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Spectrophotometric Simultaneous Determination of Dexketoprofen and Thiocholchicoside in Combined Tablet Dosage Form by Absorbance Corrected Method and First Order Derivative Method.

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ABSTRACT

Two simple, economical, precise and accurate methods are described for the simultaneous determination of Dexketoprofen (DKP) and Thiocolchicoside (TCS) in combined tablet dosage form. The first method (Method A) is Absorption Corrected Method and second method (Method B) is First Order Derivative Spectrophotometry.The amplitudes at 300.0 nm and 373.45 nm in the Absorption Corrected Method and 244.14nm and 268.022nm in the first order derivative Spectrophotometry were selected to determine DKP and TCS, respectively in combined formulation. The methods were validated by following the analytical performance parameters suggested by the International Conference on Harmonization (ICH). All validation parameters were within the acceptable range.
Under experimental conditions described, calibration curve, assay of tablets and recovery studies were performed. A critical evaluation of proposed methods were performed by statistical analysis of data where slope, intercept, correlation coefficient is shown in Table (1). As per the ICH guidelines, the method validation parameters checked. Beer's law is Obeyed in the concentration range of 6.25-75 µg/ml for DKP and 1-12 µg/ml for TCS by both the methods.

Key words: Absorbance corrected, Derivative Spectrophotometry, Thiocholchicoside, Dexketoprofen.

INTRODUCTION

Dexketoprofen (DKP), (S)-2-(3-benzoylphenyl) propionic acid (Fig 1), is a non-opioid, propionic non-steroidal anti-inflammatory drug (NSAID) which has analgesic, antiinflammatory, and antipyretic properties [1]. It is mainly used to reduce inflammation and relieve pain [2]. Thiocolchicoside (TCS) (Fig 2), is N-[(7S)-3-(beta-Dglucopyranosyloxy)-1,2-dimethoxy-10- (methylsulfanyl)-9-oxo-5,6,7,9- tetrahydrobenzo[a]heptalen-7-yl]acetamide [2]. It is a muscle relaxant with anti-inflammatory and analgesic actions. Thiocolchicoside, displaces both [3H] gama aminobutyric acid (3H) (GABA) and [3H] strychnine binding, suggesting an interaction with both GABA and strychnine-sensitive glycine receptors. It is used topically for the treatment of muscular spasms and for rheumatologic, orthopedic, and traumatologic disorders [3, 5].

Few analytical methods that have been reported for the individual determination of TCS in biological fluids and pharmaceutical formulations which include liquid chromatography coupled with spectrofluorimetric, UV [2, 3]. Detailed literature survey reveals that, it was found that DKP can be estimated by spectrophotometry [4, 6] and

HPTLC [7, 10] methods individually or in combination with other drugs [11, 19]. To date, there have been no published reports about the simultaneous estimation of DKP and TCS by UV spectrophotometer in standard drug and in pharmaceutical dosage forms. This present study reports for the first time simultaneous UV spectrophotometric estimation of DKP and TCS by first order derivative and absorbance corrected methods in standard drug and in pharmaceutical dosage forms. The proposed methods were optimized and validated as per the International conference on harmonization (ICH) guidelines [20].

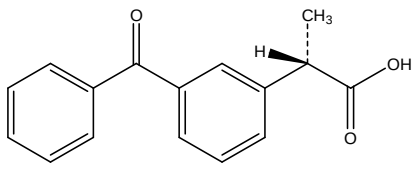

Fig 1: Structure of dexketoprofen

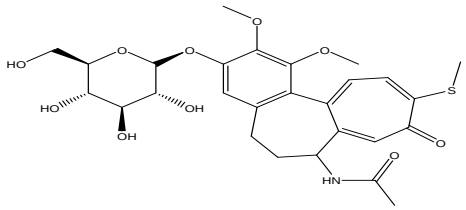

Fig 2: Structure of thiocolchicoside

MATERIALS AND METHODS

Instrumentation

An UV-Visible double beam spectrophotometer (Varian Cary 100) with 10mm matched quartz cells was used. All weighing were done on electronic balance (Model Shimadzu AUW-220D).

Reagents and chemicals

Pure drug sample of DKP, % purity 99.86 and TCS, % purity 99.92 was kindly supplied as a gift sample by Emcure Pharmaceutical Pvt.Ltd. Pune, India and Medley pharmaceuticals pharmaceuticals pvt.Ltd Jalgaon, India. These samples were used without further purification. Tablet used for analysis was INFEN-MR (Batch No.01A11001) manufactured by Emcure Pharmaceutical Pvt. Ltd. Pune, containing DKP 25 mg and TCS 4 mg per tablet.

Preparation of Standard Stock Solutions and Calibration Curve

Standard stock solutions of pure drug containing 1000 µg/mL of DKP and TCS were Prepared separately in methanol. Standard stock solutions were further diluted with methanol to get working standard solutions of analytes in the concentration range of 6.25-75 µg/mL and 1-12µg/mL of Dexketoprofen (DKP) and Thiocolchicoside (TCS), respectively and scanned in the range of 200-400nm. First derivative amplitudes of spectrum, by using the above mentioned procedures, were used to prepare calibration curves for both the drugs.

Preparation of Sample Solution and Formulation Analysis

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 25 mg of DKP (TCS 4 mg) was weighed and dissolved in the 30 mL of methanol with the aid of ultrasonication for 10 min and solution was filtered through Whatman paper No. 41 into a 100 mL volumetric flask. Filter paper was washed with solvent, adding washings to the volumetric flask, volume was made up to the mark with methanol. The solution was suitably diluted with methanol to get 25 µg/mL DKP and 4 µg/mL of TCS.

Theoretical aspects

Method A: Absorption Corrected Method

λ max of DKP and TCS was determined by scanning the drug solution in UV Spectrophotometer in the range 200 - 400 nm at 0.5 band width and 600 nm/min scan speed and was found to be at 300.00 nm and 373.45 nm respectively. TCS also showed absorbance at 373.45 nm, while DKP did not show any interference at 373.45nm [Fig 3]. To construct Beer's plot for DKP and TCS, stock solutions of 1000 µg/ml of both the drugs were prepared in methanol and working standard dilutions were made in methanol using stock solution of 1000 µg/ml. Also Beer's plot was constructed for DKP and TCS in solution mixture at different concentration (6.25:1, 12.5:2, 25:4, 50:8, 75:12 µg/ml) levels. Both the drugs followed linearity individually and in mixture within the concentration range 6.25-75 µg/ml and 1-12 µg/ml for DKP and TCS, respectively.

Method B: Derivative Method
The method involves obtaining the first derivative spectra of the series of the solution of mixtures of DKP + TCS in ascending and descending concentration. From the observations of the derivative spectrum, derivative amplitudes responsible for DKP and TCS were selected and wavelength for each amplitude was noted. These wavelengths were further confirmed by checking the first order derivative amplitude of the mixed standard solutions of these drugs in the given ratio. Mixed standard solutions were prepared in the range of 6.25-75 µg/ml and 1-12 µg/ml for DKP & TCS respectively were used for the study [Fig 4]. Wavelengths 244.14 nm and 268.02 nm were selected for the quantification of DKP in DKP + TCS mixture and TCS in DKP + TCS mixture, respectively [Fig 5] [21]

Recovery studies
The accuracy of the proposed methods were checked by recovery studies, by addition of standard drug solution to preanalysed sample solution at three different concentration levels (80 %, 100 % and 120 %) within the range of linearity for both the drugs. The basic concentration level of sample solution selected for spiking of the drugs standard solution was 25 µg/ml of DKP and 4 µg/ml of TCS for both the methods.

Solution stability
Method stability was checked by analyzing solution kept in fridge and at room temperature by both methods. Solution at room temperature was stable for 12 hours and solution.

Method sensitivity (LOD and LOQ)
The values of LOD and LOQ were calculated by using σ (standard deviation of response) and b (slope of the calibration curve) and by using equations, $LOD = (3.3 \times \sigma)/b$ and $LOQ = (10 \times \sigma)/b$.

Precision of the Method
Method repeatability was determined by six times repetitions of assay procedure. For intra-day precision method was repeated 5 times in a day and the average % RSD was determined. Similarly the method was repeated on five different days for inter-day precision and average % RSD was determined Table (1). Precision of analyst was determined by repeating study by another analyst working in the laboratory.

Specificity
Specificity is a procedure to detect quantitatively the analyte in the presence of component that may be expected to be present in the sample matrix. Commonly used excipients in tablet preparation were spiked in a pre-weighed quantity of drugs and then absorbance was measured and calculations done to determine the quantity of the drugs.

Robustness:
The robustness of the proposed methods was tested by changing parameters such as wavelength range, slit width, temperature, shaking time etc. None of these variables significantly affected the absorbance of the drugs indicating that the proposed methods could be considered as robust.

RESULTS AND DISCUSSION

Under experimental conditions described, calibration curve, assay of tablets recovery studies, solution stability were performed. Using appropriate dilutions of standard stock solution, two solutions were scanned separately for method A and combined dilutions were used for method B. A critical evaluation of proposed methods were performed by statistical analysis of data where slope, intercept, correlation coefficient are shown in Table (1). As per the ICH guidelines, the method validation parameters checked were linearity, accuracy and precision, specificity, roubustness & method sensitivity (LOD & LOQ). Beer's law is obeyed in the concentration range of 6.25-75 µg/mL and 1-12 µg/mL for DKP and TCS, respectively. Correlation coefficient was greater than 0.999 for both the drugs. The proposed methods were also evaluated by the assay of commercially available tablets containing DKP and TCS. The results of formulation analysis were in the range of 100 ± 2 % and are presented in Table (1). Recovery was found in the range of 99.90 to 100.39% for DKP and 98.89 to 99.45 %, for TCS by absorbance corrected method and 98.63%-99.66 % for DKP and 99.13-99.75 % for TCS by first order derivative method (Table 2). The accuracy is evident from the data as results are close to 100 % and standard deviation is low. All the statistical data analysis was performed by using MIP Pharmasoft 1.0, software developed and validated in the Institute.

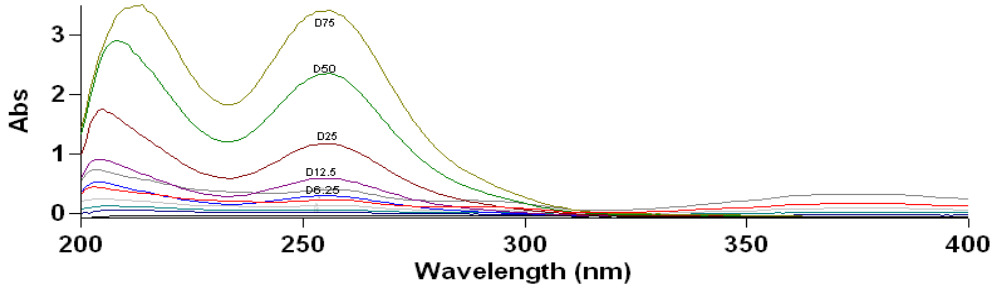

Fig 3: Simple overlay spectra of DKP (6.25-75 µg/ml) and TCS (1-12 µg/ml) in methanol.

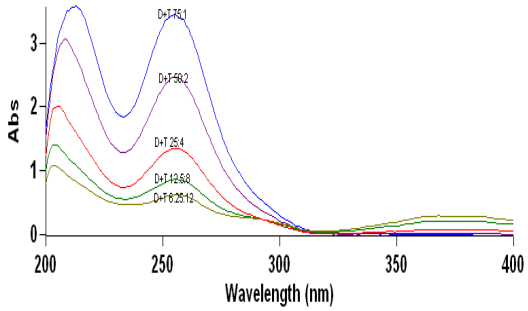

Fig 4: Overlain spectra mixture of DKP (6.25-75µg/mL) and TCS (1-12 µg/mL) in methanol.

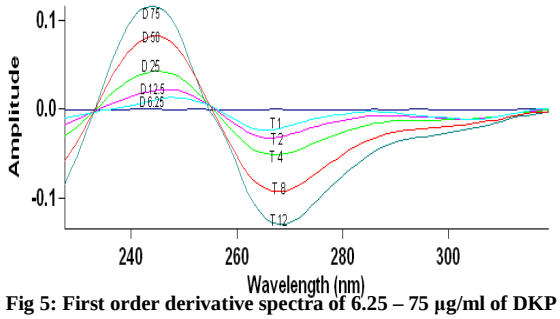

Fig 5: First order derivative spectra of 6.25 – 75 µg/ml of DKP and TCS in mixture, when 1-12 µg/ml of TCS.

Table 1: Optical characteristics of the proposed methods and result of formulation analysis

Parameter		Dexketoprofen		Thiocolchicoside	
		Method A	Method B	Method A	Method B
wavelength (nm)		300.00	244.14	268.022	373.45
Beer's law limit (µg/mL)		6.25-75	6.25-75	1-12	1-12
Regression Equation*	Slope (m)	0.0040825	0.015387	0.0098592	0.02976
	Intercept (c)	-0.03291	0.01830	0.01175	-0.03920
Correlation coefficient (r)		0.999	0.999	0.997	0.997
Precision (%RSD)	Repeatability (n=5)	0.71	0.60	0.79	0.55
	Intra-day(3x5 9)))times0)0))	1.25	1.11	1.12	1.26
	Inter-day(3x5 days)	0.91	1.04	0.79	1.18
Formulation Analysis (% Assay, %RSD), n=6		100.44 ±0.45	99.81 ± 0.81	101.82 ± 0.4	99.21 ± 0.29
LOD	(µg/mL)	0.538	0.7131	0.656	1.0171
LOQ	(µg/mL)	1.564	2.1611	2.565	3.0824
Ruggedness (%RSD)	Analyst I	0.55	0.72	0.45	0.63
	Analyst II	0.56	0.85	0.83	0.48

RSD = Relative Standard Deviation, Y = mX + c, where Y is the absorbance and X the concentration in micrograms per milliliter*

Table 2: Result of accuracy study

Recovery Level	Analyte name	Amount Spiked (µg/mL)	% Mean Recovery, % RSD, n=6	
			Method A	Method B
80%	DKP	20	100.39 0.79	99.45, 0.38
	TCS	3.2	99.45, 0.97	98.75, 1.05
100%	DKP	25	99.90, 1.03	98.63, 0.92
	TCS	4	98.89, 1.57	99.13, 1.72
120%	DKP	30	100.05, 0.19	101.75, 0.74
	TCS	4.8	98.93, 1.34	99.66, 0.93

CONCLUSION

The validated spectrophotometric methods employed here proved to be simple, economical, precise and accurate. Thus it can be used as IPQC test and for routine simultaneous determination of DKP and TCS in tablet dosage form.

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