

Development And Validation Of RP-HPLC Method For Simultaneous Estimation Of Thiocolchicoside And Dexketoprofen In Bulk And Tablet Dosage Form

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Abstract: A simple, fast, accurate and precise method has been developed for the simultaneous determination of thiocolchicoside and dexketoprofen from pharmaceutical formulation by reversed-phase high performance liquid chromatography. The separation was carried out on C₁₈ column using mobile phase consisting of a mixture of methanol: phosphate buffer and pH adjusted to 4.5 with orthophosphoric acid in the ratio (65:35 v/v). The flow rate was maintained at 1.0 ml/min. The UV detection was carried out at a wavelength of 260 nm. The retention time for thiocolchicoside and dexketoprofen was found to be 3.02 min and 8.91 min respectively. Linear response obtained for thiocolchicoside was in the concentration range 4-24 µg/ml ($r^2 = 0.9998$) and dexketoprofen in the range 5-30 µg/ml ($r^2 = 0.9990$). The relative standard deviation in the tablets was found less than 2% for six replicates. The method was validated according to the ICH guidelines with respect to linearity, precision, accuracy, limit of detection, limit of quantification and robustness. Thus, proposed method can be successfully applicable to the pharmaceutical preparation containing the above mentioned drugs without any interference of excipients.

Key words: Thiocolchicoside (THC); Dexketoprofen (DKP); RP-HPLC; Validation.

INTRODUCTION

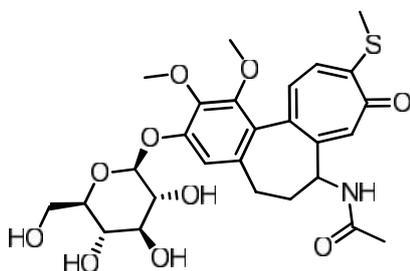
Thiocolchicoside chemically, *N*-[(7*S*)-3-(beta-D-glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfonyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide. It is a semi-synthetic derivative of the naturally occurring compound colchicoside with a relaxant effect on skeletal muscle, which has been found to displace both [³H] gamma-amino butyric acid ([³H] GABA) and [³H] strychnine binding, suggesting an interaction with both GABA and strychnine-sensitive glycine receptors. THC is potent competitive antagonist of GABA function, thereby acting as potent muscle relaxant and displays anti-inflammatory and analgesic properties¹.

Dexketoprofen chemically, (2*S*)-2-[3-(benzoyl)phenyl]propanoic acid. It is a (s)-(+)-enantiomer of Ketoprofen with anti-inflammatory

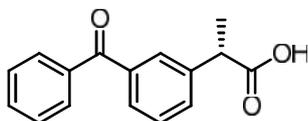
and analgesic property. It is one of the most potent *in vitro* inhibitors of prostaglandin synthesis; by blocking cyclo-oxygenase therefore it reduces inflammation and pain. Both drugs are marketed as combined dose tablet formulation (4:25mg THC: DKP)².

Literature survey reveals that THC can be estimated by HPLC³⁻⁹, spectrophotometry¹⁰⁻¹², TLC¹³ and HPTLC¹⁴⁻¹⁵ methods individually or in combination with other drugs. DKP is reported to be estimated by HPLC¹⁶⁻¹⁷, spectrophotometry¹⁸ and HPTLC¹⁹ methods individually or in combination with other drugs. However, there is no analytical method reported for the estimation of THC and DKP in a combined dosage formulation. Aim of present work was to develop and validate simple, economic, rapid, accurate and precise RP-HPLC method for determination of these drugs in

fixed dose combination. The proposed methods was optimized and validated as per the International Conference on Harmonization (ICH) guidelines²⁰.



Thiocolchicoside



Dexketoprofen

Figure 1. The chemical Structure of Thiocolchicoside and Dexketoprofen

EXPERIMENTAL

Materials and reagents

Active pharmaceutical ingredient of THC and DKP standards were received as gift samples from Emcure Pharmaceuticals Ltd, Pune. Methanol (HPLC grade), water (HPLC grade), potassium dihydrogen ortho-phosphate buffer (AR grade) and orthophosphoric acid (AR grade) were procured from Merck Chemicals, India. Tablet containing THC and DKP (4:25 mg) were purchased from local pharmacy shop (Infen MR, Emcure Pharmaceuticals Ltd, Pune, India).

Equipment and chromatographic conditions

The HPLC system used was a Waters 510 HPLC system equipped with a Rheodyne injector (20 μ l) and UV detector. Chromatographic separation was carried isocratically at room temperature with a Purosphere STAR RP-18e (250 mm \times 4mm i.d., 5 μ m) column from Merck KGaA 64271 Darmstadt, Germany. Data acquisition was made with Data Ace software.

The mobile phase consisted of methanol and phosphate buffer (0.035M) in the ratio 65:35 v/v (pH adjusted to 4.5 with orthophosphoric acid). The mobile phase was premixed and filtered through a 0.45 μ m nylon filter and degassed. The injection volume was 20 μ l and eluted at a flow

rate of 1 ml/min. The detection wavelength was 260 nm.

Preparation of standard solutions

Standard stock solutions (100 μ g/ml) of THC and DKP were prepared by dissolving accurately weighed 10 mg of each drug separately in mobile phase in 100 ml volumetric flask and filtered through 0.45 μ nylon filter. The working standard solutions of these drugs were further diluted with mobile phase to get required concentration of THC (4 μ g/ml) and DKP (25 μ g/ml).

Preparation of sample solutions

Twenty tablets were weighed and crushed to a fine powder. The quantity of the powder equivalent to 4 mg of THC and 25 mg of DKP was weighed accurately and then transferred to 100 ml volumetric flask containing 70 ml of mobile phase. It was then sonicated for 15 min. The solution was filtered through a 0.45 μ nylon filter and volume was made up to the mark with mobile phase. The final dilution made with mobile phase, contained about 4 μ g/ml and 25 μ g/ml of THC and DKP respectively.

Method validation

The method of analysis was validated as per the recommendations of ICH for the parameters like linearity, accuracy, limit of detection, limit of quantitation, intra-day and inter-day precision, repeatability and robustness²⁰.

To establish the linearity a series of dilutions ranging from 4-24 μ g/ml for THC and 5-30 μ g/ml for DKP were prepared separately and calibration graph was plotted between the mean peak area Vs respective concentration and regression equation was derived.

The ICH document defines specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. For this diluent was used as blank. Standard and sample were prepared as per test procedure. Check for the interference of blank with the analyte peak. In the case of assay, demonstration of specificity requires that it can be shown that the procedure is unaffected by the presence of impurities or excipients.

The accuracy of the method was determined by calculating percent recovery of THC and DKP by the standard addition method. The recovery experiments were carried out in triplicate (80, 100 and 120 %) by spiking previously analyzed samples of the tablets with three different concentrations of standards. The basic concentration level of sample solution selected for

spiking of the drugs standard solution was 4 µg/ml of THC and 25 µg/ml of DKP for both the methods. The results are reported in term of percent recovery.

Precision of estimation of THC and DKP by proposed method was ascertained by replicate analysis of homogenous samples of tablet powder at different time intervals on the same day (Intra-day precision) and on second day (Inter-day precision). The relative standard deviation (% RSD) was determined to assess the precision of the assay and it was found to be not more than 2.0 %.

Repeatability of the method was performed by injecting 100% concentration of THC and DKP of the regular analytical working value consecutively for six times and the effects on the results were examined. Results were reported in terms relative standard deviation.

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated for the proposed method which was based on the standard deviation of the y intercept and the slope of the calibration curves. LOD and LOQ were calculated using following formulae: $LOD = 3.3(SD)/S$ and $LOQ = 10(SD)/S$. Where, SD = standard deviation of response (peak area) and S = average of the slope of the calibration curve.

To evaluate robustness of a HPLC method, few parameters were deliberately varied. The parameters included variation of flow rate ± 1 , PH of mobile phase ± 1 and percentage of methanol in the mobile phase ± 2 .

RESULTS AND DISCUSSION

The proposed method for simultaneous estimation of THC and DKP in bulk as well as in pharmaceutical preparation was found to be simple, accurate, economical and rapid. The method was validated as per the ICH guidelines.

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of methanol: phosphate buffer (PH 4.5 adjusted with orthophosphoric acid) in the ratio of 65:35 v/v at 1 ml/min flow rate. The optimum wavelength for detection was set at 260 nm at which much better detector responses for both drugs were obtained. The retention time for THC and DKP was found to be 3.02 min and 8.91 min respectively.

System suitability testing

To know reproducibility of the method, system suitability test was employed to establish the parameters such as retention time, tailing factor, etc. The results obtained for system suitability are summarized in Table 1.

Linearity

THC and DKP showed a linearity of response between 4-24 µg/ml and 5-30 µg/ml with a correlation coefficient of 0.9998 and 0.9990 respectively. The results obtained for linearity of THC and DKP are summarized in Table 1.

Precision

The precision of this method was determined by intra-day and inter-day precision. The % R.S.D was found less than 2 this indicate that the method is precise. The results of precision studies are shown in Table 1.

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

The sensitivity of method is described in terms of LOD and LOQ. LOD and LOQ values for THC were found to be 0.6µg/ml and 2.0 µg/ml and that for DKP were found to be 1.5µg/ml and 5.0µg/ml respectively. The results of LOD and LOQ studies are shown in Table 1.

Table 1. Method validation and system suitability parameters

Parameter		THC	DKP
Linearity range		4-24 µg/ml	5-30 µg/ml
regression coefficients (r)		0.9998	0.9990
Limit of detection		0.6 µg/ml	1.5 µg/ml
Limit of quantitation		2.0 µg/ml	5.0 µg/ml
Precision	Intra-day (%RSD)	0.6644	0.5226
	Inter-day (%RSD)	1.2011	1.1236
Retention time (min)		3.02	8.91
Tailing factor		1.6	1.4

Accuracy

The accuracy was evaluated by the recovery of THC and DKP at three different levels (80, 100 and 120%). The percentage recovery was found to be 99.82% and 100.16% for THC and DKP respectively, % RSD was found to be less than 2, ensuring that the method is accurate. The results of accuracy studies are shown in Table 2.

Repeatability

The experimental values obtained for the repeatability of THC and DKP in samples is presented in Table 3. The result obtained shows % R.S.D. < 2, indicating good repeatability of method.

Robustness

Robustness of the method was carried out by deliberately made small change in the flow rate ± 0.1 , pH of mobile phase ± 0.1 and mobile phase

composition (methanol: phosphate buffer) ± 2 . The results of robustness studies are shown in Table 4.

Specificity

Specificity was observed that the excipients present in the formulation and diluents did not interfere with detection of thiocolchicoside and dexketoprofen.

Label claim recoveries from tablets

The proposed method was evaluated in the assay of commercially available tablets containing THC (4 mg) and DKP (25 mg). Six replicate determinations were carried out on an accurately weighted amount of the pulverized tablets equivalent to 4 mg of THC and 25 mg of DKP. The results of label claim studies are shown in Table 5. Chromatogram of the sample is shown in Figure 3.

Table 2. Result of recovery study of THC and DKP

Formulation	Label Claim mg/tablet	Amount added (%)	Total amount added (mg)	% Recovery* \pm SD	% RSD
THC	4	80	3.2	98.74 \pm 0.0070	0.0071
		100	4.0	101.05 \pm 0.0141	0.0139
		120	4.8	99.68 \pm 1.4424	1.4470
DKP	25	80	20	98.57 \pm 0.0070	0.0071
		100	25	100.83 \pm 1.0535	1.0448
		120	30	101.09 \pm 1.5877	1.6051

* Average value \pm SD of three determinations, SD is standard deviation and %RSD is relative standard deviation

Table 3. Result of repeatability study of THC and DKP

Parameter	THC	DKP
% Mean*	100.92	99.79
SD	0.0178	0.0089
% RSD	0.0176	0.0089

* Average of six determinations, S.D. is Standard deviation; RSD is the Relative Standard deviation

Table 4. Result of Robustness study

Parameter	Variation	SD		% RSD	
		THC	DKP	THC	DKP
Mobile phase composition (MeOH : Phosphate buffer) ± 2 *	63:37	0.0070	0.9121	0.0072	0.8726
	67:33	1.2020	1.5556	1.2277	1.5494
pH ± 0.1 *	4.4	1.0182	0.0141	1.0339	0.0136
	4.6	0.0141	0.3252	0.0145	0.3251
Flow rate (ml/min) ± 0.1 *	0.9	1.4071	0.9263	1.4607	0.8983
	1.1	1.3293	0.8768	1.3432	0.8939

* Average of three determinations, S.D. is Standard deviation; RSD is the Relative Standard deviation

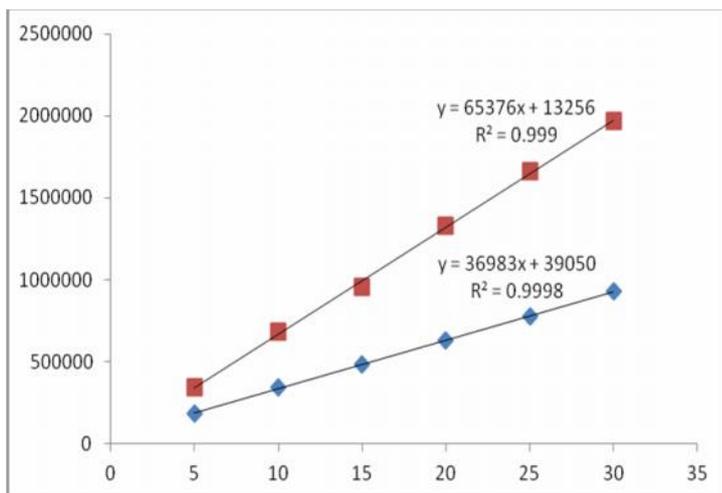


Figure 2. Calibration curves for Thiocolchicoside and Dexketoprofen showing linearity

Table 5. Result of assay of Tablet formulation

Formulation	Label claim mg/tablet	Amount found in mg	% Label claim * mean \pm SD
THC	4	4.0	101.25 \pm 0.6708
DKP	25	24.7	99.26 \pm 0.5187

* Average of six determinations, S.D. is Standard deviation

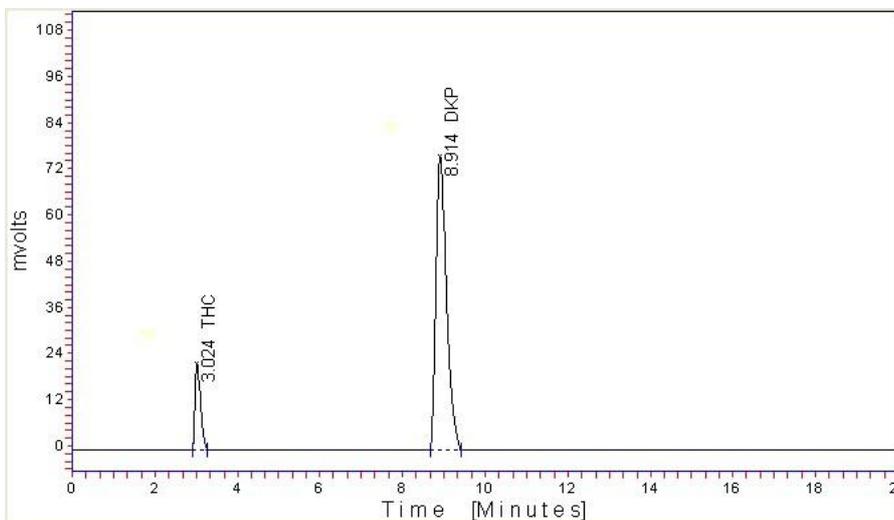


Figure 3. A HPLC chromatogram of Thiocolchicoside (RT 3.02) and Dexketoprofen (RT 8.91) of marketed formulation.

CONCLUSION

All these factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive, rugged and rapid and can be applied successfully for the estimation of THC and DKP in pharmaceutical formulations without interference and with good sensitivity; hence it can be used for the routine analysis in quality control department.

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