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Development and Validation of UV-Visible Spectrophotometric Methods for Simultaneous Estimation of Thiocolchicoside and Dexketoprofen in Bulk and Tablet Dosage Form

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ABSTRACT

Development and validation of two simple, accurate, precise and economical UV Spectrophotometric methods for simultaneous estimation of Thiocolchicoside and Dexketoprofen in bulk and in tablet dosage form. The methods employed were Method-1 Absorbance correction method and Method-2 First order derivative spectroscopic method. In method-1 Absorbance is measured at two wavelengths 370nm at which Dexketoprofen has no absorbance and 255nm at which both the drug have considerable absorbance. In method-2, two wavelengths 232nm for Thiocolchicoside (zero cross for Dexketoprofen) and 242nm for Dexketoprofen (zero cross for Thiocolchicoside) were selected. The beers law obeyed in the concentration range 4-40µg/ml and 5-50µg/ml for Thiocolchicoside and Dexketoprofen respectively in methanol. The suggested method is validated by using ICH validation parameters like accuracy, precision, linearity, LOD and LOQ respectively. Recovery study values of Thiocolchicoside and Dexketoprofen was found 99.8% and 100.2% respectively for both the methods. Standard deviation and RSD for intra-day and inter-day precision studies was found to be less than ± 2. The linearity coefficient was found 0.9996 at 242nm and 0.999 at 255 nm for Dexketoprofen and 0.9997 at both 232nm and 370nm for Thiocolchicoside. The LOD and LOQ for Method-1 were found to be 0.0067µg/ml and 0.020µg/ml for Thiocolchicoside, 0.043µg/ml and 0.132µg/ml for Dexketoprofen and for Method-2 were found to be 0.0093µg/ml and 0.028µg/ml for Thiocolchicoside, 0.055µg/ml and 0.168µg/ml for Dexketoprofen respectively. The developed methods were successfully applied to estimate the amount of Thiocolchicoside and Dexketoprofen in bulk and tablet dosage form.

Keywords: Thiocolchicoside, Dexketoprofen, Absorbance correction method, First order derivative spectroscopic method.

INTRODUCTION

Thiocolchicoside (THC) chemically, *N*-[(7*S*)-3-(beta-D-glucopyranosyloxy)-1, 2-dimethoxy-10-(methylsulfanyl)-9-oxo-5, 6, 7, 9-tetrahydrobenzo[a]heptalen-7-yl]acetamide. [1] It is 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. THC is potent competitive antagonist of GABA function, thereby acting as potent muscle relaxant and displays anti-inflammatory and analgesic properties. [1]

Dexketoprofen (DKP) chemically, (2S)-2-[3-(benzoyl)phenyl]propanoic acid. It is a (s)-(+)-enantiomer of Ketoprofen with anti-inflammatory and analgesic property. [2] It is one of the most potent *in-vitro* inhibitors of prostaglandin synthesis; by blocking cyclo-oxygenase therefore it reduces inflammation and pain. Both the drug is marketed as combined dose tablet formulation (4:25mg THC: DKP).

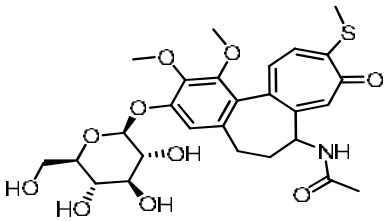

Fig. 1: Structure of Thiocolchicoside

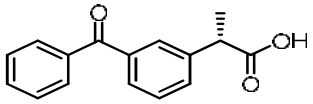

Fig. 2: Structure of Dexketoprofen

Literature survey reveals that THC can be estimated by spectrophotometry [3-11], HPLC [12-17] HPTLC [18-21] and by Mass spectroscopy [22] methods individually or in

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combination with other drugs. DKP is reported to be estimated by spectrophotometry [23-24], HPLC [25] and HPTLC [26] individually or in combination with other drugs. However, there is no analytical method reported for the estimation of THC and DKP in a combined dosage formulation. Aim of present work was to develop and validate simple, rapid, accurate and precise spectrophotometric methods for determination of these drugs in fixed dose combination. The proposed methods were optimized andvalidated as per the International Conference on Harmonization (ICH) guidelines. [27]

MATERIAL AND METHODS

Chemical and reagents

Active pharmaceutical ingredient of THC and DKP were received as gift samples from Emcure Pharmaceuticals Ltd, Pune, India.Marketed formulation containing THC and DKP (4: 25 mg) were purchased from local pharmacy shop. AR grade methanol was procured from Merck, Mumbai, India.

Instrumentation

A Jasco UV- Visible V-530 spectrophotometer with spectral bandwidth of 2 nm and wavelength accuracy ± 0.5 nm was used for all absorbance measurements with automatic wavelength correction with a pair of 1 cm matched quartz cells at a scan speed 100nm per min.

Analytical method development

Preparation of standard stock solution

Standard stock solutions (100µg/ml) of THC and DKP were prepared by dissolving accurately 10 mg of each drug separately in methanol in 100 ml volumetric flask. The working standard solutions of these drugs were further diluted with methanol to get required concentration of THC and DKP.

Preparation of sample solution for combined dosage form

Twenty tablets were weighed and crushed to a fine powder. The quantity of the powder equivalent to 4 mg of THC and 25 mg of DKP was weighed and then transferred to a 100 ml volumetric flask containing 70 ml of methanol. It was then sonicated for 2min and 45 sec. The solution was filtered through Whatmann filter paper No. 41 and volume was made up to the mark with methanol. The final dilution contained about 4µg/ml and 25µg/ml of THC and DKP respectively. Results of analysis of tablet formulation are shown in Table 1.

Method 1: Absorbance correction method

In Absorbance correction method the λ_{max} of THC and DKP was determined by scanning the drug solution was found to be at 370nm and 255 nm respectively. THC also showed absorbance at 255 nm, while DKP did not show any interference at 370 nm. Therefore, the wavelengths selected for analysis were 370nm and 255nm for THC and DKP respectively which exhibited linearity in the range of 4-40µg/ml and 5-50µg/ml respectively. Quantitative estimation of THC and DKP was carried out using following equation:

$$C_{THC} = A_2 / ax_2 \quad (1)$$

Where, A_2 is absorbance of tablet sample solution at 370 nm and ax_2 is absorptivity value of THC at 370 nm. The concentration of DKP is calculated by using the formula obtained by rearranging the equation (1)

$$C_{DKP} = A_1 - ax_1 C_{THC} / ay_1 \quad (2)$$

Where, A_1 is the absorbance of tablet sample solution at 255 nm. ax_1 is absorptivity value of THC at 255 nm. ay_1 is absorptivity value of DKP at 255 nm.

Method 2: First order derivative spectroscopic method

The working standard solutions of THC (20µg/ml) and DKP (20µg/ml) were prepared separately. THC and DKP initially scanned for determining sampling wavelength in range 200 nm to 400 nm against methanol as a blank; λ_{max} was obtained at 370 and 255 nm respectively. The zero order spectra thus obtained was then processed to obtain first derivative spectrum. From the overlain spectra of both drugs, wavelengths are selected for quantitation was 232 nm for THC (zero crossing point for DKP) and 242nm for DKP (zero crossing point for THC). Calibration graphs for THC (4-40µg/ml) and DKP (5-50µg/ml) were constructed from the absorbance at respective wavelength. The concentration of both the drugs present in mixture was calculated using regression equation derived from calibration curve.

Validation of the proposed methods

The developed method was validated in terms of linearity, accuracy, intra-day and inter-day precision, limit of detection, limit of quantification.

Accuracy

The accuracy of the method was determined by calculating percent recovery of THC and DKP by the standard addition method. The recovery experiments were carried out in triplicate (80, 100 and 120 %) by spiking previously analyzed samples of the tablets with three different concentrations of standards. The basic concentration level of sample solution selected for spiking of the drugs standard solution was 4µg/ml of THC and 25µg/ml of DKP for both the methods. The results are reported in term of percent recovery in Table 2.

Linearity

The linearity of measurement was evaluated by analyzing different concentration of the standard solution of THC and DKP. For Method-1and Method-2, the graphs of absorbance verses concentration found to be straight line over the concentration range of 4-40µg/ml and 5-50µg/ml for THC and DKP respectively. The regression equations were calculated. The linearity coefficient was found 0.9996 at 242nm and 0.999 at 255 nm for DKP and 0.9997 at 232nm and 370nm for THC.

Precision

Precision of estimation of THC and DKP by proposed method was ascertained by replicate analysis of homogenous samples of tablet powder at different time intervals on the same day (Intra-day precision) and on second day (Inter-day precision). The results are reported in terms relative standard deviation in Table 3.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated for the proposed method which was based on the standard deviation of the y intercept and the slope of the calibration curves. For method 1-LOD and LOQ were found to be 0.0067µg/ml and 0.020µg/ml for THC,0.043µg/ml and 0.132µg/ml for DKP and for method 2-LOD and LOQ were found to be 0.0093µg/ml and 0.028µg/ml for THC, 0.055µg/ml and 0.168µg/ml for DKP respectively.

RESULTS AND DISCUSSION

The proposed methods for simultaneous estimation of THC and DKP in combined dosage form were found to be simple, rapid, accurate and economic. Since not a single method is reported for simultaneous analysis of the two drugs earlier by

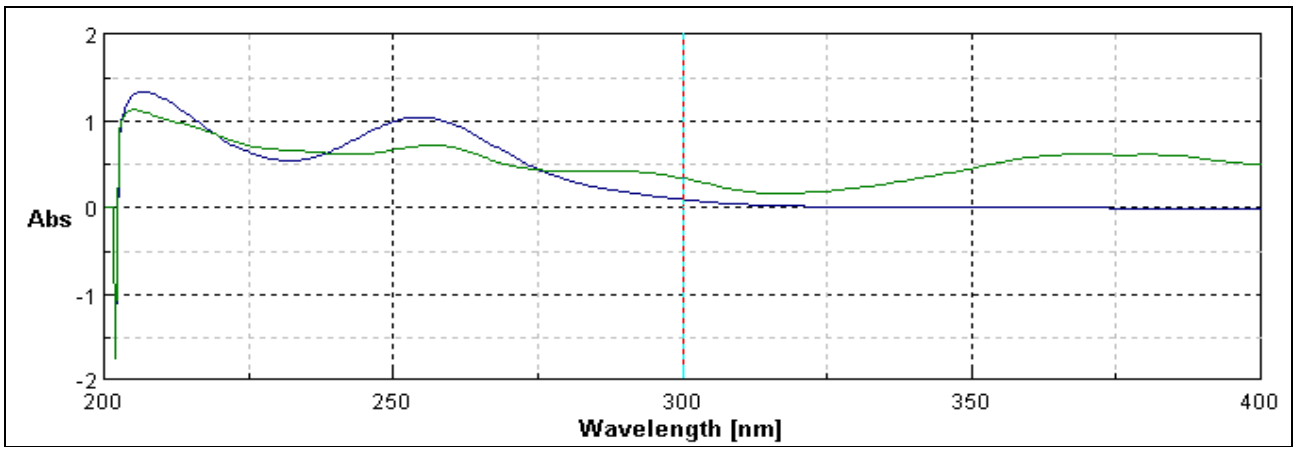

Fig. 3: Overlay Spectra of THC and DKP for Absorption Correction Method

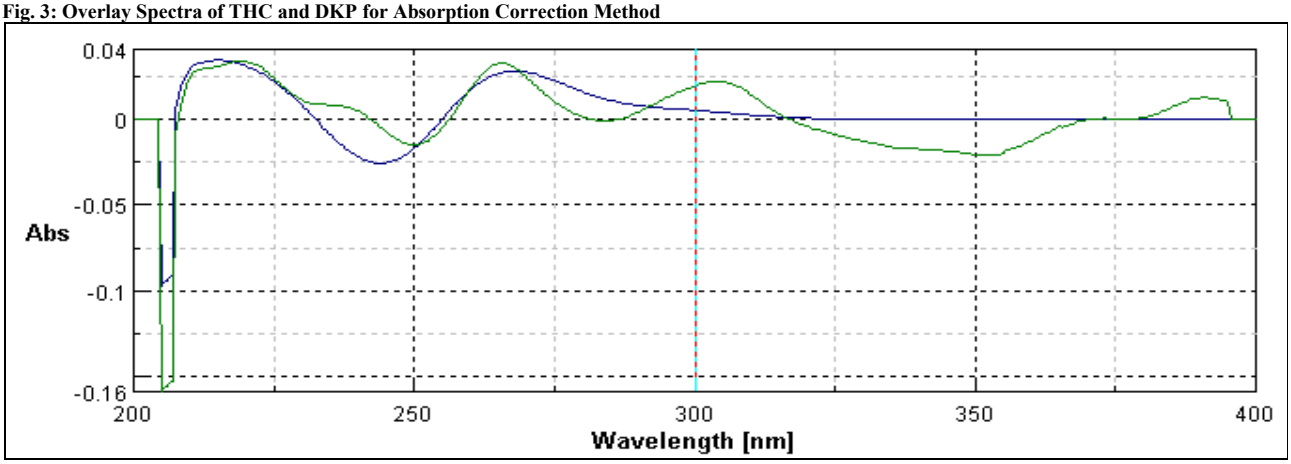

Fig. 4: Overlay spectra of THC and DKP for First order derivative spectroscopic method

Table 1: Result of analysis of tablet formulation

Method	Label claim		Amount of Drug (mg/tab) Estimated*		% Label Claim*		S.D.		%RSD	
	THC	DKP	THC	DKP	THC	DKP	THC	DKP	THC	DKP
Method 1	4	25	3.98	24.93	99.54	99.72	0.1441	0.2037	0.1447	0.2042
Method 2	4	25	3.97	25.08	99.4	100.3	0.1609	0.2126	0.1618	0.2119

*Average of six determinations, S.D.: Standard deviation, R.S.D.: Relative standard deviation

Table 2: Results of Recovery Studies

Method	Level of % recovery	%Mean Recovery*		S.D.		%RSD	
		THC	DKP	THC	DKP	THC	DKP
Method 1	80	99.76	100.2	0.1788	0.1788	0.1792	0.178
	100	99.70	100.1	0.3130	0.1341	0.3139	0.1339
	120	100.0	100.5	0.2683	0.2236	0.2683	0.2224
Method 2	80	99.60	100.0	0.2236	0.1654	0.2244	0.1654
	100	100.1	100.4	0.1788	0.2236	0.1786	0.2227
	120	99.70	100.2	0.2012	0.3130	0.2018	0.3123

*Average of six determinations, S.D.: Standard deviation, R.S.D.: Relative standard deviation

Table 3: Results of intraday interday precision

Method	Drug	%RSD Intraday	%RSD Interday
Method 1	THC	0.2141	0.3787
	DKP	0.1438	0.1924
Method 2	THC	0.2150	0.3177
	DKP	0.1045	0.3568

Average of six determinations, R.S.D.: Relative standard deviation

UV spectrophotometric method, the developed methods can be used for routine analysis of two drugs in combined dosage forms.

In Method-1 absorbance are measured at two wavelengths 255nm for DKP and 370nm for THC. In Method-2 absorbance are measured at two wavelengths 232nm for THC (zero cross for DKP) and 242nm for DKP (zero cross for THC). For both the methods linearity was observed in the concentration range of 4-40and 5-50µg/ml for THC and DKP respectively in methanol. The linearity coefficient was found 0.9996 at 242nm and 0.999 at 255 nm for DKP and 0.9997 at 232nm and 370nm for THC. Marketed brand of tablet was analysed and amount of drug determined by proposed method ranges from 99.8 to 100.2% as shown in Table 1. Recovery study values of THC and DKP was found 99.8% and 100.2% respectively for both the methods with RSD less than 2%. Standard deviation and RSD for intra-day and inter-day precision studies was found to be less than ± 2 indicating precision of proposed method. LOD and LOQ for Method-1 were found to be 0.0067µg/ml and 0.020µg/ml for THC, 0.043µg/ml and 0.132µg/ml for DKP and for Method-2 were found to be 0.0093µg/ml and 0.028µg/ml for THC, 0.055µg/ml and 0.168µg/ml for DKP respectively. The

results of the methods lie within the prescribed limit, showing that method is free from interference from excipients.

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