of PCM and DKP.TRIS from the pharmaceutical formulation.

MATERIALS AND METHODS

Experimental Instrumentation

Shimadzu UV/Visible spectrophotometer, model 1700 (Japan) with spectral bandwidth of 2 nm and wavelength accuracy of ± 0.5 nm, with automatic wavelength correction employing a pair of quartz cells was used for analysis. Shimadzu electronic analytical balance (AX-200) was used for weighing the samples.

Reagents and Chemicals

Gift samples of standard Paracetamol and Dexketoprofen Trometamol in bulk form were obtained from Advanced Vital Enzymes Ltd., Thane and Emcure Pharmaceutical Ltd. (R & D Centre), Pune, India. Methanol (AR) provided by Qualigens Fine Chemicals Ltd.

Standard Stock Solution

Standard stock solutions (100µg/ml) of PCM and DKP.TRIS were prepared by dissolving accurately about 10 mg of each drug separately in methanol in 100 ml volumetric flask. The working standard solutions of these drugs were further diluted to get different concentration ranges for calibration curves.

Method – I: Simultaneous Equation Method

Appropriate dilutions of the standard solutions were prepared and scanned in the UV range from 400nm-200 nm and the absorbance maxima (λmax) for PCM was found to be 248.0 nm and 253.0 nm for DKP.TRIS. As the overlay spectral pattern at absorbance maxima exhibited interference of the two components, the working wavelength selected were 243.0 nm for PCM and 253.0 nm for DKP.TRIS which exhibited linearity in the range of 8-28 µg/mL and 0.4-1.4 µg/ mL, respectively. A set of two simultaneous equations were established using the mean of absorptivity coefficients of PCM and DKP.TRIS at the selected sampling wavelengths.

$A1 = 80.55 \times C_{PCM} + 76.62 \times C_{DKP.TRIS}$ .....(1)

$A_2 = 44.80 \times C_{PCM} + 46.90 \times C_{DKP.TRIS}$..... (2)

Where, C_1 and C_2 are concentrations of PCM and DKP.TRIS respectively in g/ L. A_1 and A_2 are absorbances of sample solution measured at 243.0 nm and at 253.0 nm of PCM and

DKP.TRIS, respectively while 80.55 and 44.80 are the absorptivity coefficients of PCM and DKP.TRIS at 243.0 nm and 76.62 and 46.90 are the absorptivity coefficients at 253.0 nm of PCM and DKP.TRIS respectively.

Mixed standard solutions of both the drugs in the ratio of 20:1 were prepared and the absorbances of the mixed standard solutions were measured at the selected wavelengths.

(Fig.1) The concentration of C_{PCM} and $C_{DKP.TRIS}$ in mixed standard solutions were obtained by solving equation (1) and (2).

Fig. 1. Simultaneous equation method

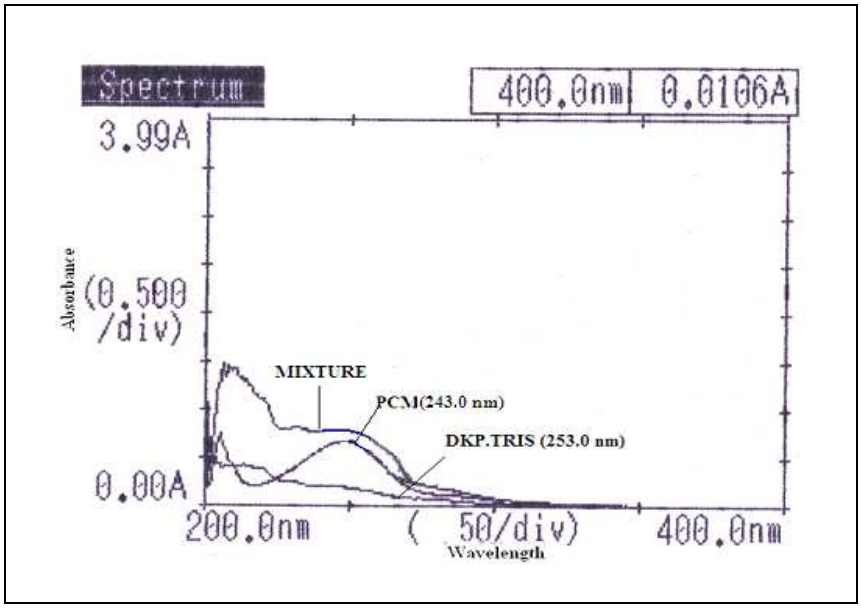
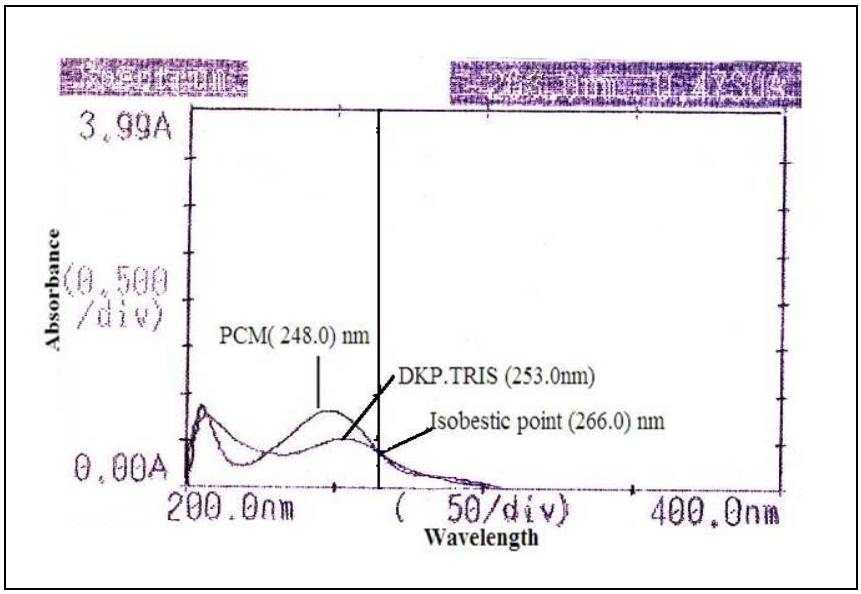


Fig. 2. Q-Absorbance method



Method – II: Q-Absorbance Method

The sampling wavelengths selected for the estimation of PCM and DKP.TRIS were 248.0 nm and 266.0 nm. Suitable dilutions of standard stock solution of the PCM and DKP.TRIS were

prepared and scanned in the spectrum mode from the wavelength range 400–200 nm and their overlay spectra were obtained. The isobestic point was obtained at 266.0 nm. Analytical wavelengths selected were the isobestic point and other being the wavelength of maximum absorption of one of the two components 248.0 nm (λmax of PCM). PCM and DKP.TRIS exhibited linearity in the range of 8-28 µg/ mL and 0.4-1.4 µg/ ml, respectively at the selected wavelengths. Mixed standard solutions of both the drugs in the ratio of 20:1 were prepared and the absorbances of the mixed standard solutions were measured at the selected wavelengths. (Fig.2)

The concentration of C_{PCM} and $C_{DKP.TRIS}$ in mixed standard solutions obtained by solving equation (3) and (4).

$C_X = Q_M-Q_Y / Q_X - Q_Y \quad x \quad A_1 /a_{X1}$ ---------- (3)

$C_X = Q_M-Q_X / Q_Y - Q_X \quad x \quad A_2 /a_{X1}$ ---------- (4)

Where $Q_X = a_{X2} /a_{X1}$ $Q_Y = a_{Y2} /a_{Y1}$ $Q_M = A_2 / A_1$

Standard Absorptivity values of Paracetamol and Dexketoprofen Trometamol:
Absorptivity at 266.0 nm ($a_{X1=} a_{Y1}$) for PCM and DKP.TRIS was 36.19 (g/lit). Absorptivity at 248.0 nm(a_{X2} and a_{Y2}) for PCM and DKP.TRIS was 81.28 and 46.50 (g/lit) respectively.

Assay of marketed formulation:
The marketed formulation (Infen-P) containing PCM and DKP.TRIS in the ratio of 500:25mg respectively is available. From this mixture amount equivalent to 20:1µg/ml of PCM and DKP.TRIS was taken respectively and diluted up to 10 ml with methanol in volumetric flask. The solution was kept for sonication for about 15 min. then filtered through Whatman filter paper No.41. Absorbances of sample solutions were recorded at 243.0 nm and 253.0 nm and the concentration of two drugs in the sample solution were determined by using equations (1) and (2) for simultaneous equation method. The same sample solutions were subjected to analysis by the Q-absorbance method where absorbances of sample solutions were recorded at 266.0 nm and 248.0 nm. The concentration of each drug was determined by using equation (3) and (4). (Table 1)

Table 1: Statistical Validation Data for Tablet Assay

Methods	% Mean*		S.D*		% R.S.D*		S.E*	
	PCM	DKP. TRIS	PCM	DKP. TRIS	PCM	DKP. TRIS	PCM	DKP. TRIS
I	100.17	99.94	0.676	1.220	0.675	1.220	0.276	0.497
II	99.72	99.18	0.450	1.107	0.451	1.116	0.183	0.451

** Mean of three determinations*
S.D: Standard Deviation, R.S.D: Relative Standard Deviation, S.E: Standard Error
Where, Method-I- Simultaneous equation method
Method-II – Q-absorbance method

Method validation [10-11]
Linearity:
Aliquots of standard stock solution of PCM and DKP.TRIS were taken in 10 ml volumetric flasks and diluted up to the mark with methanol to get final concentration of PCM and DKP.TRIS in the range 8-28 µg/ml and 0.4-1.4µg/ml respectively The concentration range

for DKP.TRIS for the linearity was selected so as to get the working range for the two drugs in the ratio of of 20:1.

Limit of detection (LOD) and limit of quantitation (LOQ):
The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The standard deviation of y-intercept and slope of the calibration curves were used to calculate the LOD and LOQ.

LOD = 3.3 * standard deviation / slope
LOQ = 10 * standard deviation / slope

The values of LOD for Method I were 0.2586 and 0.0193 for PCM and DKP.TRIS respectively and that for Method II were 0.2785 and 0.0193 for PCM and DKP.TRIS respectively. The values of LOQ for Method I were 0.7838 and 0.0857 for PCM and DKP.TRIS respectively and that for Method II were 0.8440 and 0.0585 for PCM and DKP.TRIS respectively.

Table 2: Statistical Validation Data of Precision

Precision Studies	Methods	% Mean*		S.D*		% R.S.D*		S.E*	
		PCM	DKP. TRIS	PCM	DKP. TRIS	PCM	DKP. TRIS	PCM	DKP. TRIS
Repeatability	I	100.13	99.70	0.684	1.505	0.683	1.053	0.279	0.428
	II	99.82	99.39	0.624	0.974	0.625	0.980	0.254	0.397
Intra-day	I	100.32	100.92	0.955	0.880	0.952	0.872	0.377	0.359
	II	99.62	99.89	0.365	1.293	0.366	1.294	0.149	0.527
Inter-day	I	99.81	100.08	0.632	1.036	0.633	1.035	0.365	0.598
	II	99.84	100.44	0.226	1.336	0.227	1.330	0.092	0.545

** Mean of six determinations*
S.D: Standard Deviation, R.S.D: Relative Standard Deviation, S.E: Standard Error
Where,
Method-I- Simultaneous equation method
Method-II – Q-absorbance method

Table 3: Statistical Validation Data of Recovery Studies

Level of % recovery	Methods	*% Recovery		%S.D*		*%RSD		*SE	
		PCM	DKP. TRIS	PCM	DKP. TRIS	PCM	DKP. TRIS	PCM	DKP. TRIS
80	I	100.31	99.66	0.070	0.819	0.069	0.821	0.040	0.472
	II	99.65	101.36	0.132	0.812	0.132	0.801	0.076	0.469
100	I	100.95	99.90	0.050	0.099	0.049	0.092	0.029	0.053
	II	99.40	101.13	0.350	0.665	0.352	0.348	0.202	0.383
120	I	101.51	100.48	0.315	0.791	0.031	0.078	0.182	0.451
	II	99.51	99.89	0.448	0.370	0.451	0.451	0.259	0.213

** Mean of three determinations*
S.D: Standard Deviation, R.S.D: Relative Standard Deviation, S.E: Standard Error
Where,
Method-I- Simultaneous equation method
Method-II – Q-absorbance method

Precision:
Precision of the method was done by intraday and interday study. Suitable aliquots of the tablets solutions (100µg/ml) of PCM and DKP.TRIS were pipetted out and transferred to 10

ml volumetric flasks. The volume was made upto the mark to obtain PCM and DKP.TRIS in the concentration ratio of 20:1 as done in the synthetic mixture. Intra day and inter day precision was determined by repeating the assay every two hours on same day for intraday precision and after 24 hrs and 48 hrs for inter day precision studies. (Table 2)

Accuracy (recovery studies):

To study the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120% of the test concentration as per ICH guidelines). (Table 3)

RESULTS AND DISCUSSION

Paracetamol and Dexketoprofen Trometamol are available in combined dosage form for the treatment of acute musculoskeletal pain. UV spectrophotometric method has not been reported so far for the estimation of both the drugs in combination dosage form. Here two simple UV spectrophotometric methods (Simultaneous equation method and Q-absorbance method) were developed for their simultaneous analysis. The methods can be applied for the assay of formulations based on the absorptivity coefficients obtained in the method. The methods were validated as per ICH guidelines. Standard Deviation. RSD and standard error calculated for both the methods are within limits, indicating high degree of precision and accuracy of the methods. The correlation coefficient (r^2) values for PCM were 0.9965 and 0.9961 for method I and II respectively and for DKP.TRIS were 0.9988 and 0.9968 for method I and II respectively. The paired t-test gave P values greater than 0.05 with t value of 1.9960 for PCM and 0.8470 for DKP.TRIS with 5 degrees of freedom The mean difference between the two methods was 0.4467and 0.7583 for PCM and DKP.TRIS. The 95% confidence interval for Method I was 99.458 - 100.88 and 98.658-101.22 for PCM and DKP.TRIS respectively and that for Method B was 99.249-100.19 and 98.018-100.34 for PCM and DKP.TRIS respectively.

CONCLUSION

The combination of paracetamol with dexketoprofen trometamol is recommended for rheumatoid arthritis. Spectrophotometric methods were developed and validated as per ICH guidelines. The assays done by both methods were statistically validated and were found to have P values >0.05 and were considered non significant.

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