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Development and Validation of Spectrophotometric Method for Simultaneous Estimation of Ketoprofen and Thiocolchicoside in combined Solid Oral Dosage Form

Toral Joshi*[1], Ankita Bhavsar[1] and Arvind Senta[2]
1, Sat Kaival College of Pharmacy, Sarsa-388365 Ta, Di. Anand, (Gujarat) - India
2 Aum Research laboratories, Rakanpur, Ahmedabad, (Gujarat) - India

Abstract

A newer, simple, rapid, accurate, precise and cost-effective Q-Absorbance ratio method has been developed for the simultaneous estimation of Ketoprofen and Thiocolchicoside in solid oral dosage form and validated as per International Conference of Harmonisation guidelines. Absorbance ratio method was developed using two wavelengths which are 275 nm and 251.5 nm respectively. Methanol was used as a solvent. The linearity was found in the range of 5-25 µg/ml and 4-20 µg/ml for Ketoprofen and Thiocolchicoside respectively. The linearity coefficient was found to be 0.995 and 0.990 for Ketoprofen and 0.986 and 0.990 for Thiocolchicoside at 251.5 nm 275 nm respectively. % RSD studies were found to be less than ± 2. The Limit of Detection and Limit of Quantitation were found to be and 16.58 µg/ml and 50.25 µg/ml for Ketoprofen and 13.33 µg/ml and 40.40 µg/ml for Thiocolchicoside respectively. The % recovery was found to be 99.84% and 99.20% for Ketoprofen and Thiocolchicoside respectively. The developed method is simple, precise and accurate and it can be used for routine analysis of both drugs in their combined solid oral dosage form.

Key-Words: Ketoprofen, Thiocolchicoside, UV Spectrophotometry, Q-absorbance , Ratio Method, Validation

Introduction

Ketoprofen (KET) , chemically 2-(4-isobutylphenyl) propionic acid, as shown in fig.1is a nonsteroidal anti-inflammatory and analgesic agent. Ketoprofen is used for its antipyretic, analgesic, and anti-inflammatory properties by inhibiting cyclooxygenase-1 and -2 (COX-1 and COX-2) enzymes reversibly, which decrease production of proinflammatory prostaglandin precursors. [1] Ketoprofen is official in IP 2014. [2]

Thiocolchicoside (THC) chemically, N-[3-(B-D-glucopyranoxyloxy) - 5, 6, 7, 9-tetrahydro-1, 2-methoxy-10-(methylation) -9-oxobenzo[a]heptalen-7yl] acetamide, as shown in fig.2. It has selective affinity for γ-amino- butyric acid (GABA) receptors and acts on the muscular contracture by activating the GABA- inhibitory pathways thereby acting as a potent muscle relaxant. Its mode of action includes modulation of chemokine and prostanoid production and inhibition of neurophil and endothelial cell adhesion molecules by which it interferes with the initiation and amplification of the joint inflammation. [3-4] Thiocolchicoside is official in IP 2014. [5]

*** Corresponding Author**
E.mail: joshi.toraljoshi@gmail.com

In the present study the standard mixture of Ketoprofen and Thiocolchicoside was being taken for Q-Absorbance Ratio by UV method. This present investigation describes a rapid, accurate and precise UV method for Ketoprofen and Thiocolchicoside in combination using Methanol as a solvent. In which two wavelengths are used 275 nm and 251.5 respectively.

Material and Methods

Instrumentation

A UV-Visible spectrophotometer: Shimadzu, Model-UV-Vis Spectro was used. Absorption and overlain spectra of both test and standard solutions were recorded over the wavelength range of 200-400 nm. Ultra sonicator: PEI, Model UC-300 was used and Analytical weighing scale balance was used for weighing purpose.

Chemicals and reagents

Active pharmaceutical ingredient of Ketoprofen is gifted as a sample from Uma microns, Ranoli and Thiocolchicoside was obtained from Swiss parentrals, Ahmedabad. The commercial fixed dose combination. Marketed formulation, Lupiflex 4 was procured from the local market. Methanol was used as solvent and

calibrated glasswares were employed throughout the work throughout the experiment.

Method Development

Preparation of stock solution of KET (50 µg/ml)

Accurately weighed quantity of KET (31.2 mg) was transferred into 25ml volumetric flask, dissolved and diluted up to mark with methanol to obtain concentration 1248 µg/ml and from that stock solution (1 ml) was transferred into 25 ml volumetric flask, diluted up to the mark with methanol to get the concentration of solution 50 µg/ml.

Preparation of stock solution of THC (50 µg/ml)

Accurately weighed quantity of THC (10 mg) was transferred into 100 ml volumetric flask dissolved and diluted up to mark with methanol to obtain concentration of 100 µg/ml and that stock solution (1 ml) was transferred into 25 ml volumetric flask, diluted up to the mark with methanol to get the concentration of solution 4 µg/ml.

Selection of Wavelength

To determine the wavelength for measurement, KET (50 µg/ml) and Thiocolchicoside (4 µg/ml) solutions were scanned in the range of 200-400 nm. Overlain spectra of both the drugs show the isoabsorptive point at 272 nm for Ketoprofen and Thiocolchicoside.

Preparation of calibration curve of Ketoprofen and Thiocolchicoside

By appropriate different dilutions of standard stock solution, different dilutions were prepared in the range of 5-25 µg/ml for Ketoprofen and 4-20 µg/ml for Thiocolchicoside respectively. Absorbance of all the dilutions was plotted against the respective concentration to obtain calibration curve. The absorbance - overlain spectrum of Ketoprofen and Thiocolchicoside is shown in fig.6 and 7.

Method Validation

The developed method was validated with respect to linearity, precision, limit of detection ant Quantitation in accordance with the ICH guidelines.

Linearity and range

The linearity of Ketoprofen and Thiocolchicoside was found to be in the range of 5-25 µg/ml and 4-20 µg/ml respectively as shown in figure 2 and 3. Linearity of both the drugs was checked in terms of slope, intercept and correlation coefficient as shown in table 1 and 2.

Precision

Precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision study is carried out in terms of absorbance, concentration and assay as shown in table 3. Precision may be considered at three levels: Intermediate (Intraday) precision, Interday precision and repeatability as shown in table 4.

1) **Intraday precision**
   Solution containing 15 µg/ml of KET and 12 µg/ml of THC were analyzed six times on the same day and % RSD was calculated.
2) **Interday precision**
   Solution containing 15 µg/ml of KET and 12 µg/ml of THC were analyzed six times on the different successive days and % RSD was calculated.
3) **Repeatability**
   Solution containing 15 µg/ml of KET and 12 µg/ml of THC were analyzed six times and % RSD was calculated.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Accuracy of the developed method was confirmed by doing recovery study as per ICH guidelines at three different concentration levels 80%, 100%, 120% and the values were measured at all wavelengths for Ketoprofen and Thiocolchicoside. This performance was done in triplicate. The amount of Ketoprofen and Thiocolchicoside were calculated at each level and % recoveries were calculated by measuring the absorbance and fitting the values in equation as shown in table 5.

Limit of detection (LOD)

Limit of detection can be calculated using following equation as per ICH guidelines.

$$LOD = 3.3 * (\sigma / S)$$

Where, σ = standard deviation of the Y intercept of calibration curve.

S = mean slope of corresponding calibration curve.

Limit of Quantification (LOQ)

Limit of quantification can be calculated using following equation as per ICH guidelines.

$$LOQ = 10 * (\sigma / S)$$

Where, σ = standard deviation of the Y intercept of calibration curve.

S = mean slope of corresponding calibration curve.

Analysis of the marketed formulation

To determine the content of commercial formulation, 20 tablets (KET 50 mg: THC 4mg) were weighed, their mean weight was determined and finely powdered. An

accurately weighed quantity of tablet powder equivalent to 100.82 mg of KET and 8.06 mg of THC was taken and diluted to 50 ml up to marked volume with methanol, sonicated for 15 min and diluted to 10 ml with methanol. The analysis was repeated in triplicate and % Recovery was studied as shown in table 7.

Results and Discussion

The method was validated as per the ICH guidelines. The linearity was found in the range of 5-25 µg/ml and 4-20 µg/ml for Ketoprofen and Thiocolchicoside respectively. Standard calibration curves for KET and THC were linear with correlation coefficients (R^2) as they were found to be 0.995 for Ketoprofen and 0.986 for Thiocolchicoside. Recovery study values of Ketoprofen and Thiocolchicoside were found to be 99.84 % and 99.20% respectively. So, the proposed method is accurate. % RSD for intra-day and inter-day precision studies was found to be less than ± 2. The Limit of Detection and Limit of Quantitation were found to be 16.58 µg/ml and 50.25 µg/ml for Ketoprofen and 13.33 µg/ml and 40.40 µg/ml for Thiocolchicoside respectively. Hence, the developed method is simple, accurate, rapid, economical and precise in nature and LOD and LOQ obtained is in range as per ICH guidelines.

Acknowledgement

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Fig. 1 Chemical structure of Ketoprofen

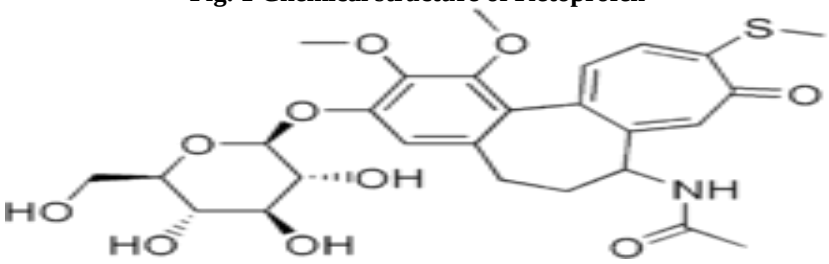

Fig. 2 Chemical structure of Thiocolchicoside

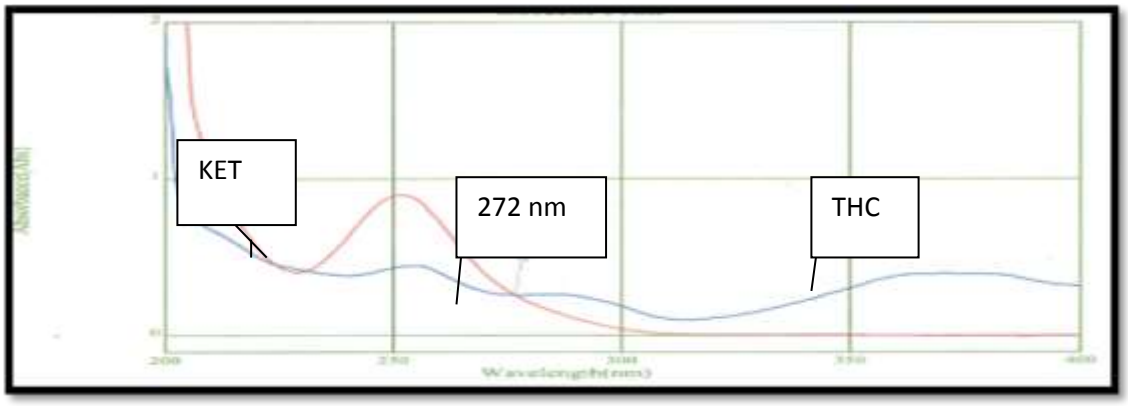

Fig. 3 Overlain spectra of KET and THC

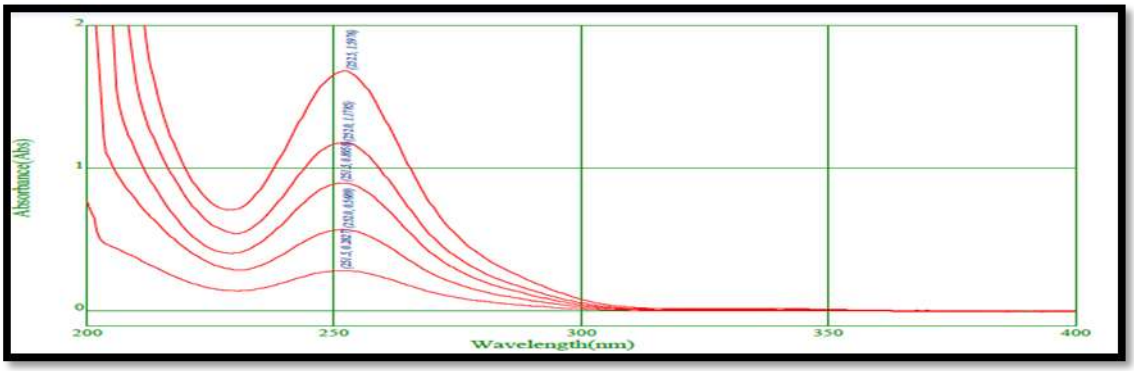

Fig.4 Overlain spectra of KET for linearity

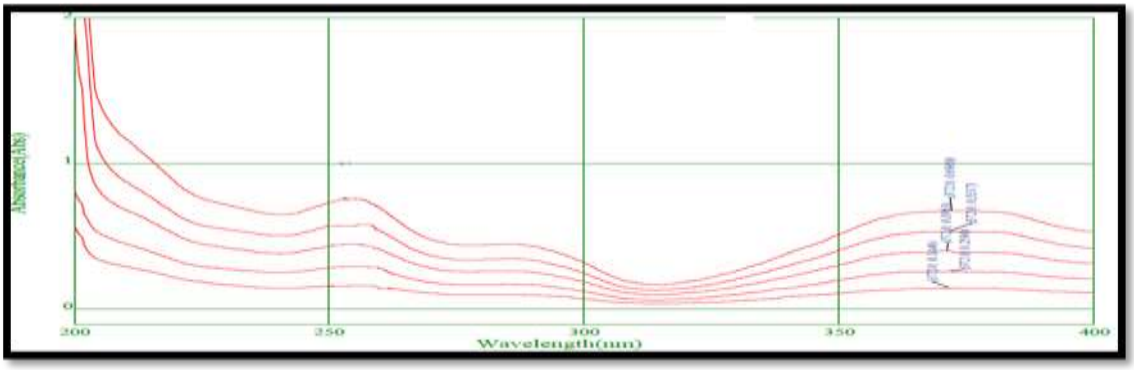

Fig.5 Overlain spectra of THC for linearity

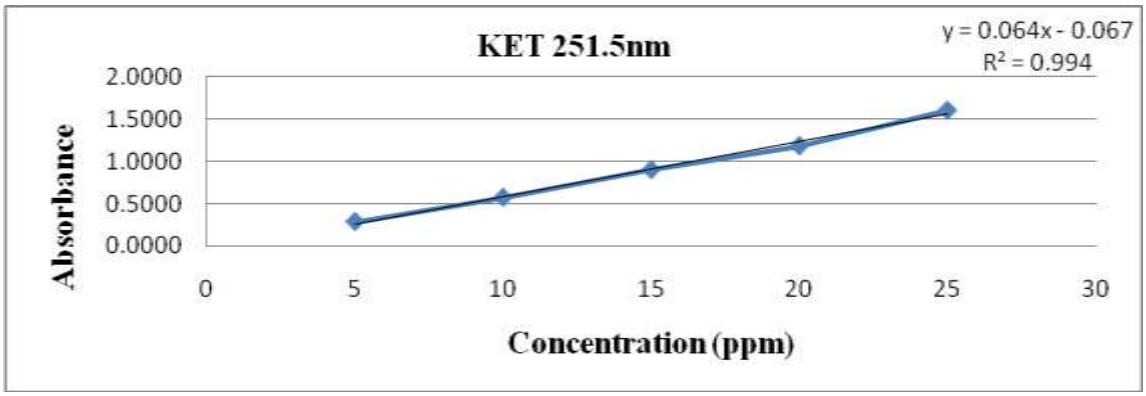

Fig.6 Calibration curve for Ketoprofen

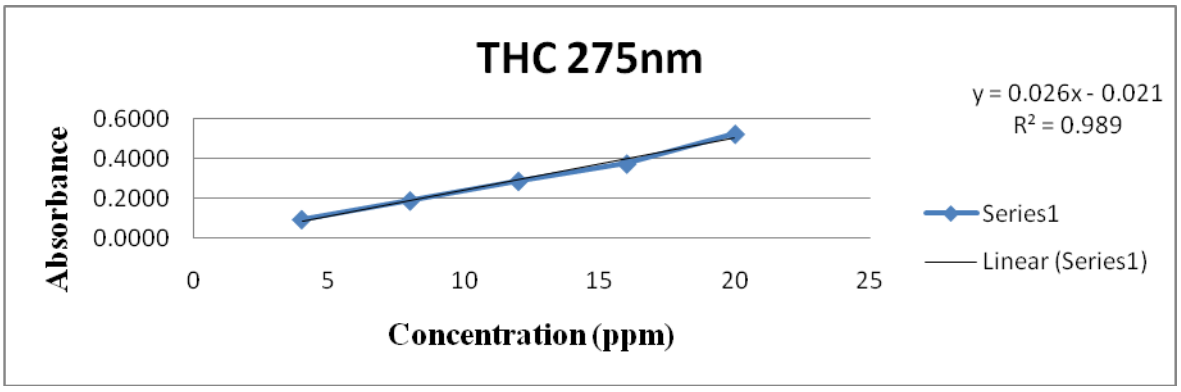

Fig.7 Calibration curve for Thiocolchicoside

Table 1 Linearity Data of Ketoprofen

ml	Conc. (µg/ml)	Absorbance (A) 275 nm	A (1%, 1cm)	Absorbance (A) 251.5 nm	A (1%, 1cm)
1	4	0.0928	232.000	0.1639	409.750
2	8	0.1876	234.500	0.2906	363.250
3	12	0.2864	238.667	0.4400	366.667
4	16	0.3740	233.750	0.5815	363.438
5	20	0.5240	262.000	0.8134	406.700
		ax_1=	240.183	ax_2=	381.961
		R^2=	0.990	R^2=	0.986

Table 2 Linearity Data of Thiocolchicoside

ml	Conc. (µg/ml)	Absorbance (A) 275 nm	A (1%, 1cm)	Absorbance (A) 251.5 nm	A (1%, 1cm)
1	5	0.0928	185.600	0.2827	565.400
2	10	0.1876	187.600	0.5689	568.900
3	15	0.2864	190.933	0.8958	597.200
4	20	0.3740	187.000	1.1785	589.250
5	25	0.5240	209.600	1.5976	639.040
		ay_1 =	192.147	ay_2 =	591.958
		R^2 =	0.990	R^2 =	0.995

Table 3 Precision Study of KET and THC (% Assay)

Wt. (mg)	Dilution (ml)	A_2 KET (251.5 nm)	A_1 THC (275 nm)	Cy (ppm) KET	Cx (ppm) THC	%Assay KET	%Assay THC
250.1	2500	1.5570	0.5286	25.01	2.00	100.84	100.63
250.2	2500	1.5527	0.5275	24.92	2.02	100.44	101.87
250.3	2500	1.5395	0.5228	24.72	1.99	99.59	100.03
250.1	2500	1.5439	0.5245	24.78	2.01	99.91	101.29
250.2	2500	1.5483	0.5260	24.85	2.02	100.15	101.56
250.2	2500	1.5352	0.5214		1.99	99.34	100.05
% RSD	0.48			0.53			

Table 4 Precision Study of KET and THC

KET (15 µg/ml)			THC(12 µg/ml)		
Interday Precision	**Intraday Precision**	**Repeatability**	**Interday Precision**	**Intraday Precision**	**Repeatability**
Abs.(A) (n=6)	**Abs.(A) (n=6)**	**Abs.(A) (n=6)**	**Abs.(A)**	**Abs.(A)**	**Abs.(A)**
0.8973	0.8895	0.8704	0.4012	0.3989	0.3994
0.8958	0.887	0.8727	0.4018	0.3943	0.3947
0.8945	0.8846	0.8750	0.3948	0.3942	0.3915
0.8908	0.8774	0.8636	0.3949	0.3913	0.3944
0.8920	0.8822	0.8681	0.4016	0.3945	0.3951
0.8933	0.8798	0.8658	0.3953	0.3946	0.3968
0.27	0.51	0.49	0.4012	0.62	0.67

Table 5 Accuracy Study of KET and THC

Name of drug	% Level of recovery	Wt. (mg)	Mean absorbance	S.D.	% RSD	% Recovery
KET	80	12.5	0.2619	0.00056	0.21	101.57
	100	37.5	0.8928	0.00245	0.27	98.78
	120	62.5	1.5500	0.00733	0.47	99.84
THC	80	10	0.0843	0.00103	1.2	101.05
	100	30	0.2899	0.00309	1.0	99.03
	120	50	0.4985	0.00579	1.1	99.20

Table 6 LOD and LOQ for KET and THC

Drug	LOD (µg/ml)	LOQ (µg/ml)
KET	16.58	50.25
THC	13.25	40.40

Table 7 Analysis of marketed formulation

Sr.no.	Actual conc.		Conc. Found		Mean % Recovery	
	KET (mg/ml)	THC (mg/ml)	KET (mg/ml)	THC (mg/ml)	KET (%)	THC (%)
1	50	4	50.2	3.9	100.4	97.5
2	50	4	50.3	4.1	100.6	102.5
3	50	4	49.8	4.0	99.6	100.0
Mean					100.4	97.5

Table 4 System Suitability Parameters

Parameters	KET	THC	Acceptance Criteria
Correlation coefficient	0.995	0.986	0.990-1.0
% RSD	0.58	0.43	< 2
Standard deviation	0.00667	0.0031	< 2
Slope	0.995	0.986	0.999-1.0
% Assay	100.4	97.5	95-101 %
LOD (µg/ml)	16.58	13.33	Within limit
LOQ (µg/ml)	50.25	40.40	Within limit

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