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Spectrophotometric Methods for Simultaneous Estimation of Thiocolchicoside and Dexketoprofen Trometamol in Pharmaceutical Dosage Form

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ABSTRACT

Two simple spectrophotometric methods have been developed for simultaneous estimation of Thiocolchicoside and Dexketoprofen trometemol 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 368 nm (λ-max of Thiocolchicoside) and 258 nm (λ-max of dexketoprofen trometamol). For Q-absorbance method, the absorbances of the standard solutions were taken at two wavelengths 258 nm (λ-max of dexketoprofen trometamol) and 281 nm (Isoabsorptive point), in methanol. Linearity range was found to be 2-24 µg/ml for dexketoprofen trometamol and Thiocolchicoside in 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. Both methods showed good reproducibility and recovery with RSD less than 2. Proposed methods were found to be rapid, specific, precise and accurate and can be successfully applied for the routine analysis of dexketoprofen trometamol and Thiocolchicoside in pharmaceutical dosage form.

KEYWORDS

Thiocolchicoside, DexketoprofenTrometamol, Simultaneous equation method, Q-absorbance method.

INTRODUCTION

Dexketoprofen trometamol (DKP) 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[1,2]. 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.

Thiocolchicoside (THC) chemically, *N*-[(7*S*)-3-(beta-D-glucopyranosyloxy)-1, 2-dimethoxy-10-(methylsulfanyl)-9-oxo-5, 6, 7, 9-tetra hydro benzo [a]heptalen-7-yl] acetamide, a semi-synthetic derivative of the naturally occurring compound colchicoside with a relaxant effect on skeletal muscle, has been found to displace both [3H]gamma-amino butyric acid ([3H]GABA) and [3H]strychnine binding, suggesting an interaction with both GABA and strychnine-sensitive glycine receptors[3,4,5], potent competitive antagonist of GABA function. THC is also shows musclerelaxant and displays anti-inflammatory and analgesic properties. The combination of 4mg of Thiocolchicoside and 25mg of dexketoprofen trometamol is available in tablet dosage form.

Thiocolchicoside is official in IP[6] and dexketoprofen trometamol is not official in any pharmacopoeia. A deep Literature survey shows that combination of these two drugs is not official in any pharmacopoeia and no official or

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reported method is available for simultaneous estimation of THC and DKP in combined dosage form. Various reported methods are available for estimation of THC such as U.V[7,8,9], HPTLC[10], HPLC[11,12], and U.V[13], HPLC[13,14,15,16], HPTLC[17] for DKP. In the present investigation an attempt has been made to develop simple, rapid, economic and accurate spectrophotometric method for simultaneous estimation of THC and DKP from the pharmaceutical formulation.

MATERIALS AND METHODS

APPARATUS

A shimadzu model 1600 (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 system software (UV Probe version 2.10). A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

REAGENTS AND CHEMICALS

THC and DKP bulk powder and The commercial fixed dose combination product was procured from the local market. Methanol (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India) and Whatman filter paper no. 41 (Millipore, USA) were used in the study.

STANDARD STOCK SOLUTION

Standard stock solutions (100 µg/ml) of THC and DKP were prepared by dissolving accurately about 5 mg of each drug separately in methanol in 50 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 400 nm-200 nm and the absorbance maxima (λ-max) for THC was found to be 368.0 nm and 258.0 nm for DKP which exhibited linearity in the range of 2-24 µg/ml for both the drugs (Figure-1). A set of two simultaneous equations were established using the mean of absorptivity coefficients of THC and DKP at the selected sampling wavelengths.

$C_X = A_2ay_1 - A_1ay_2 / ax_2ay_1 - ax_1ay_2$ ----------(1)

$C_Y = A_1ax_2 - A_2ax_1 / ax_2ay_1 - ax_1ay_2$ ----------(2)

Where, A_1 =absorbance of mixture at 258nm

A_2 = absorbance of mixture at 368nm

ax_1 =absorptivity of THC at 258nm

ax_2 = absorptivity of THC at 368nm

ay_1 = absorptivity of DKP at 258 nm

ay_2 = absorptivity of DKP at 368 nm

Where, C_X and C_Y are concentrations of THC and DKP respectively in g/ L. A1 and A2 are absorbances of sample solution measured at 258.0 nm and 368.0 nm of DKP and THC respectively while $ax_1$and ax_2 are the absorptivity coefficients of THC and ay_1 and ay_2 are the absorptivity coefficients of DKP. The concentration of THC and DKP in mixed standard solutions was obtained by solving equation (1) and (2).

METHOD – II: Q- ABSORBANCE METHOD

Suitable dilutions of standard stock solution of the THC and DKP 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 281 nm (Figure-1). Analytical wavelengths selected were the isobestic point and other being the wavelength of maximum absorption of one of the two components 258 nm (λmax of DKP). THC and DKP exhibited linearity in the range of 2-24 µg/ mL at the selected wavelengths.The concentration of THC and DKP in mixed standard solutions obtained by solving equation (3) and (4).

CX = QM-QY / QX - QY x A1 /aX1 --------- (3)

CX = QM-QX / QY - QX x A2 /aX1--------- (4)

Where, A1 and A2 are absorbances of mixture at 281 nm and 258 nm; aX1 and aY1 are absorptivities of DKP and THC at 281 nm; aX2 and aY2 are absorptivities of DKP and THC respectively at 258 nm; QX = aX2 /aX1 QY = aY2 /aY1 QM = A2 / A1.

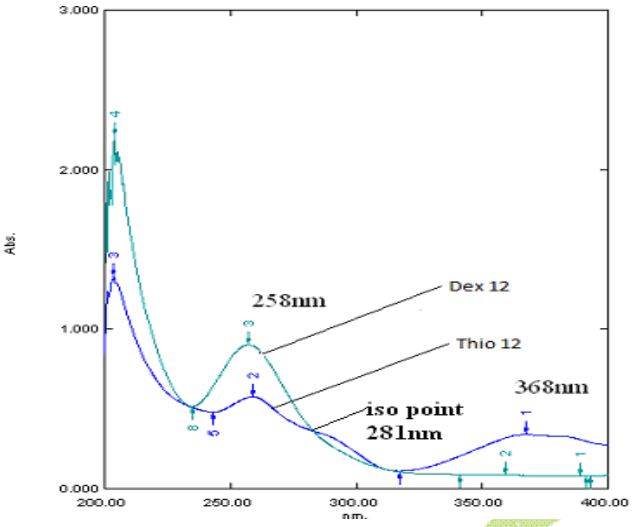

Figure: 1 Overlain Spectra of Thiocolchicoside and Dexketoprofen trometemol (12 µg/mL)

ASSAY OF MARKETED FORMULATION

Twenty tablets were weighed and the average weight was calculated. The tablet powder equivalent to 4 mg of THC and 25 mg of DKP were weighed and transferred to 100 ml volumetric flask. Methanol (30 ml) was added and sonicated for 20 min. The volume is adjusted up to the mark with methanol. The solution was then filtered through Whatman filter paper no. 41. The solution was suitably diluted with methanol to get a final concentration of 2 µg/ml of THC and 12.5 µg/ml of DKP. Absorbance of sample solutions were recorded at 258 nm and 368 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 absorbance of sample solutions was recorded at 281 nm and 258 nm. The concentration of each drug was determined by using equation (3) and (4).

METHOD VALIDATION[18]

Linearity:

Aliquots of standard stock solution of THC and DKP were taken in 10 ml volumetric flasks and diluted up to the mark with methanol to get final concentration of THC and DKP in the range 2-24 µg/ml.

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.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response

And, S = slope of the calibration curve.

Precision

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 6) for THC and DKP (12 µg/ml for both drugs) without changing the parameter of the proposed spectrophotometry method. 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 THC and DKP (4, 8, 12 µg/ml for THC and DKP). The result was reported in terms of relative standard deviation (% RSD).

Accuracy

The accuracy of the method was determined by calculating the recoveries of THC and DKP by the standard addition method. Known amounts of standard solutions of THC and DKP were added at 50, 100 and 150 % level to prequantified sample solutions of THC and DKP (2 µg/ml for THC and 12.5 µg/ml DKP).

The absorbance was measured at corresponding wavelength and the percentage recovery was then calculated.

RESULTS AND DISCUSSION

Proposed methods were found to be simple, sensitive, rapid, accurate, precise and economic for the routine simultaneous estimation of two drugs. The linearity range for Thiocolchicoside and Dexketoprofen Trometemol was found to be 2-24 µg/ml. Regression analysis data and summary of all validation parameters are given in Table 1.

Precision was calculated as repeatability (% RSD) and intra and inter day variation (% RSD) for both the drugs. The LOD and LOQ were found to be 0.52 and 1.59 µg/ml respectively for THC and 0.47 and 1.43 µg/ml respectively for DKP by Method-I and 0.59 and 1.8µg/ml respectively for THC and 0.44 and 1.48 µg/ml respectively for DKP by Method-II indicates sensitivity of the proposed method.

Accuracy was determined by calculating the recovery study at 3 different concentration levels.

Table: 1 Validation Parameter for Proposed Method

Parameters	Simultaneous method		Q-absorbance method		
	THC	DKP	THC	DKP	THC & DKP
Wavelength (nm)	368	258	258	258	281
Beer's Law Limit (µg/ml)	2-24µg/ml	2-24µg/ml	2-24µg/ml	2-24µg/ml	2-24µg/ml
Regression equation (y = mx +c)	y=0.022x+ 0.026	y=0.071x+ 0.044	y=0.036x+ 0.0588	y=0.066x+ 0.0773	y=0.023x+ 0.036
Slope (m)	0.022	0.071	0.036	0.066	0.023
Intercept (c)	0.0269	0.0449	0.0588	0.0773	0.036
Correlation Coefficient (r^2)	0.999	0.999	0.999	0.998	0.997
Sandell's sensitivity (µg/cm^2/0.001 AU)	0.037	0.012	0.0225	0.013	0.035
Repeatability (%RSD, n=6)	0.376	0.239	0.154	0.22	0.418
Interday (n=3) (% RSD)	1.06	0.447	0.154	0.45	0.418
Intraday(n=3) (% RSD)	0.38	0.114	1.46	0.12	0.94
LOD	0.52	0.47	0.59	0.44	0.14
LOQ	1.59	1.43	1.8	1.48	0.45

Known amounts of standard solutions of THC and DKP were added at 50%, 100% and 150 % level to prequantified sample solutions of THC and DKP (2 µg/ml for THC and 12.5 µg/ml DKP), and the mixture were analyzed by the proposed method and the result was shown in the Table-2.

Table:2 Analysis of THC and DKP by Proposed Method

Method	**Drug**	**Label claim (mg/tab)**	**Amount Found (mg/tab)**	**%Label claim ± RSD (n=6)**
Simultaneous method	THC	4	3.91±0.255	97.76±0.03
	DKP	25	24.90±0.04	99.67±0.01
Q-absorbance method	THC	4	4.06±0.49	101.5±0.19
	DKP	25	26±0.07	104.5±0.19

Table:3 Recovery Data of Praposed Method

Method	**Drug**	**Level**	**Amount taken (µg/ml)**	**Amount added (%)**	**% Recovery ± S.D. (n = 3)**
Simultaneous method	THC	1	4	50	97.88±0.02
		2	4	100	102.4±0.19
		3	4	150	102.7±0.19
	DKP	1	25	50	97.17±0.02
		2	25	100	102.8±0.19
		3	25	150	99.15±0.02
Q-absorbance method	THC	1	4	50	101.93±1.64
		2	4	100	101.33±1.33
		3	4	150	101.26±1.15
	DKP	1	25	50	104.5±0.33
		2	25	100	103.86±0.29
		3	25	150	101.73±0.46

CONCLUSION

Proposed spectrophotometric methods were found to be simple, sensitive, accurate and precise for determination of THC and DKP in tablet dosage form. Both methods utilizes easily available and cheap solvent for analysis of THC and DKP hence were also economic for estimation of THC and DKP from tablet dosage form. The common excipients and other additives are usually present in the tablet dosage form do not interfere in the analysis of THC and DKP using both methods, hence can be conveniently adopted for routine quality control analysis of the drugs in combined pharmaceutical formulation

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The film sample with an area of 1 cm^2 was cut from each formulation, and it was analysed for drug content at the end of every week.

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