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Research Article

REVERSE – PHASE HPLC METHOD FOR SIMULTANEOUS ANALYSIS OF THIOCOLCHICOSIDE AND KETOPROFEN IN TABLET FORMULATIONS

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ABSTRACT

Objective: A Simple, precise, and accurate method is developed and validated for the analysis of Thiocolchicoside and ketoprofen in tablet formulations.

Methods: The method has been shown adequate separation of both ingredients from each other. The chromatographic separation was carried out on a reverse phase column-C_{18} (250 mm x 4.6 mm,5µ), with a mobile phase consisting of 0.05 M ammonium acetate buffer (adjusted to pH 6 with glacial acetic acid), acetonitrile and methanol in the ratio (50:30:20,v/v) at a flow rate of 1.2ml/min and UV detection at 310 nm. This new method is validated, which include assay determination, accuracy, precision, selectivity, linearity and range, robustness and ruggedness.

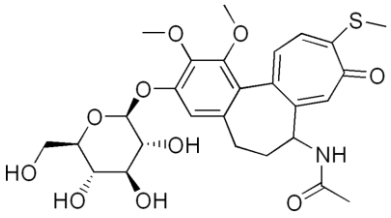
Results: The current method demonstrates good linearity over range of 64-96%µg/ml of Thiocolchicoside with r^2 of 0.9999 and in the range of 800-1200% µg/ml of ketoprofen with $r^2$of 0.9955. The average recovery of the method is 98.88% and 100.07% for thiocolchicoside and ketoprofen respectively. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters and by changing analytical operator indicating that the method is sufficiently robust and rugged.

Conclusion: A simple, accurate, precise RP-HPLC method is developed and validated for simultaneous determination of thiocolchicoside and ketoprofen in tablet formulation.

Keywords: Thiocolchicoside, Ketoprofen, RP-HPLC Method development, Validation

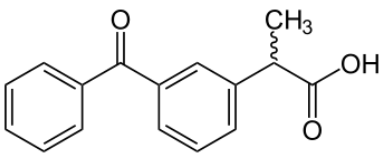
INTRODUCTION

Thiocolchicoside (THC) chemically, N-[(7S)-3-(beta-D-glucopyranosyloxy)-1, 2-dimethoxy-10-(methylsulfanyl)-9- oxo-5, 6, 7, 9-tetrahydrobenzo[a]heptalen-7-yl] acetamide (Fig 1a). It is a semi-synthetic derivative of the naturally occurring compound colchicoside with a relaxant effect on skeletal muscle, 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 properties [1].



(1a) Thiocolchicoside

Ketoprofen (2-(3-benzoylphenyl)-propionic acid (KET)(Fig 1b), is a derivative of propionic acid. It is a non-steroidal anti-inflammatory drug (NSAID) and cyclooxygenase inhibitor, which also interferes with the bradykinin pathway and stabilizes lysosomal enzymes. Oral administration of KET is effective in treating fever, pain and inflammation. As a group, NSAIDs are non-narcotic relievers of mild to moderate pain of many causes, including injury, menstrual cramps,arthritis,and other musculoskeletal conditions. [2].



(1b) Ketoprofen

Literature survey reveals that THC can be estimated by spectrophotometry [3-5], by RP-HPLC method in combined dosage forms [6-9] and by HPTLC method [10] in individual formulation and in combination with other drugs. Several methods have been described for KET determination in pharmaceutical formulations and in serum including UV spectrophotometry [11],HPLC method [12-15] for individual formulation and in combination with other drugs. However, there is no analytical method reported for the estimation of THC and KET in a combined dosage formulation .Our study attempt to develop an accurate, precise, specific, linear, simple, rapid, validated and cost effective analytical method for thiocolchicoside and ketoprofen in tablet dosage form by RP-HPLC method based on the International Conference on Harmonization (ICH) guidelines [16-18].

MATERIALS METHODS

Instrumentation and chromatographic conditions

The HPLC system, used for the method development and method validation was shimadzu (Japan) Model, equipped with LC-2010 HPLC pump, online degasser, column heater, SPD-20A UV detector, Rheodyne injector and Spinchrome software. Chromatographic separation was performed isocratically at room temperature using Column – C_{18} (Enable C_{18} G)(250 mm x 4.6mm,5µ) with mobile phase composition of 0.05M Ammonium acetate buffer(pH 6.0 was adjusted to with glacial acetic acid),acetonitrile and methanol (50:30:20,v/v) at a flow rate of 1.2 mL/ min. The injection volume was 20µL.Eluent was monitored with a UV detector at 310 nm.

Material and Reagents

HPLC grade methanol was purchased from Merck(Mumbai, India).Analytical regent grade glacial acetic acid was purchased from Merck (Mumbai, India), Acetonitrile was purchased from Merck (Mumbai, India).HPLC grade water was obtained from a Milli –Q water purification system was used throughout the analysis. Pure drug sample of THC, %purity 99.67% and KET, % purity 99.52% was procured as a gift sample from Firstmed Therapeutics Pvt. Ltd, Pondicherry. These samples were used without further purification. Tablet formulations (Brand Name-RELAXEN-4) were used for analysis containing THC 4mg and KET 50mg per tablet.

Standard solutions and HPLC conditions

Acetate buffer was prepared by dissolving3.8 gm of ammonium acetate in 1000ml of water and adjusted to pH 6 with glacial acetic acid. A filtered and degassed mixture of buffer, acetonitrile and methanol (50:30:20, v/v) was employed as a mobile phase at a flow rate of 1.2ml/min at a detection wavelength of 310 nm.

Standard Stock solution was prepared by dissolving 40.1mg of thiocolchicoside and 500.3mg of ketoprofen in 50ml of mobile phase. Working standard solution was prepared by diluting 2.5 ml of each standard stock solution to 25 ml with mobile phase to obtain a solution having a known concentration of 80 µg/ml of thiocolchicoside and 1000 µg/ml of ketoprofen.

Sample Stock solution of the formulated tablets (Brand Name-RELAXEN-4) were prepared by dissolving a quantity of the powdered tablet equivalent to 500mg of ketoprofen in 50 ml of mobile phase and further diluted to get the same concentration as in the standard solution.

RESULTS AND DISCUSSION

Method development

Preliminary studies involved trying $C_{18}$and C_8 columns and testing several mobile phases containing buffers like phosphate and acetate with different pH (4 to 8) and using organic modifiers like acetonitrile, methanol and ethanol. For the effective separation of thiocolchicoside and ketoprofen C_{18}(250x4.6 mm, 5u) column eluted with a mobile phase of ammonium acetate buffer (adjusted to pH 6 with glacial acetic acid), acetonitrile and methanol (50:30:20,v/v) at a flow rate of 1.2ml/min and a detection wavelength of 310 nm afforded the best separation of these analytes (Fig2 and Table 2). In this developed method for assay of thiocolchicoside and ketoprofen no internal standard was used because no extraction or separation step was involved.

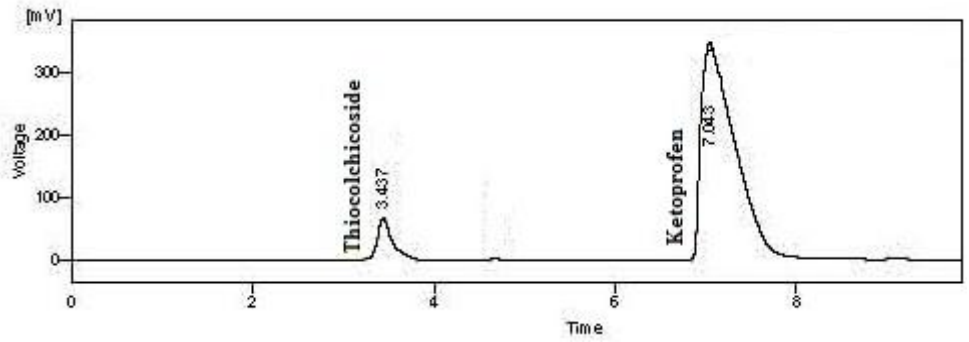


Fig. 2: Chromatogram of Thiocolchicoside and Ketoprofen. Mobile phase :Ammonium acetate buffer:acetonitrile:methanol(50:30:20,v/v).Flow rate:1.2 ml/min,injectionvolume:20µl column:C18 (Enable C18 G)(250 mm x 4.6mm,5µ).UV detection 310

Peak shape of the thiocolchicoside and ketoprofen were found to be symmetrical in this optimized chromatographic conditions. Good separation is seen as the retention times of thiocolchicoside and ketoprofen were3.4 and 7.0 min respectively, with resolution factor greater than 5. The results of the analysis of tablet formulations are reported in Table 1.

Table 1: The estimation of Thiocolchicoside and ketoprofen in tablet dosage form

Drug	Label claim	Amount present*	% Assay*
Thiocolchicoside	4mg	3.98±0.015	99.66±0.45
Ketoprofen	50mg	49.53±0.17	98.99±0.33

*Each value is the mean ± standard deviation from three determinations

Method Validation

After method development, the validation of the current method has been performed in accordance with ICH guidelines which include accuracy, precision, selectivity, linearity and range, limit of detection and limit of quantifi**c**ation, robustness and ruggedness.

Linearity and range

To evaluate linearity of the method, different concentrations of the two analytes in the range 64-96 µg/ml for thiocolchicoside and 800-1200 µg/ml for ketoprofen were analyzed by linear regression analysis. The results obtained show that the current method is linear for the range specified above with a correlation coefficient of 0.9999 and 0.9955 for thiocolchicoside and ketoprofen respectively (Table 2).

Accuracy (Recovery)

The accuracy of sample preparation was determined by recovery study, which was determined by spiking the sample with 80%, 100% and 120%. These mixtures were analyzed by the proposed method. The experiment was performed in triplicate and recovery (%), RSD (%) and standard error of mean (SEM) of spiked drugs were calculated. Results have shown that the mean recovery of the assay is within 100±2.0 % for each ingredient, and the RSD is lower than 2 % (Table 3).

Table 2: Chromatographic parameters of the separated analytes

Content	Thiocolchicoside	Ketoprofen
Linearity range(µg/ml)	64-96	800-1200
Slope	7.9582	88.015
Intercept	2.2479	161.1
Regression coefficient	0.9999	0.9955
Limit of detection(µg/ml)	53.10	42.78
Limit of quantification(µg/ml)	160.93	129.64
Retention time(min)	3.437	7.043
Tailing factor	1.864	1.978
Resolution factor	-	5.773
Theoretical plate	2687	2141

Precision

System precision

The system precision of this method was evaluated by calculating the RSD of the peak areas of six replicate injections of the standard solution, which was found to be 0.560 % and 0.397 % for thiocolchicoside and ketoprofen respectively (Table4).

Method precision

The method precision of this method was evaluated by calculating the RSD of the peak areas of six replicate injections of the sample solution, which was found to be 0.498 %and 0.380 % for thiocolchicoside and ketoprofen respectively (Table 4). These results show that the current method is repeatable.

Table 3: Accuracy (%recovery) of thiocolchicoside and ketoprofene in tablet formulation

S. No.	Thiocolchicoside				Ketoprofen		
	Recovery	Avg area	Amount recovery	% Recovery	Avg area	Amount recovery	% Recovery
1	80%	635.676	3.93	98.43	7455.15	50.78	101.57
2	100%	794.11	3.94	98.50	8926.27	49.98	99.97
3	120%	964.19	3.98	99.72	10602.57	49.48	98.68

Table 4: Precision results

Method precision results								
Drug	**Assay1**	**Assay 2**	**Assay 3**	**Assay 4**	**Assay 5**	**Assay 6**	**Average**	**%RSD**
THC	98.94%	99.07%	98.63%	97.78%	99.00%	98.39%	98.63%	0.498
KET	100.52%	100.33%	99.61%	99.67%	99.90%	99.71%	99.95%	0.380
System precision results								
Drug	Area1	Area 2	Area 3	Area 4	Area 5	Area 6	Average	%RSD
THC	798.493	798.893	802.089	806.301	807.093	809.240	803.680	0.560
KET	8832.347	8831.924	8894.785	8905.604	8884.639	8908.720	8876.330	0.397

Selectivity

Selectivity of the current method was demonstrated by good separation of the two analytes from each other, see Fig.2.Furthermore, excipient of the tablet formulation did not interfere with the active ingredients of the drug product.

Limit of detection and Limit of quantification

Limit of detection (LOD) and limit of quantification (LOQ) were estimated from the signal-to-noise ratio. The detection limit was determined as the lowest concentration level resulting in a peak area with signal-to –noise ratio of 3.The quantification limit was determined as the lowest concentration level that provided a peak area with signal-to –noise ratio of 10 (Table 2).

Robustness and ruggedness

Robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters, and provides an indication of its reliability during normal usage. Robustness of the current method was investigated by analyzing samples of the drug product using the same chromatographic conditions, method development but with a small change in the following chromatographic parameters (a) flow rate: 1.0 and 1.4 ml/min instead of 1.2ml/min, (b) detection wavelength 308 and 312 nm instead of 310 nm and p^H5.8 and p^H 6.2 instead p^H 6.RSD of thiocolchicoside and ketoprofen assay under these conditions is calculated as less than 2% (Table 5).

Table 5: Robustness of Thiocolchicoside and Ketoprofen %RSD at different flow rates, different nm& different p^H

Thiocolchicoside			Ketoprofen	
Parameter	Avg area	% RSD	Avg area	% RSD
Flow rate(1.0)	899.52	1.406	9897.93	1.28
Flow rate(1.4)	683.27	0.005	7673.83	0.105
Wavelength(-2)	804.50	0.642	8946.70	0.105
Wavelength(+2)	796.48	0.785	8919.82	0.125
P^H (-0.2)	1049.285	1.73	10524.749	0.954
P^H (+0.2)	848.41	1.45	8723.876	0.567

The ruggedness of the method was estimated by carrying out the experiment as per proposed method in duplicates using different column and analyst on different days. The method was carried out on INERTSIL-ODS C18 and phenomenex-Gemeni C18 columns. It was observed that there were no marked changes in the chromatograms and the RSD was calculated to be less than2 % thiocolchicoside and ketoprofen, respectively which demonstrated that the RP-HPLC method developed was sufficiently robust for normal expected variations in chromatographic conditions (Table 6).

Table 6: Ruggedness of the drugs on different days and different analysts

Day 1	Thiocolchicoside(%)	Ketoprofen (%)
Analyst 1,Inst 1	99.07	99.61
Analyst 2,Inst 1	99.00	99.90
Analyst 1,Inst 2	99.25	100.32
Analyst 2,Inst 2	99.59	99.20
Day 2		
Analyst 1,Inst 1	98.63	98.66
Analyst 2,Inst 1	99.62	99.67
Analyst 1,Inst 2	99.58	99.71
Analyst 2,Inst 2	99.23	100.66

System suitability

To know reproducibility of the method, system suitability test was employed to establish the parameters such as tailing factors, theoretical plates, repeatability and resolution.

CONCLUSION

A simple, accurate, precise RP-HPLC method is developed and validated for simultaneous determination of thiocolchicoside and ketoprofen in tablet formulation.

REFERENCE

1. The Merck Index: An Encyclopedia of chemicals, Drugs and Biological. Whitehouse station, NJ, USA, 14th Edn, 2006; 1603.
2. The Merck Index: An Encyclopedia of chemicals, Drugs and Biological. Whitehouse station, NJ, USA, 14th Edn, 2006; 918.
3. Nikhade RD, Thakur AD, Choudhari SB, Chandewar AV Simultaneous estimation of paracetamol,Thiocolchicoside and Aceclofenac by UV Spectrophotometer using multicomponent mode method. J Pharm Res. 2011; 4(7): 2297-2299.
4. Joshi RR , Gupta KR Simultaneous UV-Spectrophotometric determination of Thiocolchicoside and Diclofenac in pharmaceutical formulation. Der Pharmacia Sinica. 2010; 1(2): 44-51.
5. Achariyar SK, Rajesh Y, Panda P, Mallick P, Annapurna MM. Spectrophotometric methods for simultaneous estimation of etoricoxib and thiocolchicoside in bulk and combined pharmaceutical dosage form. J Pharma. Educ. Res. 2010; 1(1): 75-82.
6. Kumar S, Joshi A, Thakur RS, Pathak AK, Shah K. Simultaneous estimation of etoricoxib and thiocolchicoside by RP-HPLC method in combined dosage forms. Actapoloniae Pharmaceutica-Drug Res. 2011; 68(6): 839-845.
7. Sahoo M, Syal P, Ingale K, Sinde S, Sail M, Choudhari VP, Kuchekarm BS Development and validation of a RP-HPLC-PDA method for simultaneous determination of Lornoxicam and Thiocolchicoside in pharmaceutical dosage form and its

application for dissolution study. Int.J.Res.pharm.Sci. 2011; 2(1): 1-7.

8. Walash M, Belal F, Eid M, Abass S, Abo EI.Simultaneous HPLC determination of Thiocolchichoside and Glafenine as well as Thiocolchicoside and Floctafenine in their combined dosage forms.J Chromatographic Sci. 2011; 49: 159-164.
9. Patil ST, Bhusari VK, Dhaneshwar SR. Validated HPTLC method for simultaneous estimation of Thiocolchicoside and aceclofenac in bulk drug and formulation. Int. J. Pharma. Bio. Sci. 2011; 2(2): 482-490.
10. Gandhi S, Deshpande P, Sengar M.HPTLC determination diclofenac sodium and thiocolchicoside in fixed dose combination.Int.Res.J Pharm. 2010; 1(1): 220-224.
11. Ozlu C, Basan H, Satana E, Ertas N, Goger NG. Quantitative determination of ketoprofen in gel and ampules by using flow-infection UV spectrophotometry and HPLC. J.Pharm.Biomed.Anal. 2005; 39(3-4): 606-611.
12. Guo Z, Wang H, Zhang Y. Chiral separation of ketoprofen on an achiral c8 column by HPLC using norvancomycin as chiral mobile phasadditives. J.Phram.Biomed.Anal. 2006; 41(1):310-314.
13. Panus PC, Tober-meyer B, Ferslew KE. Tissue extraction and high-performance liquid chromatographic determination of ketoprofen enantiomers. J.Chromatograp, 1998; 705(2): 295-302.
14. De jalon EG, Josa M, Campanero MA, Santoyo S, Ygartua P.Determination by high-performance liquid chromatography of ketoprofen in vitro in rat skin permeation samples.J.Chromatograp.A. 2000; 870(1-2): 143-149.
15. Dvorak J, Hajkova R, Matysova L, Novakova. Simultaneous HPLC determination of ketoprofen and its degradation products in the presence of preservatives in pharmaceuticals.J.Pharm.Biomed.Anal. 2004; 36(3):625-629.
16. ICH Guidelines Q2A, Text on validation of analytical procedures.In:International Conference of Harmonization,Geneva1994.
17. ICH Guidelines Q2A, Text on validation of analytical procedures: methodology.In:International Conference of Harmonization,Geneva1996.
18. ICH Guidelines Q2 (R1), Validation of analytical procedures:Text and methodology,Geneva 2005