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Simultaneous estimation of thiocolchicoside and ketorolac tromethamine using UV spectroscopy

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Thiocolchicoside and ketorolac are potent anti-inflammatory and pain relieving agents. They impart synergistic action when delivered in combination. In the present study a simple accurate, precised and sensitive method was developed for the simultaneous estimation of both drugs. The drug thiocolchicoside and ketorolac have absorption maxima at 259 nm and 323 nm respectively. Both of the drugs in the proposed method showed linearity in the range of 5–25 μg/mL with an r^2 value of 0.997. The accuracy study results data showed recovery of the standard drug in the range of 98.84–100.67% with a standard deviation of 0.92 for thiocolchicoside and 98.20–101.69% with a standard deviation of 1.96 for ketorolac. Intra-day and inter-instrument variability study results also found within the standard limits, showing the precision of the methodology. The LOD and LOQ values for thiocolchicoside was 1.11 and 3.390 μg/ml, respectively, whereas, the LOD and LOQ values for ketorolac were 0.986 and 2.99 μg/ml, respectively. Thus, it could be concluded that the developed method was accurate, precised, specific with enough sensitive and successfully implied for the simultaneous estimation of thiocolchicoside and ketorolac in their combined dosage during the routine analysis work.

Keywords: Simultaneous estimation, accuracy, precision, selectivity, LOD, LOQ.

Introduction

Thiocolchicoside (Fig. 1A) is a potent muscle relaxant having analgesic and anti-inflammatory activity[1]. It is available in the market with the brand name of Muscoril, Myoril, Neoflax[2,3]. It is chemically *N*-[(7*S*)-1,2-dimethoxy-10-methylsulfanyl-9-oxo-3-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-6,7-dihydro-5*H*-benzo[*a*]heptalen-7-l]acetamide. It is a semi-synthetic sulfur-containing derivative of the colchicine (natural anti-inflammatory alkaloid) originated from the plant *Colchicum autumnale*, commonly known as autumn crocus, meadow saffron or naked ladiesz[4]. *Patients having the history of* seizures are not good candidates for thiocolchicoside because of its potent anticonvulsant activity[5]. It is the competitive antagonist of GABA receptor, glycine receptor (with similar potency) as well as nicotinic acetylcholine receptors (with lesser potency)[5,6]. It is mainly used for the treatment of stiffness in the spine, joints, nerve diseases, muscle diseases, rheumatoid arthritis, ankylosing spondylitis and for nonarticular rheumatism by inhibiting the action of cyclooxygenase enzyme and resulting in lowering the pain and swelling of body cells[7,8]. This drug can also be used for the treatment of acute and chronic lumbar and sciatic pain, post-traumatic and post-operative pain, cervicobrachial neuralgia and also persistent torticollis[8]. But at the same time, Thiocolchicoside also have some side effects like drowsiness, impaired judgment, impaired body movements etc.

Ketorolac tromethamine (Fig. 1A) is the tromethamine salt of ketorolac with anti-inflammatory, analgesic, antipyretic properties and also a derivative of synthetic pyrrolizine carboxylic acid[9,10]. It is chemically 2-amino-2-(hydroxymethyl)-propane-1,3-diol; 5-benzoyl-2,3-dihydro-1*H*-pyrrolizine-1-carboxylic acid[11]. Various studies have shown that ketorolac is a better alternative than major opioid analgesics, such as morphine, and more effective than codeine for the treatment of postoperative pain in children[12–14]. It is a non-selective inhibitor of the cyclooxygenases (COX), inhibits both COX-1 and COX-2 enzymes. It prevents the conversion of arachi-

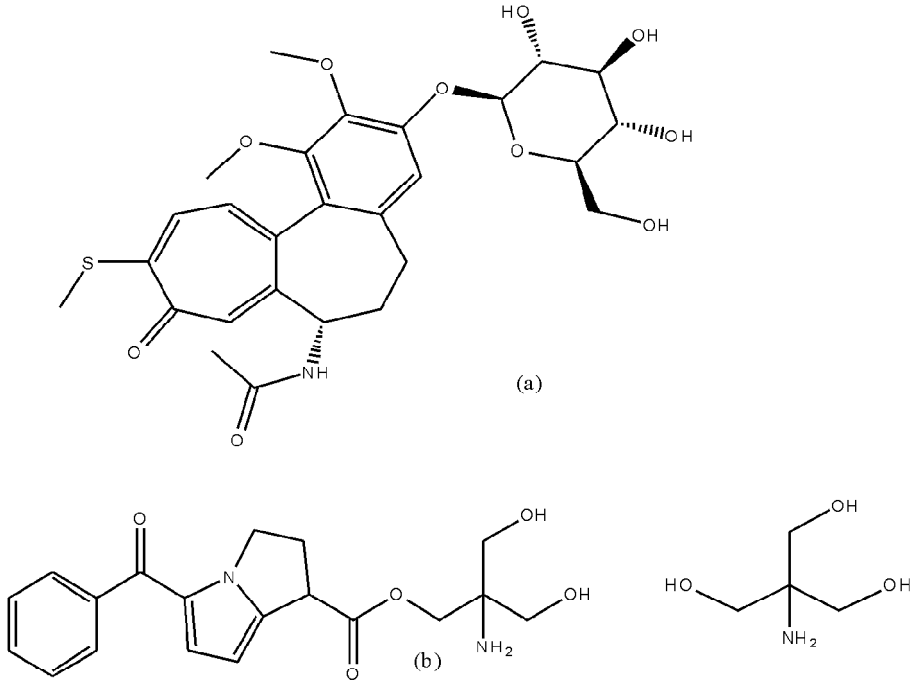

Fig. 1A. (a) Structure of thiocolchicoside, (b) structure of ketorolac tromethamine.

donic acid to prostaglandins produced at the inflammatory site[15]. Apart from being potent analgesic, it also has some major side effects such as gastric upset, nausea, vomiting, constipation, diarrhoea, gas, dizziness etc. Literature survey reveals that there are several different methods like UV spectroscopy, HPLC[15], UPLC[16], HPTLC[17] etc. has been reported for the estimation of Ketorolac tromethamine[18–21] and Thiocolchicoside separately. Simultaneous estimation of ketorolac with other drugs like ofloxacin[19], febuxostat[17], moxifloxacin[22] etc. Similarly, the UV spectrophotometric simultaneous estimation of thiocolchicoside with other drugs like aceclofenac[23], diclofenac[24], ketoprofen[25], etodolac[26] etc. have also been reported already, but there is no UV spectrophotometric method has been reported yet for the simultaneous estimation of ketorolac and thiocolchicoside in their combination dosage form. So, the development of such a method could be used for the estimation of both drugs in combined dosage form.

Material and methods:

Chemicals and reagents:

Thiocolchicoside and ketorolac tromethamine both were collected as gift sample from the Aspire Life Sciences Pvt. Ltd., Mumbai, India and Vasudha Pharma Chem Limited, Hyderabad, India respectively. All the chemicals used during the experiment were of analytical grade.

Instrument:

UV-Visible spectrophotometer (Shimadzu-1800) was used to perform simultaneous estimation of both drugs.

Experimental

Spectral analysis of both the drugs for their simultaneous estimation.

Preparation of stock solutions:

A stock solution having a concentration of 100 μg/ml of both the drugs (thiocolchicoside and ketorolac tromethamine) were prepared separately by dissolving the 10 mg drug in 100 ml of phosphate buffer (pH 7.4).

Scanning:

The absorption maxima (λ_{max}) were obtained by scanning the appropriate dilution in the range of 200–400 nm. The λ_{max} of thiocolchicoside and ketorolac were found to be 259 nm and 323 nm respectively.

Development of a simultaneous equation:

An overlay spectrum (Fig. 1) using both the drugs obtained for deriving the further equation for simultaneous es-

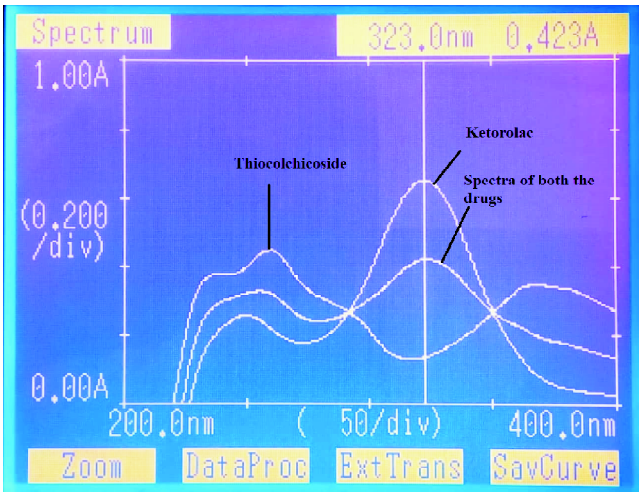

Fig. 1. Overlay spectrum of the combined dosage form.

timation of both drug in combined dosage form[19,20,25,27–29]. The scanning result confirmed the additivity of absorbance and there was no chemical interaction with drugs.

The following equation was developed for both of the drugs:

Thiocolchicoside (C_t) = (0.226A_K – 0.652A_T)/–0.2615,

Ketorolac (C_K) = (0.138A_T – 0.449A_K)/–0.2616

A_T = Absorbnce of thiocolchicoside, C_t = concentration of thiocolchicoside, A_K = absorbance of ketorolac, C_K = concentration of ketorolac.

Linearity study:

Further, different dilutions in the range of 5–25 µg/ml of both the drugs were prepared the corresponding stock solutions and calibration graph was prepared by taking the absorption at absorption maxima (λ_{max}) 259 nm of thiocolchicoside (Fig. 2) and absorption maxima (λ_{max}) 323 nm of ketorolac (Fig. 3). Details regarding the linearity study are being mentioned in Table 1.

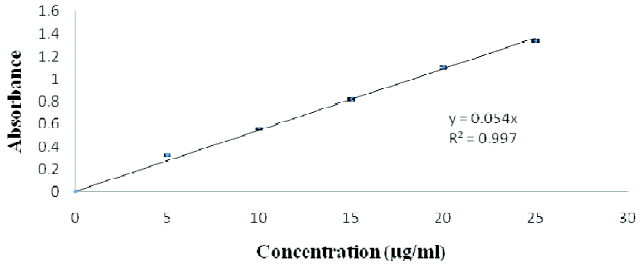

Fig. 2. Calibration graph of Thiocolchicoside in pH 7.4 buffer during simultaneous estimation.

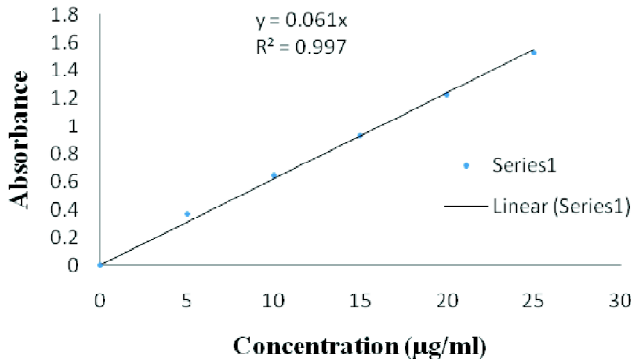

Fig. 3. Calibration graph of ketorolac in pH 7.4 buffer during simultaneous estimation.

Accuracy:

The accuracy of the method is the closeness of the measured value to the true value for the sample. To determine the accuracy of the proposed method, it was assessed by recovery studies using standard addition method was employed, which involved the addition of different concentrations of pure drug (10.4,15.6, 20.8 µg of Thiocolchicoside and 10.6, 15.9, 21.2 µg of ketorolac tromethamine) or the addition of same concentration of drug (5.2 µg/ml of Thiocolchicoside and 5.3 µg/ml of Ketorolac tromethamine) to a known pre analyzed formulation sample and the total

Table 1. Data representing the linearity

Conc. (µg/ml)	Absorbance at of thio (259) and keto (323) during simultaneous estimation									
	Thio at λ_{max} 259 nm					Keto at λ_{max} 323 nm				
Rep.	5	10	15	20	25	5	10	15	20	25
Replicate-1	0.327	0.562	0.823	1.095	1.342	0.393	0.644	0.946	1.247	1.531
Replicate-2	0.336	0.551	0.806	1.116	1.326	0.382	0.634	0.915	1.216	1.487
Replicate-3	0.344	0.566	0.827	1.087	1.353	0.407	0.654	0.938	1.228	1.568
Mean	0.336	0.560	0.819	1.099	1.340	0.394	0.644	0.933	1.230	1.528
S.D.	0.007	0.007	0.009	0.012	0.011	0.0102	0.008	0.013	0.013	0.033

concentration was determined using the proposed methods (n = 3). The % recovery of the added pure drug was calculated as:

% recovery of thio/keto = $[(C_t - C_s)/C_a] \times 100$,

where C_t is the total drug concentration measured after standard addition; C_s, drug concentration in the formulation sample; C_a, drug concentration added to the formulation. Calculated, recovered amount during the study is being mentioned in Table 2.

Table 2a. Recovery studies data showing the amount of drug recovered from the sample solution and average recovery using a fixed amount of analyte (pure drug)

Sr. No.	Sample	Amount present in formulation (μg)	Amount added (μg)	Amount recovered (μg)	% recovered
1.	Thio	15.16	10.4	10.28	98.846154
2.		20.24	10.4	10.47	100.67308
3.		30.67	10.4	10.36	99.615385
Standard deviation					0.9172492
1.	Keto	15.07	10.6	10.43	98.396226
2.		20.31	10.6	10.78	101.69811
3.		30.3	10.6	10.41	98.207547
Standard deviation					1.9630804

Table 2b. Recovery studies data showing the amount of drug recovered from the sample solution and average recovery using a different amount of analyte (pure drug)

Sr. No.	Sample	Amount present in formulation (μg)	Amount added (μg)	Amount recovered (μg)	% recovered
1.	Thio	15.16	10.4	10.22	98.2692308
2.		15.16	15.6	15.31	98.1410256
3.		15.16	20.8	20.56	98.8461538
Standard deviation					0.37560675
1.	Keto	15.07	10.6	10.54	99.4339623
2.		15.07	15.9	15.68	98.6163522
3.		15.07	21.2	20.91	98.6320755
Standard deviation					0.46757457

Precision:

Repeatability was determined using different levels of drug concentrations, prepared from independent stock solutions and analyzed (n = 3). Inter-day and inter-instrument variation were studied to determine the intermediate precision of the proposed analytical methods. Different levels of drug concentrations in triplicates were prepared three different times in a day and studied for intra-day variation. The same procedure was followed for three different days to study inter-day variation (n = 3). One set of different levels of the concentrations was reanalyzed using the UV-1800 (Shimadzu) spectrophotometer. The percent relative standard deviation (% R.S.D.) of the predicted concentrations from the equation for simultaneous estimation, was taken as precision and mentioned in the Tables 3a and 3b.

Limit of detection and limit of quantitation:

The LOD and LOQ for thiocolchicoside and ketorolac tromethamine by the proposed method were determined using calibration standards having the sample of both drugs. LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively[30]. Where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation (n = 3). The details related to LOD and LOQ is mentioned in Table 4.

Specificity and selectivity:

The estimation of the drug was performed in the presence of common excipient used in the tablet formulation; and it was found that the UV-spectrum of the combined drug was not changed significantly in the presence of common excipient used in the microemulsion formulation (Fig. 4) such as tween 80, butanol etc. The absorption spectrum of pure drug sample resembled with those of the formulation samples in the selected buffer media. This experimentation shows the specificity of the methodology.

Table 3a. Data obtained for the precision study

Sr. No.	Concentration taken (μg/ml)		Estimated concentration ± SD (n = 3)					
			Day 1		Day 2		Day 3	
	Thio	Keto	Thio	Keto	Thio	Keto	Thio	Keto
1.	10	5	9.93±0.12	4.95±0.08	10.17±0.14	5.06±0.11	10.06±0.14	4.82±0.08
2.	15	10	14.91±0.13	9.91±0.09	14.85±0.11	10.07±0.14	15.23±0.17	9.93±0.11
3.	20	15	20.26±0.16	14.88±0.17	20.08±0.13	15.18±0.15	19.85±0.24	15.12±0.17

Table 3b. Data obtained for the precision study

Sr. No.	Concentration Taken (µg/ml)		% estimated concentration of Thio ± SD (n = 3)	% estimated concentration of Keto ± SD (n = 3)
	Thio	Keto		
1.	10	05	99.2±0.087	98.91±0.87
2.	15	10	98.7±0.167	99.43±1.11
3.	20	15	101.2±0.413	98.7±0.61

thiocolchicoside and ketorolac. Using the overlay spectra of both the drugs, the equation for the calculation of thiocolchicoside (C_t = (0.226A_K – 0.652A_T)/–0.2615) and ketorolac (C_k = (0.138A_T – 0.449A_K)/–0.2616) was developed for calculating the concentration of both the drugs in combination. While recovery study by standard addition method shows the recovery of thiocolchicoside in range of 98.84–100.67% with a standard deviation of 0.92 and 98.20–101.69% and of ketorolac with a standard deviation of 1.96 indicating the accuracy of the method. For checking out the precision of the proposed methodology, intra-day variability and inter-instrument variability study were performed and the results are shown in the Tables 3a and 3b supports the precision study. The sensitivity of the method was checked by calculating LOD and LOQ and the low value of LOD and LOQ as shown in Table 4 proves the sensitivity of the said method. Spectral analysis was also done in presence of two major excipients used in the formulation of microemulsion i.e. tween 80 and butanol. During the study, it was observed that there is no significant change in the absorption maxima of both the drugs as mentioned in Fig. 4, reveals the specificity of the proposed method.

Table 4. Spectral detail regarding linearity, LOD and LOQ

Sr. No.	Parameters	Dual wavelength values	
		Thio	Keto
1.	λ_{max} (nm)	259	323
2.	Beer's law range (µg/ml)	5–25	5–25
3.	Regression equation	y = 0.052x + 0.031	y = 0.059x + 0.042
4.	Correlation coefficient	0.997	0.997
5.	Slope	0.052	0.059
6.	The standard deviation of the response	0.016405975	0.017626054
7.	LOD (µg/ml)	1.118577	0.986
8.	LOQ (µg/ml)	3.389626	2.99

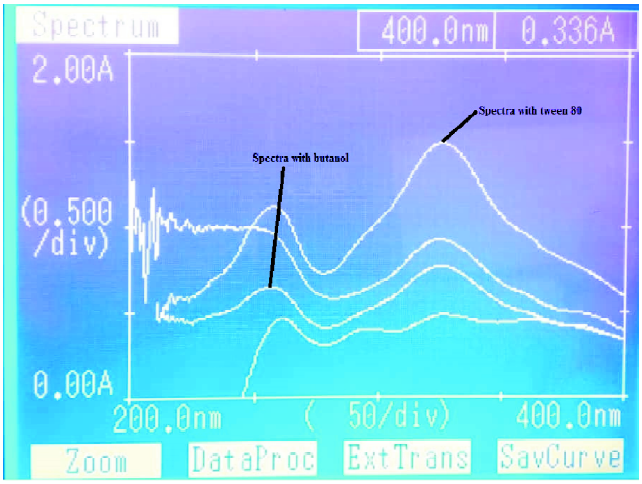

Fig. 4. Spectra of both the drugs in presence of tween 80 and butanol.

Results and discussion

Spectral analysis shows that thiocolchicoside has λ_{max} at 259 nm and ketorolac tromethamine have λ_{max} at 323 nm. During the simultaneous spectral analysis thiocolchicoside and ketorolac both have shown the linearity in the range of 5–25 µg/ml having regression coefficient value of 0.997 for both the drugs with a slope of 0.052 and 0.059 respectively. Calibration graph for both the drugs with equation has been displayed in Figs. 2 and 3 respectively for

The proposed method can be used for the routine estimation of both the drugs in their combined dosage form such as a tablet, micro-emulsions etc.

Conclusion

The developed spectrophotometric method for estimation of thiocolchicoside and ketorolac in combined dosage is found to be accurate, precised, specific, selective and sufficiently sensitive to find out the concentration of both the said drugs in combined form. The proposed spectrophotometric

methodology was validated for simultaneous estimation of thio and keto for linearity, range, accuracy and precision, LOD, LOQ, and specificity. Calculation of standard deviation for all the needed parameters was found to be within the range indicating the validity. The present study concludes that the proposed developed method can be used for the regular estimation and quantization of keto and thio in the combined dosage form.

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