AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Simultaneous Determination of Thiocolchicoside and Ketoprofen in Bulk and Pharmaceutical Formulations by Validated Stability Indicating RP-HPLC Method

Satyanarayana MV [1]*, Satyadev TNVSS [2], NageswaraRao M[1], Anuradha V[4]

1. Dept. of freshman engineering, PVP Siddhartha institute of Technology, Kanuru, Vijayawada, Andhra Pradesh India.

2. PG Centre, P.B. Siddhartha college of Arts and Science, Vijayawada, Andhra Pradesh, India

3. Head. Dept. of Chemistry, Vignan P.G. College, Pedapalakaluru, Guntur, Andhra Pradesh, India.

ABSTRACT

A new validated RP – HPLC method was developed for the simultaneous determination of Thiocolchicoside and Ketoprofen in combined dosage form. The method developed produced high sensitivity, precision and accuracy. An isocratic C18 (Inertsil ODS, 250 x 4.6 mm, 5µ) column was used with mobile phase of composition Acetonitrile: Phosphate buffer (70: 30 at pH 4.6) at a flow rate of 1.0 mL/min with UV detection at 258.2 nm for separating Thiocolchicoside and Ketoprofen. The retention time of Thiocolchicoside and Ketoprofen were 2.4 min and 3.5 min respectively. The developed method was validated for specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness as per ICH guidelines. Linearity for Thiocolchicoside and Ketoprofen were found in the range of 2.0 – 12.0 µg/ml and 6.2-38.75 µg/ml, respectively. The percentage recoveries for Thiocolchicoside and Ketoprofen ranged from 99.35- 100.21% and 98.66-99.29 %, respectively. The proposed method could be used for routine analysis of Thiocolchicoside and Ketoprofen in their combined dosage forms. All the proposed methods for Thiocolchicoside and ketoprofenare simple, selective, reproducible and specific with good precision and accuracy. The method was proved to be superior to most of the reported methods. These proposed methods for estimation of selected drugs were successfully applied either in tablet dosage form. More over the low solvent consumption along with short retention time of 2.4 and 3.5 for both Thiocolchicoside and Ketoprofen to be cost effective when compared to other developed method shown in literature reviews. The proposed method can be used as alternative methods to the reported ones for the routine determination of selected drugs under the study in tablet dosage form

Keywords: Liquid Chromatography; Thiocolchicoside, Ketoprofen, Combined dosage forms; Simultaneous estimation, Validation

*Corresponding Author Email: mvsku14@gmail.com

Received 16 March 2014, Accepted 30 March 2014

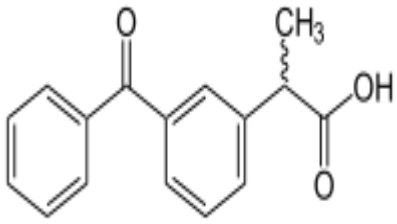
Please cite this article in press as: Satyanarayan MV *et al*., Phytochemical evaluation and Biomarker isolation from a Poly-herbal formulation. American Journal of PharmTech Research 2014.

INTRODUCTION

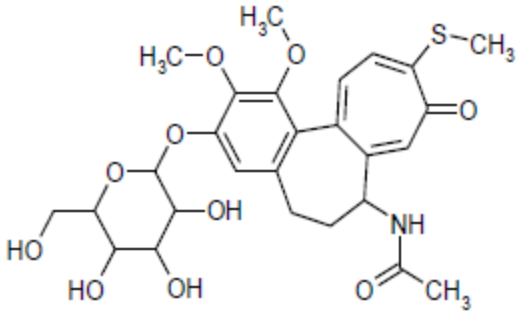
Ketoprofen[*(RS)*2(3benzoylphenyl)propionicacid]is a synthetic non steroidal anti-inflammatory drug (NSAID), has been proved to be safe and used as analgesic and antipyretic [1]

Thiocolchicoside (THIO) chemically, (s)-N-[3-(B-D-glucopyranoxyloxy)-5, 6, 7, 9-tetrahydro-1, 2-dimethoxy-10-(methylthio)-9-oxobenzo (a) heptalen-7yl] acetamide, is a muscle relaxant which has been claimed to possess GABA mimetic and glycinergic actions. It belongs to the category of Anti- Rheumatoid arthritis and Analgesic. Literature review reveals that there are few analytical methods reported for the analysis of Thiocolchicoside and Ketoprofen by simultaneous estimation. Spectrophotometer, HPLC and HPTLC are the reported analytical methods for compounds either individually or in combination with other dosage form[2-17]. Hence, it was felt that, there is a need of new analytical method development for the simultaneous estimation of Thiocolchicoside and Ketoprofen in pharmaceutical dosage form.

Present work is aimed to develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-HPLC method for the simultaneous analysis of Thiocolchicoside and Ketoprofen. The developed method will be validated according to ICH guidelines[1].



A. Structure of Ketoprofen



B. Structure of Thiocolchicoside

MATERIALS AND METHOD

Materials

HPLC grade sodium dihydrogen phosphate (NaH_2PO_4), sodium hydroxide, acetonitrile Merck India. All dilutions were performed in standard class-A, volumetric glassware. For the estimation of commercial formulation, Lupiflex-4 tablets having thiocolchicoside 4mg and 50mg Ketoprofen were procured from the local market.

Instrumentation

Waters model 2695 LC chromatographic system, with UV-Visible detector of Waters (2996) make and a fixed injector equipped with 20µL loop was used for the chromatographic separation. The chromatogram was recorded at ambient temperature and peaks quantified by means of

Empower software. Chromatographic separation was carried out on a C18 column [Inertsil ODS, 250mm x4.5mm 5µ]. Sartorious electronic balance was used for weighing the samples. Ultra-sonic bath sonicator was used for degassing and mixing of the mobile phase.

Chromatographic conditions

Chromatographic separation of Thiocolchicoside and Ketoprofen was carried on a C18 column. The mobile phase was composed of acetonitrile and phosphate buffer (pH 4.6) in the ratio of 70: 30 v/v. It was filtered through a 0.45 µ membrane filter and degassed for 15 minutes. The flow rate of the mobile phase was maintained at 1.0 ml/min. Detection was carried out at 258.2 nm at ambient temperature.

Method development

Preparation of Standard Stock Solutions

Standard stock solutions of Thiocolchicoside and Ketoprofen were prepared by dissolving 12.5 mg and 12.5 mg in two volumetric flasks of 25mL using 15mL of mobile phase respectively. Later, the volumes were made upto the mark with mobile phase to obtain a final concentration of 500 µg/mL and 500 µg/mL. From the above stock solutions, 1mL aliquots each were pipetted in to a 25mLvolumetric flask and dissolved in 15mL of the mobile phase and made up to the mark with the solvent to obtain a final concentration of 20µg/mL and 20µg/mL for Thiocolchicoside and Ketoprofen respectively.

Preparation of Sample solutions

Finely grind 20 pre weighed tablets. Transfer grinded Sample quantitatively equivalent to 80 mg thiocolchicoside and 1000 mg of ketoprofen into100 mL volumetric flask add 60 mL of diluent, sonicate to dissolve for 10 minutes and dilute to volume with diluent. Further filter the solution through 0.45µ filter paper. Dilute 1 ml of filtrate to 100 ml with mobile phase.

Method validation

The developed HPLC method for the simultaneous determination of Thiocolchicoside and Ketoprofen was validated as per the ICH guidelines.

System suitability and System Precision

System suitability for chromatographic separation was checked on each day of validation to evaluate the components of the analytical system in order to show that the performance of the system meet the standards required by the method. System suitability parameters established for the developed method include number of theoretical plates (efficiency), Resolution, Tailing factor. The HPLC system was equilibrated using the initial mobile phase composition, followed by 5 injections of the standard solution of 100% concentration containing 20 µg/mL

thiocolchicoside and 20 µg/ml Ketoprofen. These 5 consecutive injections were used to evaluate the system suitability on each day of method validation. The result was given in the Table 1.

Specificity

Blank interference

A study to establish the interference of blank was conducted. Diluent was injected into the chromatograph in the above defined chromatographic conditions and the blank chromatograms were recorded. Chromatogram of Blank solution **(**Figure 2) showed no peaks at the retention time of thiocolchicoside and Ketoprofen peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of thiocolchicoside and Ketoprofen in Thiocolchicoside and Ketoprofen tablets. Similarly typical representative chromatogram of standard is also shown (Figure-3)

Forced Degradation:

To assess the specificity of the developed method forced degradation studies were carried out. These studies include Acid degradation, Base degradation, Thermal degradation and Photo degradation. The sample solution for forced degradation studies was prepared by transferring 1mL standard solution into 10 mL standard flask. 6 mL of diluent was added to standard flask and then thoroughly shaked to dissolve the contents. The contents were made upto mark with diluent. The solution was then filtered through 0.45µm filter. 1 mL of this filtrate was diluted to 10 mL by mobile phase. For acid and base degradation studies acid and base were added to 10 mL volumetric before making it upto mark and filtering. The sample solution was also subjected to thermal and photolytic effects. The details of degradation studies were provided in the table 2.

Linearity and range

In the concentration range of 2.0-12.0µg/ml for thiocolchicoside and 6.25- 38.75µg/mL for Ketoprofen standard curve was obtained. A statistical method known as linear regression analysis was used to evaluate the linearity of the curve. To assess the linearity of the proposed method slope, intercept and correlation coefficient [r^2] of standard curve were calculated and were given in Figure-5A(For thiocolchicoside) and Figure-5B(For Ketoprofen). The result was given in the Table 3 and Table 4. From the data obtained (For thiocolchicoside and Diclofenac) the method was found to be linear within the proposed range.

Accuracy

Accuracy is defined as the closeness of results obtained by that method to the true value for the sample. Accuracy is expressed in terms of percentage recovery. Recovery % is determined by the

standard addition method. In the present study recovery studies were carried out at 50%, 100% and 150% spiked levels. The results of Recovery % were given in Table 5.

Precision

The closeness of replicate results obtained from analysis of the same homogeneous sample is known as precision of the method. The precision of the method was assessed by six replicate injections of 100% test concentration. The precision was expressed in terms of standard deviation and %RSD. The results were given in Table 6.

LOD and LOQ

The formulae 3.3 σ/S and 10 σ/S were used to calculate LOD and LOQ respectively. σ is the mean of standard deviation of y intercepts of the three calibration curves and S is the mean of slopes of the calibration curves. The results were given in Table 7 & 8.

Robustness

The ability of the developed method to remain unaffected by the small changes in the parameters is known as Robustness. Robustness was assessed by varying the parameters such as percent organic content, pH of the mobile phase, buffer concentration, temperature, injection volume and flow rate. In the present investigation, a variation of ± 0.1 mL/min in the flow rate, change in buffer concentration were adopted to study Robustness. The results were tabulated in Table 9.

RESULTS AND DISCUSSION

In present study a new analytical method reversed phase HPLC method for the simultaneous determination of Thiocolchicoside and Ketoprofen tablets in combined dosage form. The column used in this method is Inertsil ODS C18, 100 X 4.6, 5µm with a flow rate of 1.0ml/min at a wavelength 258 nm and Coloumn temperature is 30°C. The mobile phase preparation was done by using buffer 0.01N potassium di hydrogen ortho phosphate (pH 4.6). The mobile phase combination was Buffer: ACN (60:40). The run time was set for 6 minutes. The retention time of Thiocolchicoside and ketoprofen is 2.425 and 3.523. The new HPLC method developed and validated for simultaneous determination of Thiocolchicoside and Ketoprofen in pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also determining lower concentration of each drug in its solid combined dosage form by RP-HPLC method. The linearity range for Thiocolchicoside and Ketoprofen is 0-30 µg/ml the co-relation co-efficient was found to be 0.999. The percentage RSD obtained for system precision of Thiocolchicoside and Ketoprofen are 0.021 and 0.077 respectively. The percentage RSD obtained for method precision of Thiocolchicoside and Ketoprofenare 0.085 and 0.050 respectively.

The Limit of detection values for Thiocolchicoside and Ketoprofen are 2.237 and 1.9396 for system precision respectively. The Limit of detection values for Thiocolchicoside and Ketoprofen for method precision are 0.03725 and 0.16222 respectively. The Limit of quantification values for Thiocolchicoside and Ketoprofen respectively for system precision are 6.758 and 5.8776 and for method precision are 0.112892 and 0.49160 respectively.

The Ruggedness of the method has been verified by analyzing the six samples of same batch for method precision as per test method by different analysts using different instrument, different days. The analyst's prepared six sample of the same batch by two different day's .calculated %RSD for two different days in six samples for ruggedness results with the method precision.

The system suitability was evaluated in each condition and compare the results with method precision results the method is robust for change in flow rate and, mobile phase buffer solution.

To conform that during stability or throughout shelf life any degradation product if found will not interfere with the main peak of Thiocolchicoside and Ketoprofealso the force degradation study will help to identify the type of degradation(with alkali,acidic,thermal and photolytic) for each of the degradents. No peaks are observed at the time of retention time of Thiocolchicoside and Ketoprofenthe method was found to be specific.

The sample solution was injected and the amount of Thiocolchicoside and Ketoprofen present in the formulation was calculated from the calibration curve. The amount of Thiocolchicoside and Ketoprofen present per each mL was found to be 4.021 ± 0.031 and 49..99± 0.453 respectively. The result of assay of Thiocolchicoside and Ketoprofen was found to be 100.51% and 99.97% respectively.

Table 1: System suitability parameters for Thiocolchicoside and Diclofenac by proposed method

Name of the Compound	Retention Time	Tailing factor	Theoretical plates	USP Resolution
Thiocolchicoside	2.425	1.14	3032	-
Ketoprofen	3.523	1.23	4891	5.87

Table 2 Forced degradation specificity data for Thiocolchicoside and Ketoprofen

Degradation conditions	Retention time(min)		Area (μV^2SEC)		% Area of Active drug		symmetry factor	
	TCH	KTP	TCH	KTP	TCH	KTP	TCH	KTP
Acid	2.3	3.6	249337	3112985	6.66	83.11	0.98	1.13
Alkaline	2.4	3.5	916174	4439043	13.41	64.96	1.12	1.20
Thermal	2.4	3.6	821876	4093559	16.61	82.75	2.26	1.15
Photolytic	2.4	3.5	882682	4286537	16.30	79.14	1.14	1.22

Table 3: Linearity studies for Thiocolchicoside by proposed method

Concentration(mcg/ml)	Area (μv^2sec)
2.0	234468
4.0	463845
6.0	696264
8.0	924974
10.0	1171373
12.0	1398181

Table 4 Linearity Data for Ketoprofen

Concentration	Area (μv^2sec)
6.2500	1307621
12.5000	2568337
18.7500	3839006
250000	5073831
32.5000	6282784
38.7500	7559907

Table 5 Accuracy data for Thiocolchicoside and ketoprofen

S.No	Thiocolchicoside			Ketoprofen		
	Area(μv^2sec)					
	50%	100%	150%	50%	100%	150%
Injection 1	696264	924554	1172046	3386901	4494217	5632134
Injection 2	695984	924240	1158324	3415476	4543656	5638950
Injection 3	694783	924005	1150265	3404543	4531604	5646578
Average	695677	924266	1160211	3402307	4523159	5639220
*Amount recovered (µg)	50.10	99.35	150.31	49.64	99.29	147.99
*% Recovery	100.19	99.35	100.21	99.29	99.29	98.66

Table 6 Data for System precision of Thiocolchicoside and ketoprofen

S.no	Thiocolchicoside Area(μv^2sec)	Ketoprofen
Injection 1	937422	4463759
Injection 2	937776	4478758
Injection 3	924974	4494097
Injection 4	949845	4532741
Injection 5	936543	4468363
Injection 6	939002	4507459
Average	937594	4490863
Standard deviation	7905.7	26180.4
% RSD	0.021	0.58

Table 7 Data for limit of detection of Thiocolchicoside and Ketoprofen

Drug	Limit of detection	
	System precision	Method precision
Thiocolchicoside(µg/mL)	2.237	0.03725
Ketoprofen (µg/mL)	1.93963	0.162222

Table 8 Data for limit of Quantification of Thiocolchicoside and Ketoprofen

Drugs	Limit of quantification	
	System precision	Method precision
Thiocolchicoside(µg/mL)	6.758	0.112892
Ketoprofen (µg/mL)	5.8776	0.049160

Table 9 Data for Robustness study ofThiocolchicoside and Ketoprofen

S.No.	TCZ		KTP	
	RT	Area (μv^2 sec)	RT	Area(μv^2)
Standard				
1	2.429	949845	3.531	4532741
Robust-1 Flow -1				
2	2.672	1024882	3.874	4980796
Robust-2 Flow-2				
3	2.208	852061	3.209	4107385
Robust-3 Buffer-1				
4	2.424	835725	3.498	4047879
Robust-3 Buffer-2				
5	2.431	841139	3.489	4049187

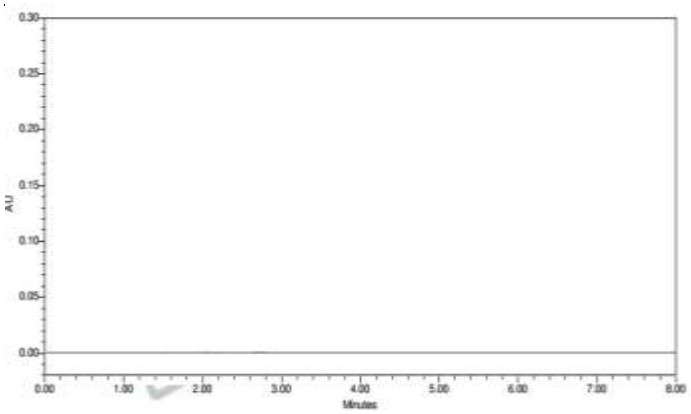


Figure 2: Chromatogram of Thiocolchicoside and Ketoprofen Blank

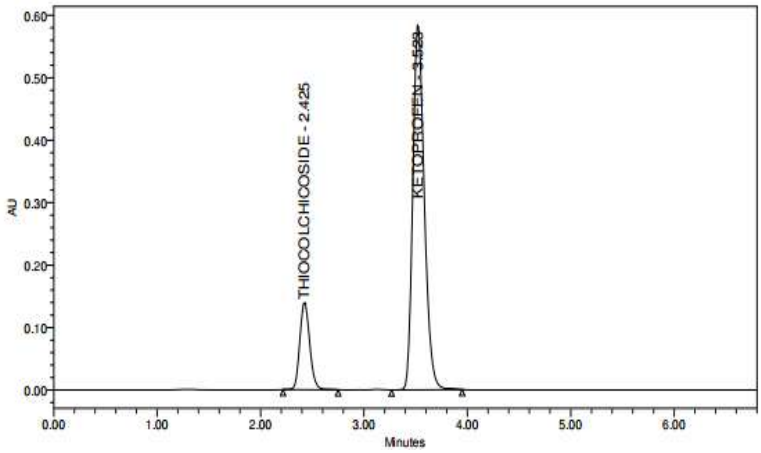


Figure 3*:* Standard Chromatogram of Thiocolchicoside and ketoprofen

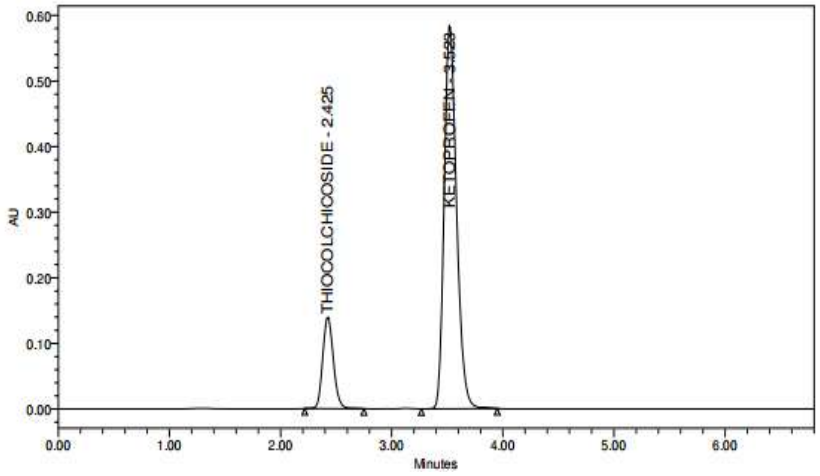


Figure 4A: Standard Chromatogram of Thiocolchicoside and ketoprofen

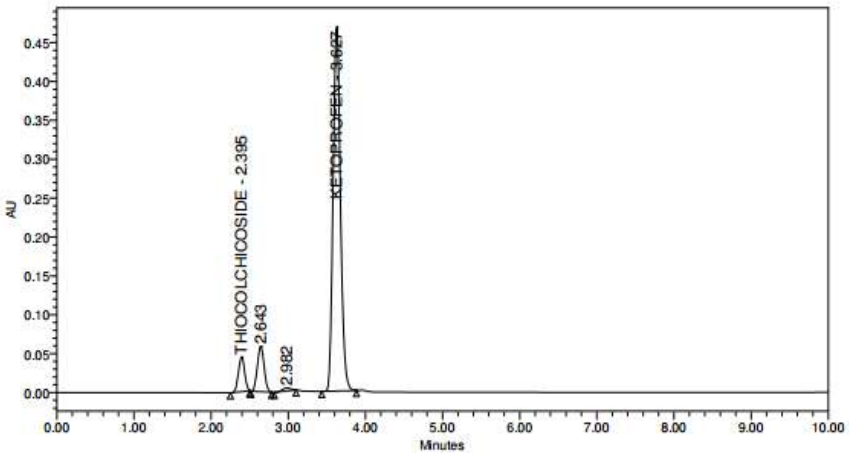


Figure 4B: Chromatogram of Thiocolchicoside and Ketoprofen Forced Degradation specificity-Acid

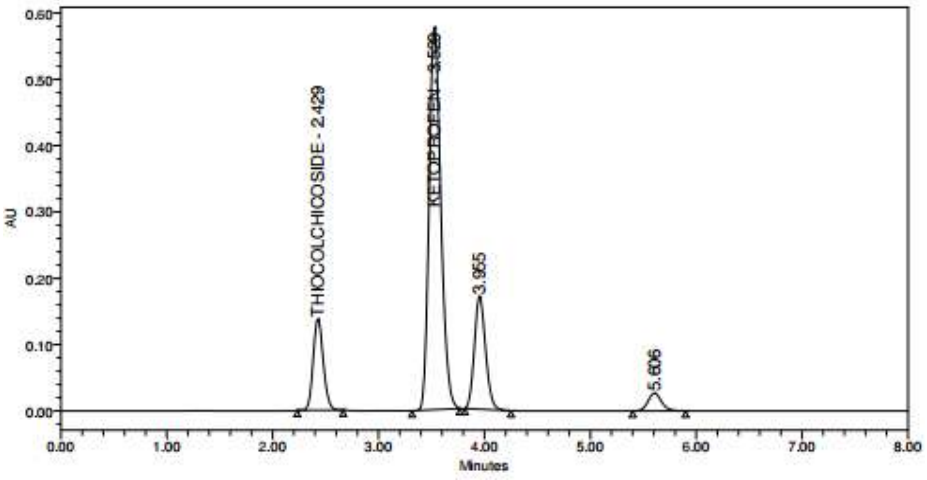


Figure 4C: Chromatogram of Thiocolchicoside and Ketoprofen Forced Degradation specificity- Alkaline

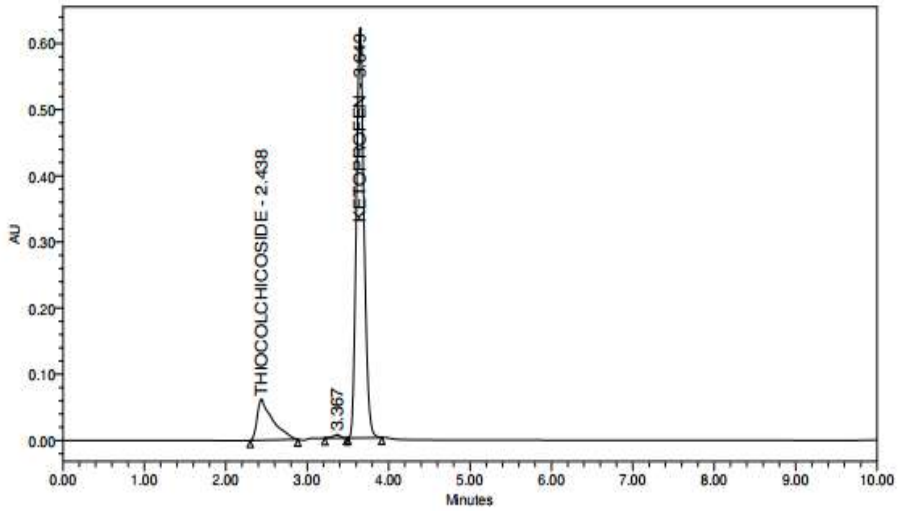


Figure 4D: Chromatogram of Thiocolchicoside and Ketoprofen Forced Degradation specificity- Heat

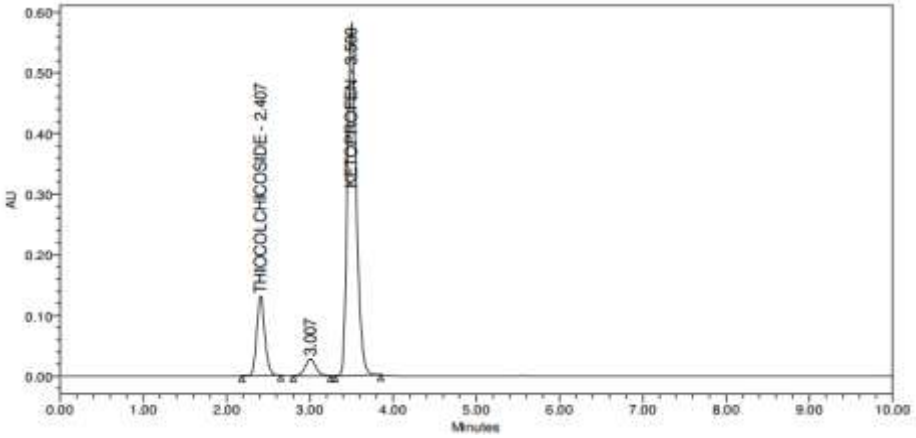


Figure 4E: Chromatogram of Thiocolchicoside and Ketoprofen Forced Degradation specificity-light

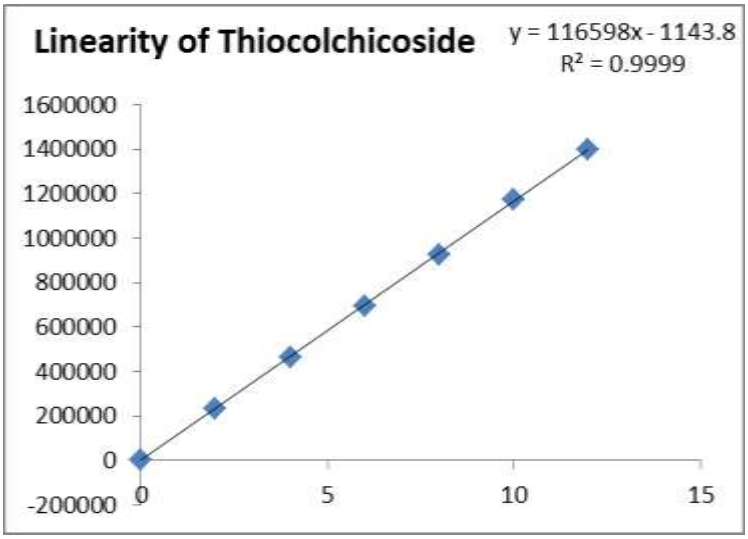


Figure 5A: Calibration curve for thiocolchicoside

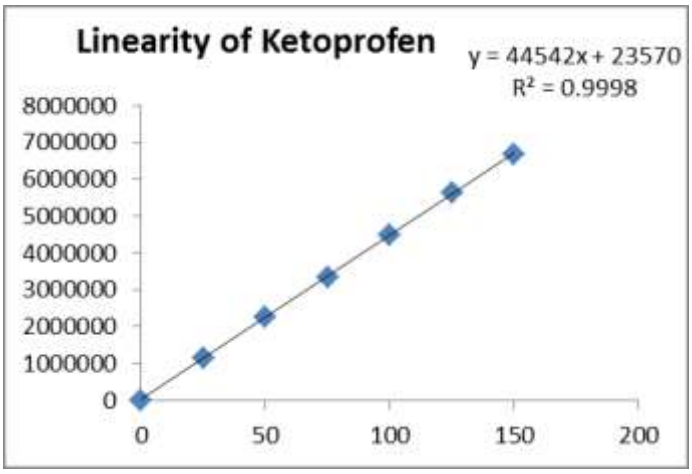


Figure 5B: Calibration curve for Ketoprofen

CONCLUSION

All the proposed methods for Thiocolchicoside and ketoprofenare simple, selective, reproducible and specific with good precision and accuracy. The method was proved to be superior to most of the reported methods. These proposed methods for estimation of selected drugs were successfully applied either in tablet dosage form. More over the low solvent consumption along with short retention time of 2.4and 3.5 for both Thiocolchicoside and Ketoprofen to be cost effective when compared to other developed method shown in literature reviews. The proposed method can be used as alternative methods to the reported ones for the routine determination of selected drugs under the study in tablet dosage form.

REFERENCES

1. International Conference on Harmonization (ICH) Harmonized Tripartite 2005. Validation of Analytical Procedures: Text and Methodology,Q2 (R1).
2. URL:http://hplc.chem.shu.edu/new/hplcbook/detector
3. http://www.chemblink.com/products/56392-17-7.htm
4. Mitesh D Phale., and Purnima D Hamrapurkar. A Validated and Simplified RP-HPLC of Thiocolchicoside from Bulk Drugs. Asian Journal of Research Chemistry 2009,2(2):119-122.
5. Patel Amit R, Chandrul Kaushal Kishor. Method Development ,validation and stability study for simultaneous estimation of thiocolchicoside and indapamide by reverse phase-high performance liquid chromatography in pure and marketed formulation. International Journal on Pharmaceutical and Biomedical Research 2011,2(1):4-16.
6. http://www.drugbank.ca/drugs/DB01140

7. Willard HH, Merritt LL, Dean JA, Settle FA. Instrumental Methods of Analysis, 7thed. New Delhi; CBS publishers and Distributors, 1986;518-521& 580-610
8. Singh Brijesh, Patel DK, Ghosh SK. Development of Reverse-Phase HPLC Method for Simultaneous Analysis of Thiocolchicoside and ketoprofen in a Tablet Formulation. Tropical J Pharma Res 2009;8(6):539-543.
9. Vijayamirtharaj R, Ramesh J, Jayalakshmi B, Hanas Bin Hashim. Development And Validation of RP-HPLC method for the simultaneous estimation of telmisartan and atorvastatin calcium in tablet dosage forms**.** Int J Comprehensive Pharm 2010;1(4):1-4.
10. HarikridhnanN, Venkatreddy P, Ravisankarreddy D, Vijayan V, Srinivasa Rao R. RP-HPLC Method for Simultaneous Estimation of Thiocolchicoside and Ramipril in Tablet Dosage Form. J Innovative Trends in Pharma Sci 2011; 2(3):106-110.
11. Moreshwar N Kulkarni, Rajeshwar V Kshirsagar, Dinesh M Sakarkar. Development and validation of spectrophotometric method for determination of thiocolchicoside. Int J ChemTech Res 2009;1(4):1273-1277.
12. Sanjay Dinkar Sawant, Minal Rushikesh Ghante, Asmita Shripad Deshpande, Bonny Shah. Three Simple Spectrometric Methods for Thiocolchicoside In Tablet Form. Int J Chemical and Analytical Sci 2010;1(9):217-218 .
13. Phale Mitesh D, Hamrapurkar, Purnima D. Optimization and Establishment of a Validated Stability-Indicating HPLC Method for Study of the Stress Degradation Behaviour of Thiocolchicoside. J AOAC Int 2010; 93:911-916.
14. Sagar Tatane. Development of UV Spectrophotometric Method of Thiocolchicoside in Tablet Formulation. J Advances in Pharm Healthcare Res 2011; 1:23-26.
15. Jaithlia Rajiv Chouhan, Raj Kumar Chouhan, Chethan Guptha, Akash Nagori BP. Development of UV Spectrophotometer method and validation for estimation of Thiocolchicoside as API and its pharmaceutical dosage form. International Journal of Research in Ayurvedha and Pharmacy 2011;2(6):1816-1818.
16. Lakshmi NarasimhamYS ,Barhate VD. Development and Validation of stability indicating UPLC method for the simultaneous determination of Beta-Blockers and diuretic drugs in Pharmaceutical dosage forms. J Chemical Metrology 2010;4:1-20.
17. Ramesh *J*, Kamala Kanan D, Jambulingam M, Ravi Shankar M, Arul Prakasam KC. Development and validation of HPTLC method for the simultaneous estimation of Atorvastatin and Thiocolchicoside in combined dosage form. Int J Xenobiotics and Pharma Technology 2011; 1(1):19-23.