Research Article

DEVELOPMENT AND VALIDATION OF A LIQUID CHROMATOGRAPHIC METHOD FOR THE ESTIMATION OF A MYORELAXANT DRUG IN PHARMACEUTICAL DOSAGE FORMS

Sasmita Kumari Acharjya*, Smaranika Pradhan, Partha Sarathi Das

Roland Institute of Pharmaceutical Sciences, Department of Pharmaceutical Analysis and Quality Assurance, Berhampur, Orissa, India, 760010.

ARTICLE INFO

Article history:
Received 29 May 2020
Revised 09 June 2020
Accepted 15 June 2020

Key Words: Thiocolchicoside, Liquid chromatography, TBAHS, Myorelaxant

ABSTRACT

The main objective of this study is to develop and validate a liquid chromatographic method for the estimation of a myorelaxant drug i.e. Thiocolchicoside in various marketed pharmaceutical formulations. In this method reverse phase liquid chromatograph having ODS C_{18} column (250×4.6mm packed with 5µ), methanol and 10 mM TBAHS (tetra butyl ammonium hydrogen sulfate) as mobile phase in isocratic mode at a flow rate of 0.8 ml/min is used to achieve chromatographic separation. Benzoic acid was employed as an internal standard (IS). The chromatograms were recorded at 259nm. The drug obeys linearity in the concentration range of 2.5–400µg/ml with correlation coefficient 0.999. Retention time of drug and internal standard was found to be 4.162 ± 0.004min and 8.447 ± 0.016min respectively.

©2020 Published by HOMES on behalf of RJPLS
This is an open access article under the CC-BY-NC-ND License.

***Corresponding author**

Sasmita Kumari Acharjya

Department of Pharmaceutical Analysis and Quality Assurance

Roland Institute of Pharmaceutical Sciences, Berhampur, Orissa, India, 760010.

E-mail: sasmita_acharjya@yahoo.com; Telephone: (91-9777235916)

INTRODUCTION

Thiocolchicoside (TC) is a potent myorelaxant which is a semi-synthetic sulphur derivative of colchicoside. It is chemically known as N-[3-(B-D-glucopyrano xyloxy)-5,6,7,9-tetrahydro-1,2-imethoxy-10-(methylthio)-9-oxobenzo [a] heptalen-7yl] acetamide (Fig. 1).

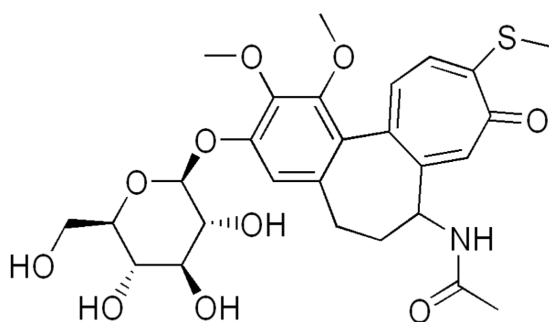

Fig. 1: Chemical structure of Thiocolchicoside

TC shows pharmacological response on the muscular contracture by activating the γ-amino-butyric acid (GABA)-inhibitory pathways, as it has selective affinity for GABA receptors [1-3].

On detailed investigation, it was summarised that TC can be quantified by several analytical techniques such as spectrophotometry[4-9], HPLC[10-15], HPTLC[16-17], spectrofluorimetric[18], LC-MS[19-20] and capillary electrophoresis[21]either alone or in presence of other drugs. The main objective of this research work is to develop and validate a new RP-HPLC method for the analysis of TC in various pharmaceutical dosage forms as per the ICH guideline.

METHODOLOGY

Chemicals and reagents

TC standard drug was obtained as gift from Sun Pharma, Vadodara India. The purity of the drug was determined by recording its ultraviolet (UV) spectra and melting point. A capsule dosage form containing 8 mg of drug was acquired from local market. HPLC grade methanol (Merck, India.) and TBAHS (tetrabutyl ammonium hydrogen sulphate) (Himedia, India) were used for preparation of mobile phase.Benzoic acid (Sigma-Aldrich, India) was used as internal standard. TKA–Gen Pure (Germany) water purification system was used to prepare purified water.Nylon membrane filter (0.45 µm) was used for solvent filtration and ultrasonicator (Enertech, India) for degassing. All solutions required for the experiment were prepared on daily basis.

Instruments and chromatographic conditions

The chromatographic separation was conducted under ambient conditions and in isocratic mode, with HPLC system (Shimadzu, Japan) connected with two pumps (LC–10AT and LC–10AT VP), a system controller (SCL 10AVP), a variable wavelength programmable UV–Vis.detector (SPD–10A) operated at 259 nm, and manual injection valve (20 µL). Shimadzu CLASS-VP V 6.14 SP1 software was used to monitor and integrate

the output signal. The separation technique was carried out on a reverse phase Hypersil ODS C18 packing material (Hibar®LiChrospher®) column (250 mm × 4.6 mm i.d., 5 μm particle size). The mobile phase was composed of mixture of methanol-10mM TBAHS (45:55, v/v) and injected at a flow rate of 0.8 mL min^{-1}.

Stock and standard solutions preparation

For the preparation of both stock and standard solutions, mobile phase was used as a solvent. For preparing stock solution (1000 μg mL^{-1}) of both the standard drug and internal standard, 25 mg of TC and 25 mg of benzoic acid was added separately in 25 mL solvent. Appropriate dilutions of the stock solution was made and spiked with 1 mL of internal standard (100μg/mL) and mobile phase was used as diluent as required to prepare various standard solutions (1.0–400 μg/mL).

Preparation of the sample solutions

Twenty TC capsule contents (each capsule contains 8 mg TC) were weighed, placed in a clean dry mortar and triturated into a fine powder using a pestle.Capsule powder equivalent to 25 mg of drug was transferred to a 25 mL volumetric flask and 20 mL of mobile phase was added. Then the mixture was allowed for 20 min sonication and mobile phase was used as diluent. Nylon membrane filter (0.45μm) was used for the filtration of the solution. An appropriate aliquot was transferred from the filtrate and spiked with internal standard in such a manner that the final concentration in 10 mL lies within the linearity range tested. The sample solution was tested (three times) under the optimized conditions as described above. The peak area ratio was calculated and sample concentration was calculated by fitting these values to the regression equation of standard curve.

Method validation

ICH Q2 (R1) guidelines are applied for the validation of the developed method.

Linearity range

The linearity range of the method was determined over the concentration range of 1.0–400 μg/mL at seven points and each solution was prepared in triplicate. A standard curve for TC was obtained by plotting peak area ratio (y) versus the theoretical concentrations of standards (x) following linear least-squares regression analysis. The correlation coefficient (R^2) value must be ≥0.999.

LOD and LOQ

The limit of detection (LOD) is the lowest absolute concentration at which the peaks of the analyte can be detected but not necessarily quantified as an exact value. The limit of quantitation (LOQ) is the lowest amount of analyte at which the

peaks of the analyte can be quantitatively determined with suitable precision and accuracy. Standard deviation of the response (y-intercept) and the slope was used to determine the LOD and LOQ of the method.

Precision

The intra and interday precision was accomplished by testing the standard samples containing internal standard, within the linearity range, at three different concentrations (50, 100 and 200 µg/mL), preparing each solution in triplicate and analyzing on the same day (intraday validation) and on three different days (interday validation). The results are reported in terms of % RSD and one-way ANOVA was applied to compare the intra and interday result of the method.

Accuracy

Accuracy of the developed method was measured as the percentage of analyte recovered in the assay. It was conducted by adding three known quantities of analyte along with internal standard (80, 100 and 120 µg/mL; ranging from 80-120% of sample solution) into a predetermined sample solution (100 µg/mL) and each solution was prepared in triplicate. The peak area ratio was calculated and by fitting these values to the regression equation of calibration curve, the percentage recovery of added drug was determined.

Robustness

To determine robustness of the method, optimised chromatographic parameters like flow rate and organic content of mobile phase were altered by ±5% and detector wavelength by ±5nm deliberately, one at a time, and test solution was examined under each condition. The effect of these changes on peak area ratio, retention time, asymmetric factor and theoretical plates for the drug was checked.

System suitability

The system suitability of the developed method was decided by calculating number of theoretical plates, resolution factor, tailing factor and precision of peak area ratio and retention time of six replicate injections.

RESULTS AND DISCUSSION

Chromatography

The purity of the standard drug TC was analysed by determining its melting point (196.0˚C) and recording UV absorption spectrum (Fig. 2).

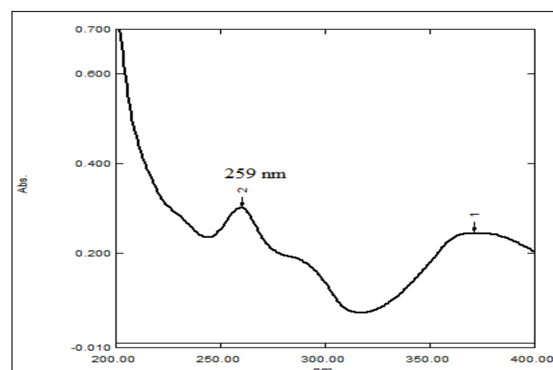

Fig. 2. UV absorption spectrum of Thiocolchicoside 10 µg/mL (λ_{max} at 259nm)

No impurities were found. To optimize the method, initially different mobile phase compositions at different flow rates was tried to obtain a simple and specific assay method for the estimation of TC. Finally, the combination of methanol and 10 mM TBAHS (45:55, v/v), at a flow rate of 0.8 mL/min was used as mobile phase based on various peak characteristics (resolution factor, theoretical plates and asymmetric factor were 6.09 ± 0.03, 2910.55 ± 46.32 and 1.35 ± 0.01, respectively), retention time (4.162 ± 0.004 and 8.447 ± 0.016 min for the standard drug and internal standard, respectively), ease of preparation and cost. Many chemicals and drugs were tried for their use as an internal standard, finally Benzoic acid was selected as internal standard as it shows different retention time as that of the drug, symmetrical peak, and can be detected in the same chromatographic conditions as that of drug. Absorption maximum (λ_{max}) of the drug was set as the detection wavelength (259 nm) because at this wavelength the drug shows more peak area ratio value as compared to detection at other wavelengths. Standards and samples were analyzed after achieving an acceptable stable base line running the mobile phase at the optimized chromatographic conditions (Table 1).

Table 1: Optimized Chromatographic Conditions

Parameters	Conditions
Column stationary phase	Hibar®LiChrospher®;C 18 (250 x 4.6 mm,5µm)
Mobile Phase composition	Methanol:10 mM TBAHS (45:55, v/v)
Flow rate (mL/min)	0.8
Column back Pressure(kgf cm^{-2})	Pump A(methanol):218 ± 3 Pump B (10 mM TBAHS):180 ± 3
Run time (min.)	12
Column temperature (°C)	Ambient
Volume of injection loop (µL)	20
Detection wavelength (nm)	259
Internal standard	Benzoic acid
Drug RT(min.)	4.162 ± 0.004
Internal standard RT(min.)	8.447 ± 0.016

Method validation

Linearity

The linearity of the method was evaluated with a seven-point standard curve (Fig. 3) over the concentration range of 1.0–400 µg/mL with a %RSD of less than 2 based on three successive readings. Linear regression analysis was used to calculate the slope and intercept of the standard curve. The regression equation of the standard curve was: $y = 0.052x - 0.109$; $R^2 = 0.999$. Correlation coefficient value of 0.999 indicates that the developed RP-HPLC method showed an excellent

linearity over the investigated linearity range.

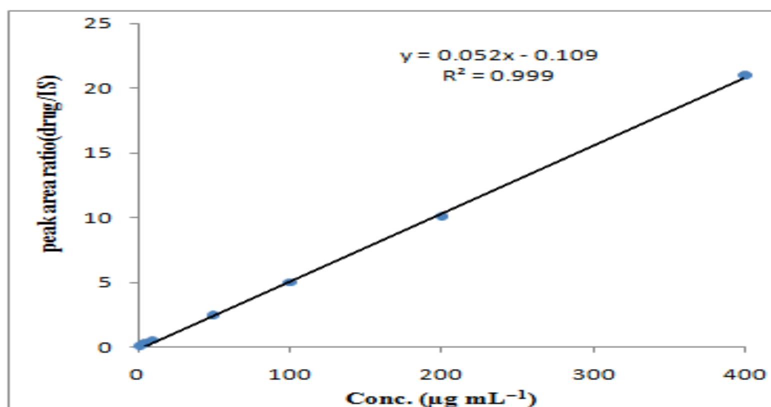

Fig. 3. Calibration curve of Thiocolchicoside by HPLC method

Limit of detection and limit of quantification

The LOD and LOQ value were obtained to be 0.176 and 0.588 µg/mL, respectively and this value indicates that the method is sensitive.

Precision

Standard samples spiked with internal standard at three different concentrations in triplicate were used for precision calculation. The observations are presented in Table 2. The %RSD value less than 2 indicates that the method is precise and reproducible. To prove that there was no difference in the results obtained in different days one-way ANOVA was applied to compare the data and p-value obtained was 0.999 ($p > 0.05$).

Table 2: Precision

Conc. (µg/mL)	Day 1 %RSD	Day 2 %RSD	Day 3 %RSD
50	0.52	0.57	0.55
100	0.52	0.54	0.58
200	0.33	0.51	0.41
ANOVA: Single Factor (95% C. L)		p-value=0.999 $F_{Calculated}$=0.0002 $F_{Tabulated}$=5.143	

Intraday: n=3; Interday: n=3 days with 3 replicates per day

Accuracy

Mean recovery and %RSD was found to be 100.56% and 0.23 (%RSD ≤2%), respectively and the observations are presented in Table 3. This study showed that the developed RP-HPLC method was accurate and absence of any interference

from the excipients present in the pharmaceutical dosage form.

Table 3: Accuracy

%Nominal value	C_{sample} (µg/mL)	$C_{standard}$ added (µg/mL)	$C_{standard}$ found (µg/mL)	%Recovery	Average recovery	%RSD
80	100	80	82.12 82.23 82.08	102.65 102.79 102.60	102.68 ± 0.10	0.09
100	100	100	98.67 99.56 99.34	98.67 99.56 99.34	99.19 ± 0.46	0.47
120	100	120	119.58 119.87 119.88	99.65 99.89 99.90	99.81 ± 0.14	0.14
Mean (n=9)					100.56 ± 0.23	0.23

n= 3; Values were expressed as mean ± SD.

Robustness

The study was conducted by using 50 µg/mL of TC standard solution, the peak area ratio values were recorded (Table 4) and the values are within the acceptance limits (95.0–105.0%) in all cases, which indicated that the method is robust.

System suitability

As per USP, the acceptance criteria for various chromatographic parameters are like less than 1 % RSD for the peak area ratio and retention time, theoretical plates (N) must be greater than 2000, resolution factor (R_s) ≥2 and USP tailing factor less than 2.0. It was conducted by using six replicate injections of the system suitability standard solutions spiked with internal standard before sample analysis and the results are presented in Table 5.

Table 4: Robustness

Parameter	Flow rate (mL min^{-1})	Organic strength (%)	Detector wavelength (nm)	Peak area ratio	Asymmetry	Theoretical plates	Retention time (min.)	% Recovery
Not altered	0.8	45	259	2.4554 ± 0.01	1.35 ± 0.01	2905.49 ± 56.02	4.162 ± 0.005	-
Organic strength	0.8	**42.75**	259	2.5039 ± 0.03	1.37 ± 0.02	2772.16 ± 15.43	4.166 ± 0.003	101.97 ± 1.25
	0.8	**47.25**	259	2.4334 ± 0.01	1.34 ± 0.01	2944.83 ± 46.00	4.153 ± 0.004	99.10 ± 0.38
Flow rate	**0.76**	45	259	2.4754 ± 0.01	1.35 ± 0.01	2853.49 ± 49.33	4.166 ± 0.005	100.81 ± 0.41
	0.84	45	259	2.4298 ± 0.03	1.33 ± 0.01	2891.83 ± 9.15	4.156 ± 0.005	98.96 ± 1.08
Detector wavelength	0.8	45	**254**	2.3754 ± 0.02	1.35 ± 0.01	2911.83 ± 57.18	4.162 ± 0.006	96.74 ± 0.80
	0.8	45	**264**	2.4351 ± 0.02	1.36 ± 0.01	2914.49 ± 55.23	4.161 ± 0.004	99.17 ± 0.84

n= 3; Values were expressed as mean ± SD

Table 5: System suitability

Injections	Peak Area ratio	Retention time (min.)	Tailing factor	Theoretical plates	Resolution factor
1	2.453	4.158	1.36	2967.83	6.09
2	2.444	4.167	1.35	2859.36	6.11
3	2.469	4.160	1.34	2889.29	6.08
4	2.442	4.163	1.36	2885.44	6.04
5	2.445	4.158	1.34	2892.39	6.10
6	2.467	4.167	1.36	2968.96	6.12
Average	2.453	4.162	1.35	2910.55	6.09
SD	0.012	0.004	0.01	46.315	0.03
%RSD	0.49	0.10	0.73	1.59	0.46

Application of the method for assay of TC in TC marketed formulations

Capsule formulation for TC was collected from the market, assay was carried out as explained in section 1.4 and the observations are summarised in the Table-6. This study showed that there was no interference from the additives found in the dosage form (Fig. 4) when compared to the control and the amount TC found in the capsule dosage form was comparable with the labelled claimed.

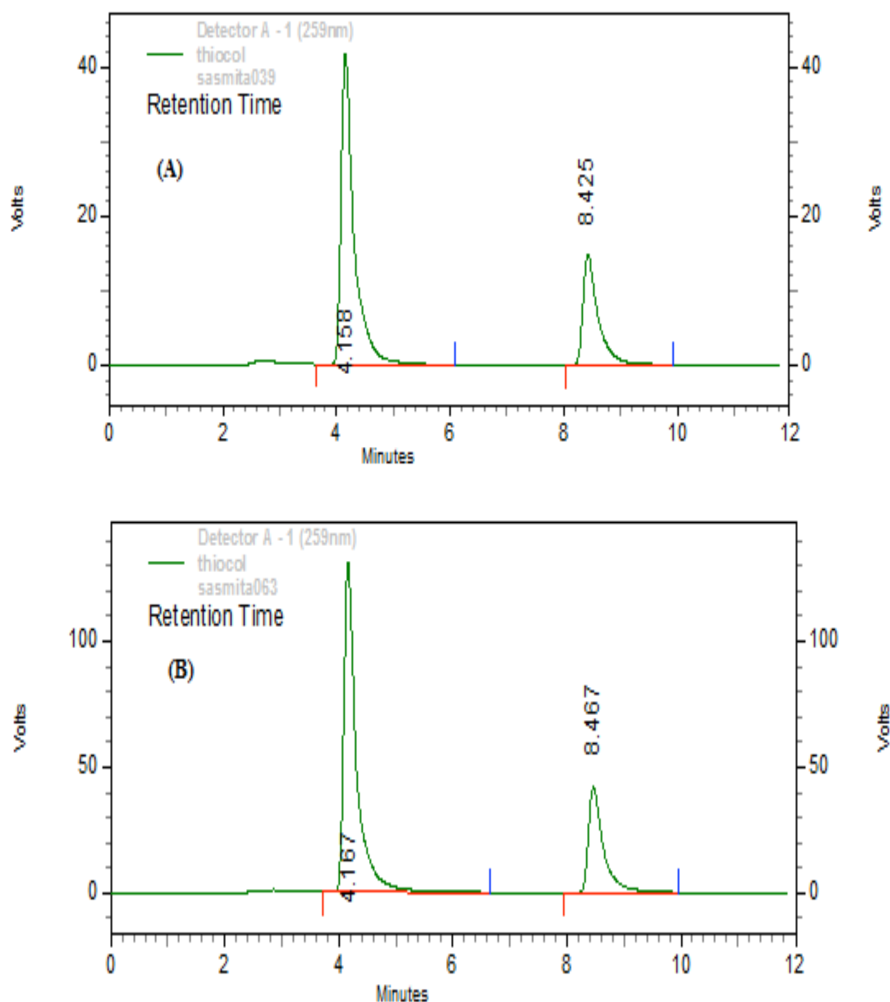

Fig. 4. Representative HPLC chromatograms (A) standard drug (50 µg/mL), internal standard (100 µg/mL) and (B) Thiocolchicoside after extraction from formulation (100 µg/mL), internal standard (100 µg/mL).

Table 6: Assay of TC

Marketed formulation	Label claim/capsule	% Recovery	Mean ± SD	%RSD
Capsule	8 mg	101.50 101.00 101.38	101.29 ± 0.26	0.26

CONCLUSIONS

As per ICH guide line a RP-HPLC method was developed and validated for TC. The developed method is simple, precise, and accurate compared to other analytical techniquesreported for the estimation of TC. Again the method has proven to be specific and the peaks due to the excipients in the TC marketed formulation did not interfere with the analysis. Recovery of TC from the marketed formulation was essentially quantitative and the method is robust with respect to variations in method parameters.

ACKNOWLEDGMENTS

The authors would like to acknowledge Alembic Ltd., (Vadodara, India) for providing the gift sample of the drug.

REFERENCES

1. The Merck Index, An Encyclopedia Of Chemicals, Drugs and Biologicals, Maryadele J.O. Neil.Eds,14th edition, Published by Merck Research Lab, Division of Merck and Co. Inc., Whitehouse Station, NJ:1603,2006.
2. Carta M, Murru L, Botta P, Talani G, Sechi GP, De Riu PL, Sanna E, Biggio G, The muscle relaxant thiocolchicoside is an antagonist of GABAA receptor function in the central nervous system, Neuropharmacology 2006; 51: 805–5.
3. Mascia MP, Bachis E, Obili N, Maciocco E, Cocco GA, Sechi GP, Biggio G, Thiocolchicoside inhibits the activity of various subtypes of recombinant GABAA receptors expressed in Xenopuslaevis oocytes, Eur. J. Pharmacol. 2007; 558:37–42.
4. Acharjya SK, Mallick P, Panda P, Annapurna MM. Spectrophotometric methods for the determination of thiocolchicoside in bulk and pharmaceutical dosage form. J Pharm Edu Res 2010; 1(1): 51-7.
5. Joshi RR, Gupta KR. UV-spectrophotometric determination of thiocolchicoside in capsule. Der Pharm Chem 2010; 2(2): 384-91.
6. Bhandari A, Nawal M, Jathalia R, Bhandari M, Solanki R, Nagori BP. UV spectrophotometric determination of thiocolchicoside from capsule dosage form. J Pharm Res 2011; 4(12): 4685-7.
7. Rajan VR. UV-spectrophotometric estimation of Thiocolchicoside by derivative method in pharmaceutical dosage form. Pharm Lett 2015; 7: 221-6.
8. Raja PC, Nirmala JP. New spectrophotometric methods for the determination of thiocolchicoside in bulk and Pharmaceutical formulations. World J Pharm Pharm Sci 2015; 4:1158-67.

9. Annapurna MM, Priya NK, Anusha N, Purneshwar PB. New derivative spectrophotometric methods for the determination of thiocolchicoside–A semisynthetic derivative of colchicoside. Int. J. Green Pharm. 2018; 12 (1): S149

10. Rosso A, Zuccaro S. Determination of alkaloids from the colchicines family by reversed-phase high-performance liquid chromatography', J Chromatogr. A, 1998; 825: 96–101.

11. HilmiIbar, Ozlem BB. Validation of Analytical Procedure for the determination of Thiocolchicoside drug by HPLC. Salon1A&B (Intercontinental at the Plaza), 2007; 281.

12. Joshi RR, Gupta KR, Jinnawar KS, Wadodkar SG. Development and validation of stability-indicating RP-HPLC and assay method for determination of thiocolchicoside in capsule. Am J Pharm Tech Res 2012; 2:590-602.

13. Arvind RU, Niki SR, Dinesh RC, Lokesh TT, Sunil BC, Mayur RB. Stability indicating RP-HPLC method for estimation of thiocolchicoside in capsule dosage forms. Res J Pharm Biol Chem Sci 2011; 2:751-6.

14. Silvio A, Rossana C, Michele B, Giorgio G, Erika DG, Development and validation of a stability indicating HPLC-UV method for the determination of Thiocolchicoside and its degradation products, J Phar. Bio Ana.http://dx.doi.org/10.1016/j.jpba.2016.09.037

15. Umarkar AR, Rewatkar NS, Chaple DR, Thote LT, Chaudhari SB, Bhurat MR. Stability Indicating RP-HPLC method for estimation of thiocolchicoside in capsule dosage forms. Res J Pharm Bio ChemSci 2011; 2(10): 750-6.

16. Pintu BP, Chandni P, Kunjan BB, Bhavin PM, Shailesh AS. Alkaline degradation kinetic study of thiocolchicoside by stability indicating high performance thin layer chromatographic method. J Liq Chrom Rel Tech 2015; 38:1767-82.

17. Rajput DK, Shirkhedkar AA, Rajput JK, Patel HM, Surana SJ. Stability studies of thiocolchicoside in bulk and capsules using RPHPTLC/ densitometry. J Anal Method Chem 2013; Article ID 142628, 1-7.

18. Suganthi A, Ravi TK. Development of validated spectrofluorimetric method for the estimation of thiocolchicoside. Int J Chem Tech Res 2012; 4(4): 1674-80.

19. Sutherland FCW, Smit MJ, Herbst L, Hundt HKL, Swart KJ, Hundt AF. Highly specific and sensitive liquid

chromatography– tandem mass spectrometry method for the determination of 3- desmethyl thiocolchicine in human plasma as analyte for the assessment of bioequivalence after oral administration of thiocolchicoside. J Chromatogr. A, 2002; 949: 71–77.

20. Del GE, Aprile S, Grosa G. Forced degradation study of thiocolchicoside: Characterization of its degradation products. J. Pharm. and Biomed. Anal. 61; 2012: 215–3. doi:10.1016/j.jpba.2011.12.008.
21. Qin L, Christine LC, Greg EC, Ultraviolet absorbance detection of colchicine and related alkaloids on a capillary electrophoresis microchip, Analytica Chimica Acta. 2006; 572: 205–11.