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Research Article

DEVELOPMENT AND VALIDATION OF UV-VISIBLE SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF KETOPROFEN AND THIOCOLCHICOSIDE IN SOLID ORAL DOSAGE FORM

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ABSTRACT

A simple, accurate, precise, economical and cost-effective UV Spectrophotometric method has been developed for simultaneous estimation of Ketoprofen and Thiocolchicoside in solid oral dosage form and validated as per International Conference of Harmonisation guidelines. Estimation of Ketoprofen and Thiocolchicoside was conducted by using simultaneous equation method based on measurement of absorbance at two wavelengths. The solvent used was methanol and the λ_{max} of Ketoprofen and Thiocolchicoside were found to be 251.5 nm and 372 nm respectively. At 251.5 nm both the drugs have considerable absorbance. 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 at 251.5 nm for Ketoprofen and 0.986 and 0.996 at 251.5 nm and 372 nm for Thiocolchicoside respectively. Recovery study values of Ketoprofen and Thiocolchicoside was found to be 99.84% and 100.57% respectively. % 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 13.25 µg/ml and 40.16 µg/ml for Thiocolchicoside respectively. The proposed method was satisfactorily validated as per the ICH guidelines and can be successfully employed for routine analysis of Ketoprofen and Thiocolchicoside in solid oral dosage form.

Keywords: Ketoprofen, Thiocolchicoside, UV Spectrophotometry, Simultaneous equation method, Validation.

INTRODUCTION

Ketoprofen (KET), chemically 2-(4-isobutylphenyl) propionic acid, as shown in fig.1 is 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 decreases 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] Thiocolchicoside is official in IP 2014.[4]

The literature review reveals that there are several analytical methods have been reported for Ketoprofen and Thiocolchicoside alone and in combination with other drugs.[5-8] However there is no method has been developed for simultaneous estimation of Ketoprofen and Thiocolchicoside in solid oral dosage form. The objective is to develop a simple, accurate, precise, economical and cost-effective analytical method developed for simultaneous estimation of Ketoprofen and Thiocolchicoside in solid oral dosage form.

MATERIALS 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. Marketed formulation containing Thiocolchicoside and Ketoprofen (4:50) was procured from local pharmacy. Methanol was used as a solvent throughout the experiment.

Method Development

Preparation of standard 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 standard stock solution of THC (4 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 200-400nm. Overlain spectra of both the drugs show the isoabsorptive point at 272 nm for Ketoprofen and Thiocolchicoside as shown in fig.3.

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 Thiocolchicoside respectively. Absorbance of all the dilutions was plotted against the respective concentration to obtain calibration curve. The calibration curve of Ketoprofen and Thiocolchicoside is shown in fig. 4 and 5.

Method Validation

The developed method was validated with respect to linearity, accuracy, precision, limit of detection and quantification 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 6-9. 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 4. Precision may be considered at three levels: Intermediate (Intraday) precision, reproducibility (Interday) precision, repeatability as shown in table 4.

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.

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.

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.

$$\mathbf{LOD = 3.3 * (\sigma / S)}$$

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.

$$\mathbf{LOQ = 10 * (\sigma / S)}$$

Where, σ = standard deviation of the Y intercept of calibration curve.
S = mean slope of corresponding calibration curve.

RESULTS

Overlain spectra of the drugs depicted occurrence of two peaks at 251.5 nm and 372 nm. Ketoprofen and Thiocolchicoside showed linearity with absorbance in the range 5-25 µg/ml and 4-20 µg/ml respectively. Coefficient of correlation was found to be 0.995 for Ketoprofen and 0.986 for Thiocolchicoside. The observations are presented in Table 1, fig.6 and fig.7. The absorptivity were found approximately same for all the concentrations hence both drugs obeyed Beer's law as indicated concentration range. The high values of correlation coefficients (R^2) also indicate good linearity of calibration curve for both the drugs. Recovery study values of Ketoprofen and Thiocolchicoside was found to be 99.84% and 100.57% 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 and 50.25 µg/ml for Ketoprofen and 13.25 and 40.16 for Thiocolchicoside respectively. So, the developed method is precise in nature and LOD and LOQ obtained is in range as per ICH guidelines.

Table 1: Linearity Data of KET

ml	Concentration (µg/ml)	Absorbance (A)372 nm	A (1%, 1cm)	Absorbance (A)251.5 nm	A (1%, 1cm)
1	5	0.0000	0.000	0.2827	565.400
2	10	0.0000	0.000	0.5689	568.900
3	15	0.0000	0.000	0.8958	597.200
4	20	0.0000	0.000	1.1785	589.250
5	25	0.0000	0.000	1.5976	639.040
ay_1 =			0.000	ay_2 =	591.958
R^2 =			00	R^2 =	0.995

Table 2: Linearity Data of THC

ml	Concentration (µg/ml)	Absorbance (A)372 nm	A (1%, 1cm)	Absorbance (A)251.5 nm	A (1%, 1cm)
1	4	0.1446	361.500	0.1639	409.750
2	8	0.2590	323.750	0.2906	363.250
3	12	0.3963	330.250	0.4400	366.667
4	16	0.5317	332.313	0.5815	363.438
5	20	0.6960	348.000	0.8134	406.700
ax_1=			339.163	ax_2=	381.961
R^2=			0.996	R^2=	0.986

Table 3: Precision Study of KET and THC (% assay)

Wt. (mg)	Dilution (ml)	A_2 KET (251.5nm)	A_1 THC (372 nm)	Cy (ppm) KET	Cx (ppm) THC	% Assay	
						KET	THC
250.1	2500	1.5570	0.0675	25.02	1.99	100.85	100.29
250.1	2500	1.5527	0.0670	24.96	1.98	100.60	99.54
250.2	2500	1.5395	0.0690	24.69	2.03	99.51	102.47
250.1	2500	1.5439	0.0681	24.79	2.01	99.92	101.18
250.2	2500	1.5483	0.0682	24.86	2.01	100.17	101.29
250.1	2500	1.5352	0.0681	24.64	2.01	99.32	101.18
% RSD		0.27	0.05				

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	0.3948	0.3944	0.3948
0.8958	0.887	0.8727	0.3951	0.3943	0.3947
0.8945	0.8846	0.8750	0.3947	0.3942	0.3945
0.8908	0.8774	0.8636	0.3949	0.3948	0.3944
0.8920	0.8822	0.8681	0.3950	0.3945	0.3951
0.8933	0.8798	0.8658	0.3953	0.3946	0.3951

Table 5: Accuracy study of KET and THC

Name of drug	% Level of recovery	Wt. (mg)	Dil. (ml)	Mean abs.	S.D.	% RSD	% Recovery
KET	80	12.5	2500	0.2619	0.00056	0.21	101.57
	100	37.5	2500	0.8928	0.00245	0.27	98.78
	120	62.5	2500	1.5500	0.00733	0.47	99.84
THC	80	10	2500	0.1312	0.00072	0.54	100.57
	100	30	2500	0.4052	0.00021	0.051	99.92
	120	50	2500	0.6748	0.0002	0.029	99.15

Table 6: System Suitability Parameters

Parameters	KET	THC	Acceptance Criteria
Correlation coefficient	0.995	0.986	0.990-1.0
% RSD	0.27	0.05	< 2
% Recovery	99.84	99.15	95-101%
Standard deviation	9.11	7.42	Within limit
Slope	0.995	0.986	<1
LOD (µg/ml)	16.58	13.25	Within limit
LOQ µg/ml	50.25	40.16	Within limit

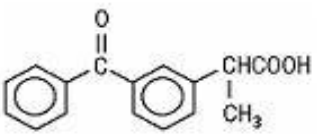

Figure 1: Chemical structure of KET

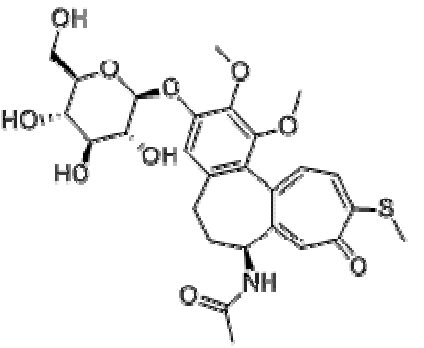

Figure 2: Chemical structure of THC

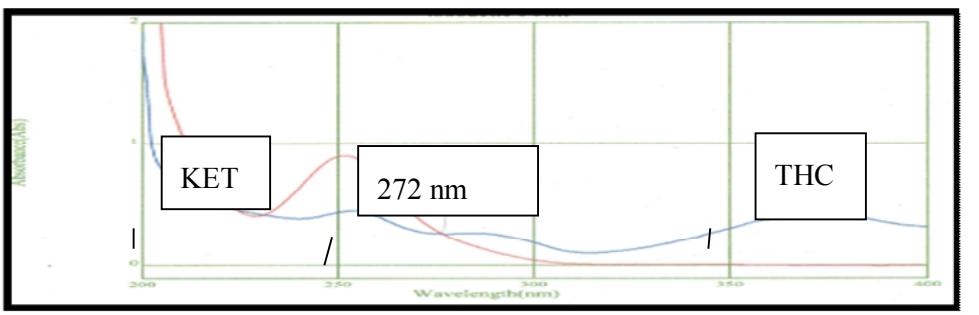

Figure 3 Overlain spectra of KET and THC

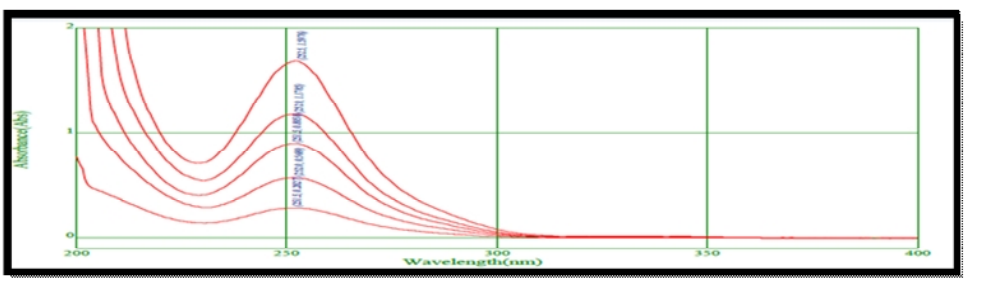

Figure 4: Overlain spectra of KET for linearity

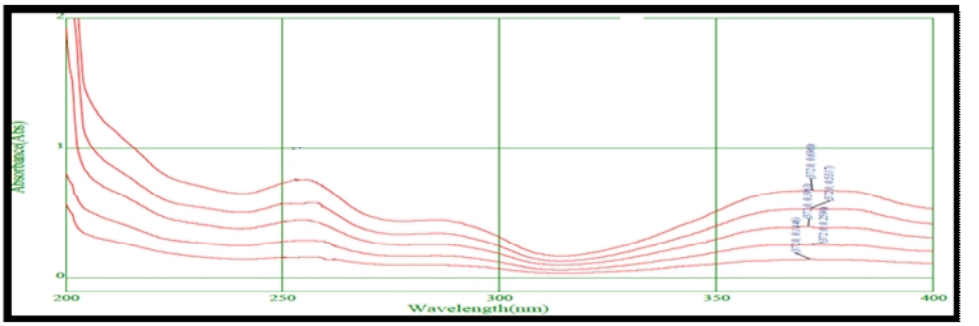

Figure 5: Overlain spectra of THC for linearity

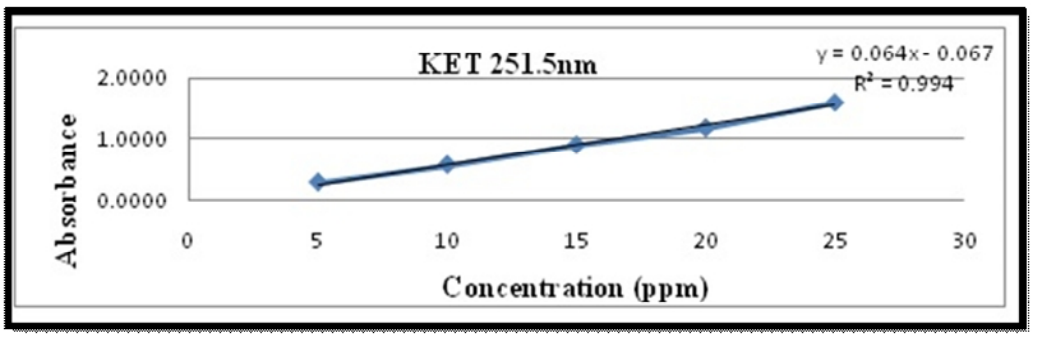

Figure 6: Calibration curve for KET

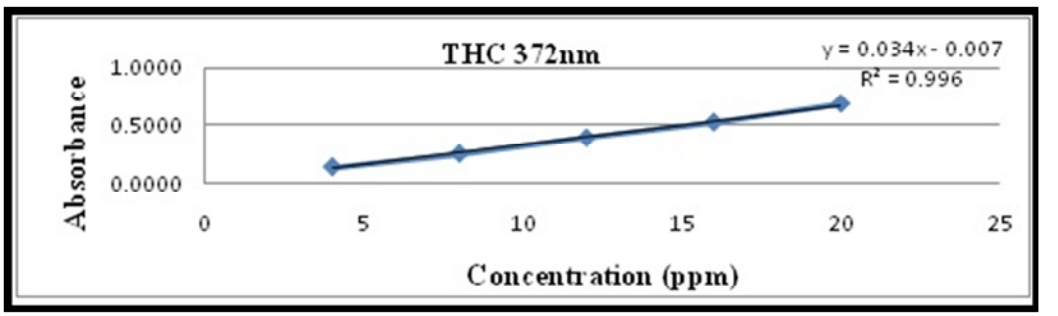

Figure 7: Calibration curve for THC

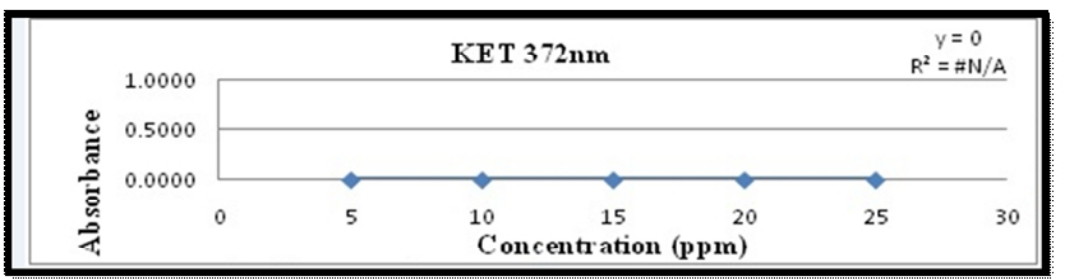

Figure 8: Calibration curve for KET

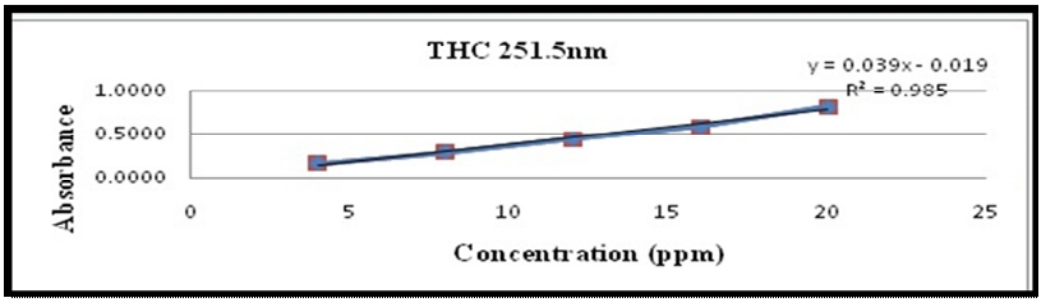

Figure 9: Calibration curve for THC

DISCUSSION

The proposed simultaneous equation method is very simple that can be performed by use of any spectrophotometer and does not require any costly instrument equipped with special package. It also shows good linearity values, precision, accuracy, LOD and LOQ.The proposed UV method was found to be simple, accurate and precise for determination of KET and THC in solid oral dosage form. This method utilizes easily available and cheap solvent for analysis of KET and THC in solid dosage form. The common excipients and other additives are usually present in solid dosage form do not interfere in the analysis of KET and THC in this method.

CONCLUSION

The results demonstrated that simultaneous equation method by UV-Visible spectrophotometer could be useful for technique for determination of Ketoprofen and Thiocolchicoside when they are given in solid dosage form. Hence it could be conveniently adopted for quality control analysis in combined pharmaceutical formulation.

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