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Der Pharmacia Lettre, 2010, 2(3): 315-320
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

RP-HPLC Method for Simultaneous Estimation of Thiocolchicoside and Ketoprofen in Combined Dosage Form

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Abstract

A RP-HPLC method has developed for simultaneous estimation of thiocolchicoside and ketoprofen in combined tablet dosage form. The mobile phase used was a combination of Acetonitrile: Water: Phosphate buffer (pH 3.0) (60:30:10, v/v/v). The detection of the combined dosage form was carried out at 260 nm and a flow rate employed was 1 ml/min. The retention time for thiocolchicoside and ketoprofen has found to be 2.70 and 4.90 min respectively. Linearity was obtained in the concentration range of 4-20 µg/ml of thiocolchicoside and 20-100 µg/ml of ketoprofen with a correlation coefficient of 0.9950 and 0.9997. The method was accurate and precise with recoveries in the range of 99.60 and 101.30 % for both the drugs and relative standard deviation (R.S.D.) < 2. The proposed method is highly sensitive, accurate and precise and hence was successfully applied for the reliable quantification of API content in the commercial formulation containing these drugs in combination. The developed method was validated according to International Conference on Harmonization guidelines.

Key Words : RP-HPLC, Thiocolchicoside, Ketoprofen.

INTRODUCTION

Thiocolchicoside (THC) chemically, N-[3-(B-D-glucopyranoxyloxy)-5,6,7,9-tetrahydro-1,2-imethoxy-10-(methylthio)-9-oxobenzo[a]heptalen-7yl]acetamide. 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[1]. Ketoprofen (KET), chemically 2-(4-isobutylphenyl) propionic acid, is a nonsteroidal-antiinflammatory and analgesic agent[2]. Both the drugs are marketed as combined dose tablet formulation (4:50 mg THC: KET). Literature survey reveals that thiocolchicoside can be estimated by spectrophotometry[3] ,HPLC[4,5,6]and by HPTLC[7] methods individually or in combination with other drugs.

Ketoprofen is reported to be estimated by spectrophotometry[8] and HPLC[9,10] The reported methods are applicable for the estimation of either for THC or KET individually or in combination with other drugs from pharmaceutical dosage forms or biological fluids. No single method was reported for the estimation in combined dosage form. The present work describes the development of a validated analytical RP-HPLC method, which can quantify these components simultaneously from a combined dosage form.

MATERIALS AND METHOD

2.1 Sample, Reagents and Chemicals:

Active pharmaceutical ingredient (API) working standards of THC and KET were received as gift samples from Sanofi- Synthelabo Ltd. Banglore and Emcure Pharmaceuticals, Pune respectively. Test samples (Tablet with composition 4 mg Thiocolchicoside and 50 mg Ketoprofen) were purchased from local market. HPLC grade chemicals were obtained from Thermo Fischer Scientific India Ltd. Mumbai.

2.2 Instrumentation:

HPLC system (Merck Hitachi) consisting of quaternary gradient pump, autosampler, column oven, and UV detector (L – 7400) was employed for analysis. Chromatographic data was acquired using Winchrome software. The column used was Thermo scientific C18, (250×4.6 mm i.d.)

2.3 HPLC conditions:

A mixture of acetonitrile, water and 0.025 M phosphate buffer (pH adjusted to 3.0 with using dilute orthophosphoric acid) in the ratio of 60:30:10 % v/v/v was used as mobile phase and was filtered before use through 0.45 µm membrane filter. A constant flow of 1.0 ml/min was maintained throughout the analysis. Detection was carried out using UV detector at 260 nm. The separation was carried out at room temperature, 25 $\pm 1^0$ C.

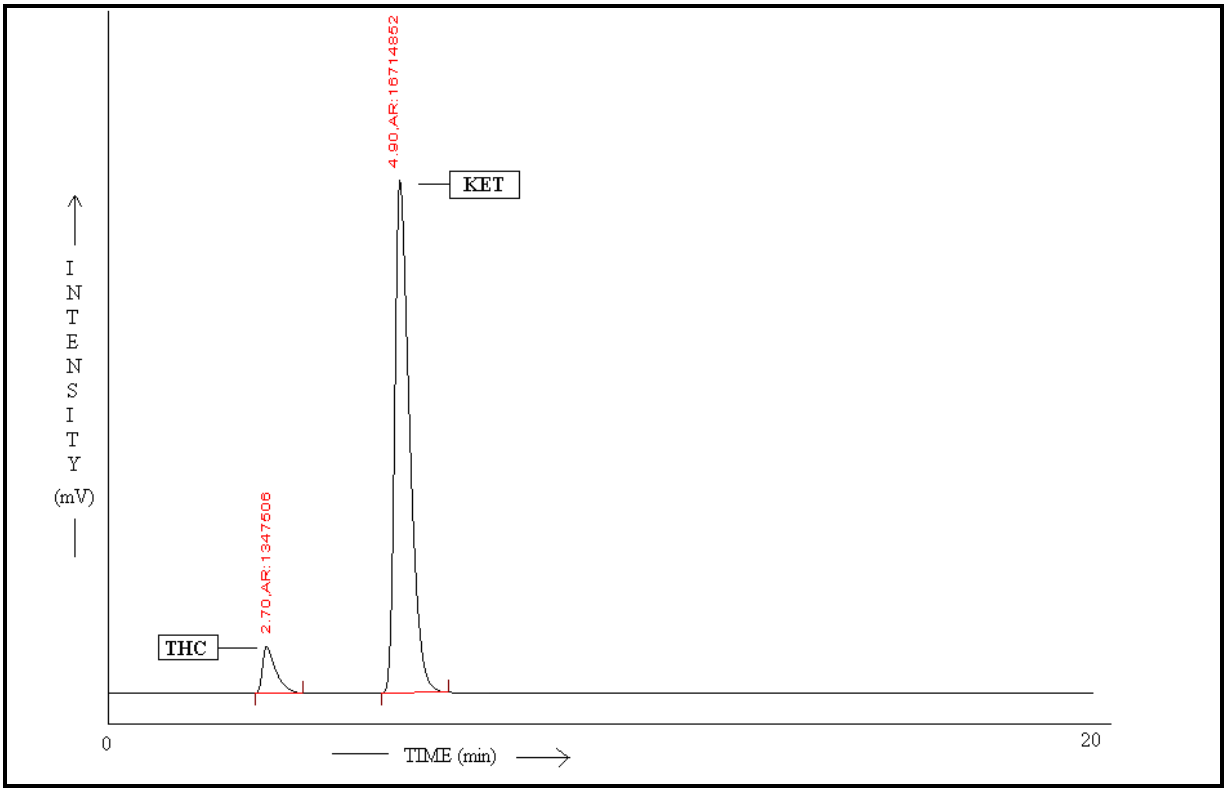
2.4 Preparation of solutions:

Standard stock solution of THC and KET were prepared separately by transferring 25 mg of each working standard in a 25 ml volumetric flask. A 20 ml portion of mobile phase was added, sonicated and remaining volume was made up to the mark with mobile phase.

2.5 Analysis of formulation:

Twenty tablets, were weighed and finely powdered. A quantity of powder equivalent to 25 mg of KET and 2 mg of THC was weighed accurately and transferred to a 50 ml volumetric flask. 30 ml mobile phase was added and sonicated for 20 min. The resulting solution was spiked with 2 ml Standard stock solution of THC and the volume was made up with the mobile Phase. The resulting solution was mixed and filtered through Whatmann filter paper and filtrate was appropriately diluted to get approximate concentration of 4 µg/ml of THC and 25 µg/ml of KET . and it was filtered using 0.2 µm membrane filter.. The 20 µl of the above solution was injected in to the column and chromatogram was recorded.

Fig-1 : Typical chromatogram of THC and KET



2.6 Linearity:

To establish the linearity a series of dilutions ranging from 4-20 µg/ml for THC and 20-100 µg/ml for KET were prepared separately and calibration graph was plotted between the mean peak area Vs respective concentration and regression equation was derived.

2.7 Method Validation:

The accuracy of the method was carried out by adding known amount of each drug corresponding to three concentration levels 80%, 100% and 120% of the label claim along with the excipients in triplicate. The samples were given the same treatment as described in Section 2.5 .Precision of the method was checked by analyzing the samples on different times of the same day as well as on different days.

Robustness was performed by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 1.0 mL/min to 0.9 mL/min and 1.1 mL/min while composition of the mobile phase was changed by ± 1 % .

LOD and LOQ are calculated by using the values of slopes and intercepts of the calibration curves for both the drugs.

Table -1: Result of recovery studies

Level of recovery	Amt. of pure drug added (mg)		Percent recovery*	
	THC	KET	THC	KET
80 %	3.2	20	100.60	99.65
100 %	4	25	101.09	99.95
120 %	4.8	30	99.89	99.57
Mean % recovery			100.52	99.72
S.D.			0.6034	0.2003
C.V			0.6002	0.2008

* Average of three determinations, SD-Standard Deviation, CV- Coefficient of Variation

RESULT AND DISCUSSION

The proposed chromatographic system was found suitable for effective separation and quantitation of THC (2.70 min) and KET (4.90 min). Chromatograms of mixed standard solutions which contained THC and KET were recorded and shown in Fig -1.

3.1System Suitability:

The system suitability test was applied to a representative chromatogram to check the various parameters such as column efficiency, resolution, precision and peak tailing. The result obtained is shown in Table 2.

Table-2: System Suitability Parameters

System Suitability Parameter	Component	
	THC	KET
Retention times (RT) min	2.70	4.90
Theoretical plates (N)	2845.49	3504.17
Tailing factor (AS)	1.37	1.20
Resolution (RS)	2.45	

3.2 Linearity:

THC and KET showed a linearity of response between 4-20 µg/ml and 20-100 µg/ml. This linearity was represented by a linear regression equation as follows.

Y_{THC} = 340753 x - 51023 (r2 = 0.9995)

Y_{KET} = 721827 x - 286312 (r2 = 0.9999)

3.3 Accuracy and Precision

The recovery experiment was carried out by spiking the already analyzed sample of the tablets with their different known concentration of standard THC and KET. The result is summarized in Table.1. The percent recovery for THC ranges from 99.89 to 101.09 % and KET ranges from

99.57 to 99.95 %. The reproducibility of the proposed method was determined by performing tablet assay at different time intervals on the same day (Intra-day assay precision) and on three different days (Inter-day precision). Results of intra-day and inter-day precision are expressed in % RSD. % RSD for Intraday assay precision was found to be 0.3519 (for THC) and 0.2493 (for KET) and that for Inter-day assay precision was found to be 0.9449 (for THC) and 0.1660 (for KET).

3.4 Robustness

In all deliberately varied conditions, the RSD of contents of ATS and MET were found to be well within the acceptable limit of 2%. The tailing factor for both the peaks was found to be <1.5.

3.5 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were determined based on the standard deviation of the y-intercept and slope of the calibration curves. Limit of detection and limit of quantitation values for THC were found to be 0.0379 µg/ml and 0.1149 µg/ml and that for KET were found to be 0.2116 µg/ml and 0.6412 µg/ml.

3.6 Assay:

The content of THC and KET found in the tablets by the proposed method are shown in Table 3. The low R.S.D indicates that the method is precise and accurate.

Table -3: Assay Results of Marketed Formulation

Component	Label claim (mg/capsule)	Amount found * (mg/capsule)	percent label claim *	S.D	C.V
THC	4	3.98	99.66	0.6646	0.6991
KET	50	50.04	100.08	0.6991	0.6985

* Average of six determinations, SD-Standard Deviation, CV- Coefficient of Variation

CONCLUSION

The proposed chromatographic system was found suitable for effective separation and quantitation of THC (RT-2.70 min) and KET (RT-4.90 min). The developed RP-HPLC method was found to be simple, rapid, selective, accurate and precise for the concurrent estimation of drugs in two-component tablet dosage form of THC and KET. The developed method was validated according to ICH guidelines for linear relation including coefficient of correlation, robustness, accuracy, reproducibility and precision. The RSD for all parameters was found to be less than two, which indicates the validity of method and assay results obtained by this method are in fair agreement. The developed method can be used for routine quantitative simultaneous estimation of THC and KET in pharmaceutical preparation.

Acknowledgement
The authors are very thankful to Dr. Avinash D. Deshpande, Director of Pharmacy, Pad. Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune for providing necessary facilities. The authors are also thankful to Sanofi-Synthelabo Ltd, Banglore. and Emcure Pharmaceuticals Pvt. Ltd. Pune. For providing gift samples of Thiocolchicoside and Ketoprofen respectively.

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