Oxidative Degradation Kinetic Study of Thiocolchicoside using Stability Indicating High Performance Thin Layer Chromatographic Method

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ABSTRACT

Stability indicating HPTLC method was developed using aluminium plates precoated with silica gel 60 F_{254} as the stationary phase and toluene: acetone: water (1.5:7.5:1.0, v/v/v) as mobile phase with detection wavelength 370 nm. Thiocolchicoside was subjected to degradation in alkaline, acidic, oxidative, neutral, dry heat and photolytic conditions. Linearity was observed in the concentration range 100-500 ng/spot with correlation coefficient (R^2) 0.9979 and 0.9960 at 370 nm. The developed method was validated for specificity, accuracy, precision, LOD and LOQ as per ICH guideline. The proposed method was successfully applied for the estimation of thiocolchicoside in pharmaceutical dosage forms and assay results was found to be in good agreement with labeled claim of pharmaceutical dosage forms of thiocolchicoside. The proposed method was also applied for degradation kinetic study of thiocolchicoside in 0.5, 1.0 and 1.5% H_2O_2 at different temperatures. The degradation of thiocolchicoside in all acidic conditions was found to be first order and highest degradation was found in 3.0 N HCl at 80°C temperature. The prediction of degradation rate constant was also done at different temperature and strengths of HCl using Design Expert software–9. The degradation products of thiocolchicoside formed in acidic medium were isolated and characterized using IR, Mass and NMR spectroscopy.

Key words: Degradation Products, Degradation Kinetic Study, Design expert software–9, Stability indicating HPTLC Method, Thiocolchicoside (THC).

INTRODUCTION

Thiocolchicoside is a semi-synthetic sulphur derivative of colchicoside, a naturally occurring glucoside present in the *Colchicum autumnale* and *Gloriosa superb* plants. Thiocolchicoside is used clinically for its muscle relaxant, anti-inflammatory, and analgesic properties.[1-4]. Thiocolchicoside is official drug in IP 2010 and chemical structure of thiocolchicoside is shown in Figure 1.[5]

The study of drug degradation kinetics is of greater importance for development of stable formulation and establishment of expiration date for commercially available drug products and also helps in deciding the routes of administration and storage conditions of various pharmaceutical dosage forms.[6-8] Extensive literature review reveals that several spectrophotometric and RP-HPLC methods have been reported for estimation of thiocolchicoside in its pharmaceutical dosage forms.[9-11] The literature also revealed stability indicating RP-HPTLC method for estimation of thiocolchicoside in its pharmaceutical dosage forms.[12,13] The reported RP-HPTLC method includes use of aluminum plates pre-coated with silica gel 60 RP-18 F_{254} S as stationary phase which is very costly as compare to aluminum plates pre-coated with silica gel 60 F_{254}.[14] The reported RP-HPTLC method did not show any degradation in neutral, dry heat and photolytic conditions for thiocolchicoside. Literature was not found which describing proper degradation kinetic data for thiocolchicoside in acidic medium. Nowadays, HPTLC is becoming a routine analytical technique because of its advantage like several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis. Mobile phase having pH 8 and above can be

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employed. Suspensions, dirty, or turbid samples can be directly applied. It facilitates automated application and scanning in situ. HPTLC facilitates repeated detection (scanning) of the chromatogram with the same or different parameters. Simultaneous assay of several components in a multicomponent formulation is possible. It is widely used throughout the pharmaceutical industry in research as well as in quality control laboratory.[15-17] In view of these points an attempt was made to develop and validate stability indicating HPTLC method for estimation of Thiocolchicoside in its pharmaceutical dosage form and its degradation kinetic study in acidic medium.

MATERIALS AND METHODS

Stability indicating HPTLC method for thiocolchicoside

Instrumentation

The HPTLC system (Camag Switzerland) consisting of Linomat V semiautomatic spotting device, TLC Scanner IV (Camag Muttenz, Switzerland), UV cabinet with dual wave length UV lamps, win CATS software were used for chromatographic study. Electronic analytical balance (Shimadzu AUX-220) was used for all the weighing purpose. Bruker Alfa FTIR-ATR instrument, Equipped with OPUS software, NMR Spectrometer (Bruker Advance III, 400 MHz and 100 MHz spectrometer), and Mass Spectrometer (410 Pro star Binary LC with 500 MS IT PDA detectors with direct infusion mass with ESI and APCI negative and positive mode ionization) used for characterization of degradation products.

Chemicals and reagents

Thiocolchicoside was procuredasagiftsample from Gufic Bioscience Pvt. Ltd., Navsari, Gujarat, India. Acetone, Toluene and Methanol were purchased from s.d. Fine-Chem Limited, Mumbai, India.Myoside, Myoril, Thiospas capsules were purchased from local market.

Chromatographic conditions

Chromatographic separation was performed on 10×10 cm aluminium plates pre coated with 250 µm layer of silica gel 60 F_{254} (E. Merk, Darmstsdt, Germany). The TLC plates were per-washed with methanol and dried in oven at 60°C for 10 min. Samples were spotted on TLC plate 15 mm from the bottom edge by Linomat V semi-automatic spotter using following parameters: band width, 6 mm; track distance, 11.6 mm; application rate, 0.1 µl/s. The TLC plate was developed in twin trough chamber using toluene: acetone: water (1.5:7.5:1.0, v/v/v) as mobile phase at temperature, 25 ± 2°C; relative humidity, 35 ± 5 %; chamber saturation time, 15 min; migration distance, 90 mm. The TLC plate was scanned and analyzed by TLC Scanner IV and Win CATS software using following parameters: slit dimension, 4×0.30 mm; scanning speed, 20 mm/sec; detection wavelength, 370 nm.

Preparation of solutions

Preparation of Working Standard Solution of THC

Accurately weighed 10 mg of THC was dissolved and diluted up to mark with methanol. From the above solution, 0.5 ml was diluted up to 25 ml with methanol to get final concentration 20 µg/ml.

Preparation of sample solutions for forced degradation study of THC

Forced degradation of thiocolchicoside was carried out under acidic, alkaline, oxidative, photolytic, dry heat and neutral conditions.

Acidic hydrolysis

Accurately weighed 50 mg of thiocolchicoside was dissolved and diluted up to 10 ml with 1.0 N HCl. The solution was heated for 3 hours at 80°C and cooled. Aliquot of 1.0 ml was transferred, neutralized with 1.0 N NaOH and diluted up to mark with methanol in 10 ml volumetric flask. From the above solution, 1.0 ml was diluted up to 10 ml with methanol to get final concentration 50 µg/ml.

Alkaline hydrolysis

Accurately weighed 50 mg of thiocolchicoside was dissolved and diluted up to 10 ml with 0.1N NaOH. The solution was heated for 3 hours at 80°C and cooled. Aliquot of 1.0 ml was transferred in 10 ml volumetric flask, neutralized with 0.1N HCl and diluted up to mark with methanol. From the above solution, 1.0 ml was diluted up to 10 ml with methanol to get final concentration 50 µg/ml.

Oxidative degradation

Accurately weighed 50 mg of thiocolchicoside was dissolved and diluted up to 10 ml with 1.0% hydrogen peroxide. The solution was heated for 15 minute at 80°C and cooled. Aliquot of 1.0 ml was transferred and diluted up to mark with methanol in 10ml volumetric flask. From the above solution, 1.0 ml was diluted up to 10 ml with methanol to get final concentration 50 µg/ml.

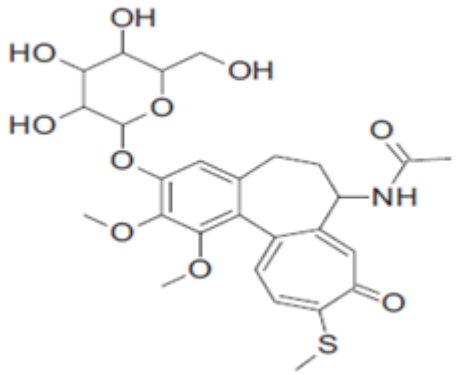


Figure 1: Chemical structure of thiocolchicoside

Photolytic degradation

Accurately weighed 50 mg of thiocolchicoside was dissolved and diluted up to 10 ml with methanol. The solution was exposed to direct sunlight for 8 hrs. Aliquot of 1.0 ml was diluted up to 10 ml with methanol. From the above solution, 1.0 ml was diluted up to 10 ml with methanol to get final concentration 50 µg/ml.

Dry heat degradation

Accurately weighed 50 mg of thiocolchicoside was taken in porcelain dish and kept in the hot air oven at 110°C for 6 hrs. After heating, it was transferred into 10 ml volumetric flask, dissolved and diluted up to the mark with methanol. Aliquot of 1.0 ml was diluted up to 10 ml with methanol. From the above solution, 1.0 ml was diluted up to 10 ml with methanol to get final concentration 50 µg/ml.

Neutral condition

Accurately weighed 50 mg of thiocolchicoside was transferred into 10 ml volumetric flask, dissolved and diluted up to the mark with distilled water. The solution was heated for 3 hours at 80°C and cooled. Aliquot of 1.0 ml was diluted up to 10 ml with methanol. From the above solution, 1.0 ml was transferred to 10 ml volumetric flask and volume was made up to the mark to get final concentration 50 µg/ml.

Preparation ofsample solution for assay of marketed formulations

For the analysis of pharmaceutical formulations, twenty capsules were accurately weighed emptied and mixed thoroughly. The capsule powder equivalent to 10 mg of THC was accurately weighed and transferred into 100 ml volumetric flask, 50 ml of methanol was added and the solution was sonicated for 20 min and filtered through What man filter paper no. 41 and diluted up to 100 ml with methanol. From the above solution, 2 ml was transferred in to 10 ml volumetric flask and diluted up to the mark with methanol.

Analysis of forced degradation samples

Fromeach forced degraded sample solutions, 10 µl was applied to same TLC plate. The plate was developed dried and analysed as described in chromatographic conditions.

Procedure for Calibration Curve

From working standard solution of THC, 5, 10, 15, 20, and 25 µl were spotted on TLC plate under nitrogen stream using Linomat V semi-automatic sample applicator. The plate was developed, dried and analysed in reflectance-absorbance mode with Camag TLC Scanner IV using Win-CATS software as described in chromatographic condition. The calibration curve was constructed by plotting peak area versus respective concentration of thiocolchicoside (100-500 ng/spot).

Method Validation

The developed method was validated for linearity, specificity, precision, accuracy, LOD and LOQ as per ICH guideline.

Procedure for assay of marketed formulations

From sample solution for assay of marketed formulation, 15 µl was applied on the TLC plate, developed, dried and analyzed as described in chromatographic condition.

Procedure for degradation kinetic study of thiocolchicoside in acidic medium

Solutions of 0.5, 1.0 and 1.5% H_2O_2 (20 ml) were transferred in series of the 25 ml volumetric flasks. The volumetric flasks were placed in controlled temperature water bath at 40 ± 2°C. Accurately weighed 25 mg of THC powder was transferred to each 25 ml volumetric flask and made up the volume with 0.5, 1.0 and 1.5% H_2O_2 to get stock solutions having strength of 1000 µg/ml. From each sample solution, 0.5 ml was withdrawn, cooled, and diluted up to 10 ml with methanol at every 15 min. interval up to 150 min. From each sample solution, 10 µl was spotted on the TLC plate. The spotted TLC plate was developed, dried and analyzed at 370 nm as described in chromatographic condition. Same procedure was carried out to perform

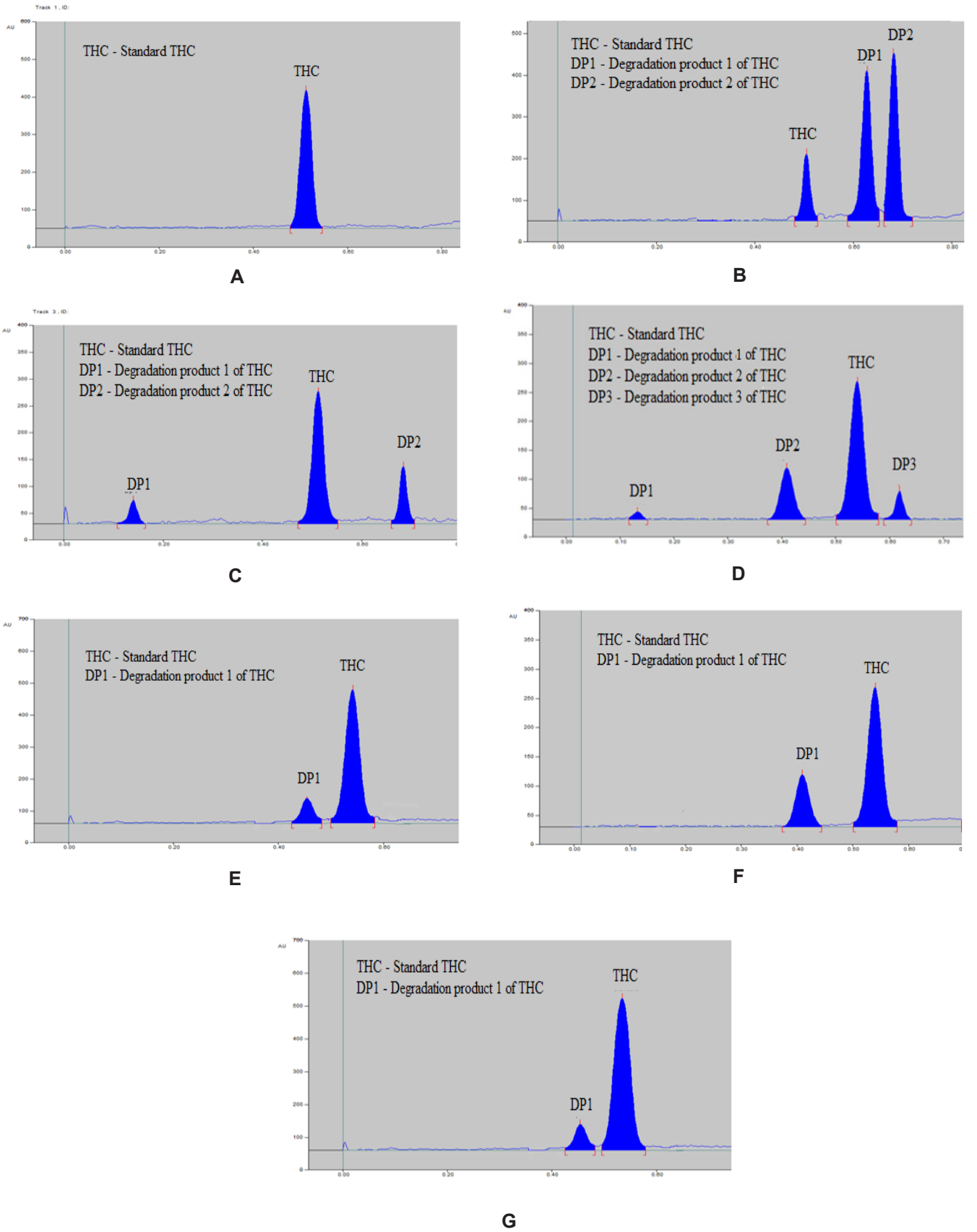


Figure 2: Chromatogram of (a) standard THC (b) acid treated THC (c) alkali treated THC (d) hydrogen peroxide treated THC (e) water treated THC (f) sun light treated THC (g) sun light treated THC

Table 1: Results of linearity for THC at 370 nm

Parameters	Results
Linearity range	100-500 ng/spot
Regression line equation	15.51X + 2355
Slope ± S.D.(n=5)	15.51 ± 0.25
Y –intercept ± S.D.(n=5)	2355.5 ± 18.98
Correlation coefficient (R2)	0.9979

degradation kinetic study of thiocolchicoside in acidic medium at 60°C and 80°C temperatures respectively. From the data of degradation kinetic study degradation rate constant, half-life and shelf life for thiocolchicoside were calculated.

Isolation and Characterization of degradation products

Procedure for isolation of degradation product (DP1) formed in acidic medium

Accurately weighted 500 mg of thiocolchicoside was heated in 25 ml of 1.0 N hydrochloric acid solution at 80°C until yellow precipitate formed. The precipitate was collected, washed and dried. The precipitate was recrystallized in methanol and it was checked for purity by TLC. The Mass spectrum, NMR spectrum, IR spectrum and melting point of precipitate of degradation product were recorded for identification and characterization of degradation product.

Procedure for isolation of degradation product (DP2) formed in acidic medium

Accurately weighted 500 mg of thiocolchicoside was heated in 50 ml of 5.0 N hydrochloric acidat 80°C. After checking completion of reaction by TLC, solution was cooled and neutralize with sodium hydroxide solution for precipitation. The precipitates was collected by filtration and dried in hot air oven. The precipitate was recrystallized in methanol and it was again checked for purity by TLC. The Mass spectrum, NMR spectrum, IR spectrum and melting point of precipitate were recorded for identification and characterization of degradation product.

RESULTS AND DISCUSSION

Stability indicating HPTLC method for estimation of THC

Optimization of Mobile Phase

The mobile phase toluene: acetone: water (1.5:7.5:1.0, v/v/v) gave good resolution and compact spots with R_f value of 0.53 for THC as shown in Figure 2a. The optimized mobile phase toluene: acetone: water (1.5:7.5:1.0, v/v/v) could separate all the degradation products of THC formed at different conditions.

Selection of wavelength for measurement

The in-situ UV spectrum of standard THC and degradation products showed reasonable absorbance at 370 nm. So, 370 nm was selected as the wavelength for measurement for estimation of THC.

Analysis of Forced Degradation Samples of THC

Thiocolchicoside was subjected to degradation in acidic, alkaline, dry heat, neutral and photolytic conditions. The chromatogram of acid treated THC shown two additional

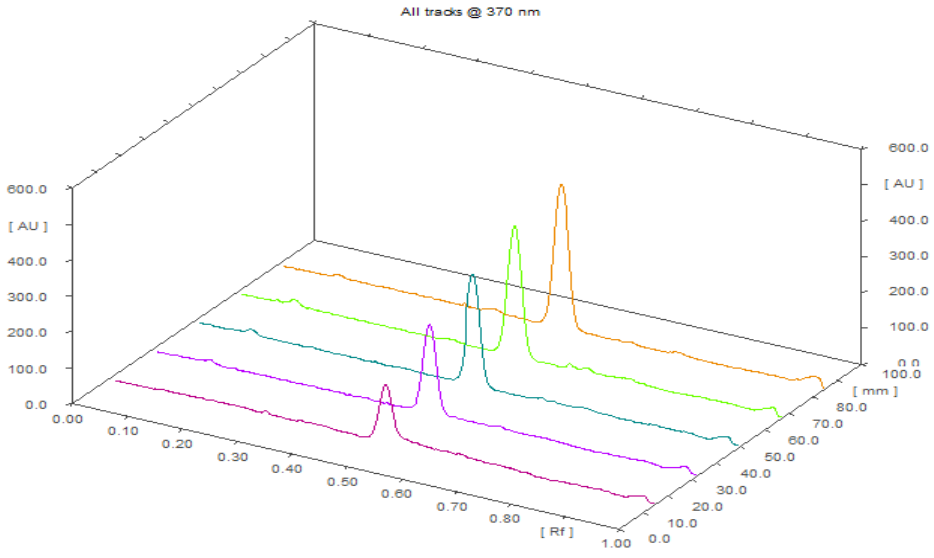


Figure 3: 3D chromatogram of THC (100-500ng/spot) at 370 nm

Table 2: Summary of validation parameters

Sr. No.	Validation parameters	Results at 370 nm
1.0	Linearity range	100-500 ng/spot
2.0	Correlation co-efficient	0.9979
3.0	Precision (% CV)	
3.1	Repeatability of peak area measurement (n=7)	0.85
3.2	Repeatability of sample application (n=7)	0.98
3.3	Intra-day precision (n=3)	1.04-1.43
3.4	Inter-day precision (n=3)	1.14–1.60
4.0	%Recovery (n=3)	99.39-101.65
5.0	Limit of Detection (LOD)	4.03
6.0	Limit of Quantitation (LOQ)	12.21
7.0	Specificity	Specific

Table 3: Summary of degradation kinetic study data of THC

Temp. (°C)	Concentration of H_2O_2 (%)	Average degradation rate constant (minute^{-1}) Kx 10^{-4}	Half life (min.)	Self-life (min.)	Order of reaction
	0.5	9.25	749.20	113.52	
40 ± 2	1.0	16.35	423.73	64.20	First
	1.5	27.42	252.71	38.29	
	0.5	32.30	216.37	32.78	
50 ± 2	1.0	57.35	120.83	18.31	First
	1.5	88.37	78.41	11.88	
	0.5	81.94	84.75	12.84	
60 ± 2	1.0	113.36	61.13	9.26	First
	1.5	164.68	42.18	6.39	

peaks at R_f 0.65 and 0.75 (Figure 2b), the chromatogram of alkali treated THC shown two additional peaks at R_f 0.15 and 0.72 (Figure 2c), the chromatogram of hydrogen peroxide treated THC shown three additional peaks at R_f 0.13, 0.42 and 0.65 (Figure 2d), the chromatogram of distilled water treated THC shown one additional peak at R_f 0.42 (Figure 2e), the chromatogram of THC treated under sun light shown one additional peak at R_f 0.42 (Figure 2f) and the chromatogram of dry heat treated THC shown one additional peak at R_f 0.47 (Figure 2g)

Method Validation

Specificity

Both the track showed only one spot having same R_f value (0.53 ± 0.01). The in-situ UV spectra of both spots were taken. There was good correlation (r=0.9999) between spectrum of standard THC and THC from dosage form. Peak purity check of THC from capsule showed high degree of correlation between spectra scanned at peak start, peak apex and peak end position (r (s,m)=0.9999, r (m,e)=0.9992) which confirm that the peak represents a pure single component i.e. THC.

Linearity and calibration curve

Calibration curve of THC was found to be linear in the range of 100-500 ng/spot with correlation co-efficient 0.9979 (Table 1). The 3D chromatogram of THC (100-500 ng/spot) is shown in Figure 3.

Precision and Accuracy

The %C.V. of precision study was found to be less than 2%. The % recovery for THC was found to be in range of 98-102%. So, developed method was found to be precise and accurate.

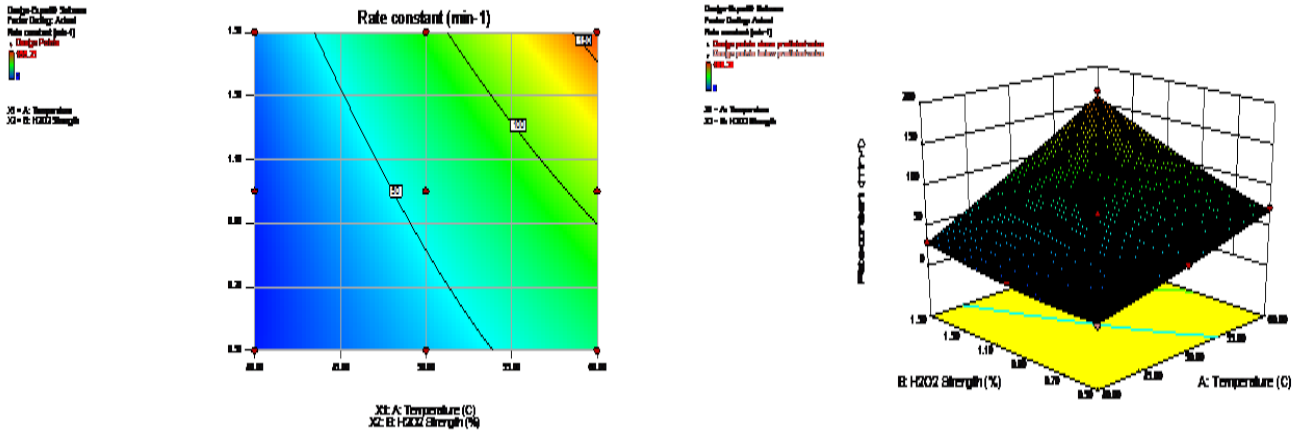
Limit of detection and quantitation

The LOD and LOQ for THC were found to be 4.03. The LOQ for THC was found to be 12.21 respectively.

The summary of validation parameters is shown in Table 2.

Assay of market formulations of THC

The proposed method was applied for assay the capsule dosage forms containing THC and % amount of THC



(a) 2D contours (b) 3D contours

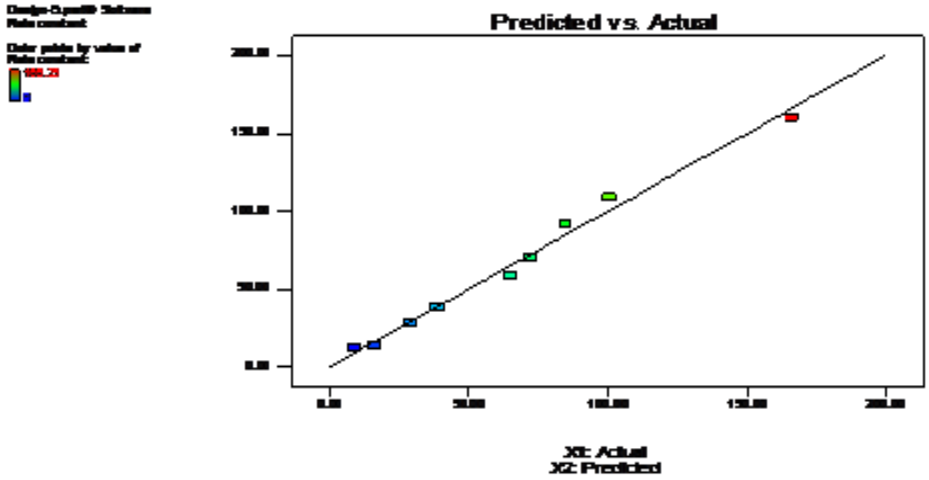


Figure 4: Plots with respect to rate constants (a) 2D contours (b) 3D contours (c)Predicted v/s actual

was found to be 99.89-100.45% of labelled claim of THC capsule. Results are shown in Table 4. The chromatogram of THC from marketed formulation showed no additional peak except THC indicate that excipient and other additives used in formulation were not interfere in assay of THC.

Degradation kinetic study of THC in oxidative condition

The acidic degradation kinetic study of THC was performed in 0.5, 1.0 and 1.5% H_2O_2 solutions at 40, 50 and 60°C. As the time increases, peak area of THC decreases and peak area of degradation product increases. The degradation rate constant, half-life and shelf-life for THC were determined for each set of data (Table 3). The acidic degradation of THC was found to follow first order kinetic and highest degradation of THC was found in 1.5% H_2O_2 at 60°C. The % degradation of THC and degradation rate constant increase either temperature increases or strength of H_2O_2 increases or both increases while the degradation half-life and shelf life decrease either strength of H_2O_2 increases or temperature increases or both increases.

Energy of Activation (Ea): A plot of ln k as a function of 1/T referred as Arrhenius plot is linear if Ea is independent of temperature. The slope of line obtained from plot of ln k versus 1/T is equal to–Ea/R. The activation energy (Ea) of the acidic degradation process of THC in 0.5, 1.0 and 1.5% H_2O_2 were calculated from Arrhenius plot and found to be 22.72, 19.87 and 18.38 kcal/mole respectively.

Prediction of degradation rate constant using design expert software - 9

3^2 level experimental designs (2 factors–3 levels) were constructed for the response surface prediction to obtain

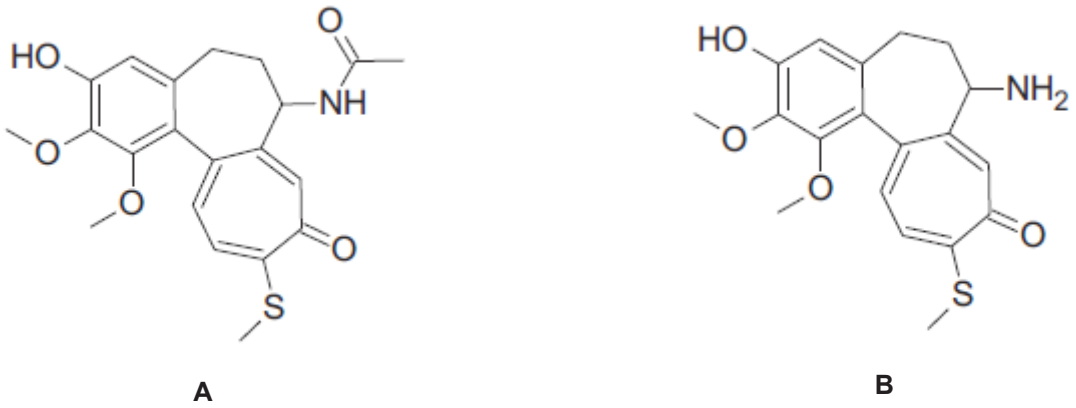


Figure 5: Chemical structure of degradation products formed in acidic medium (a) DP1 (b) DP2

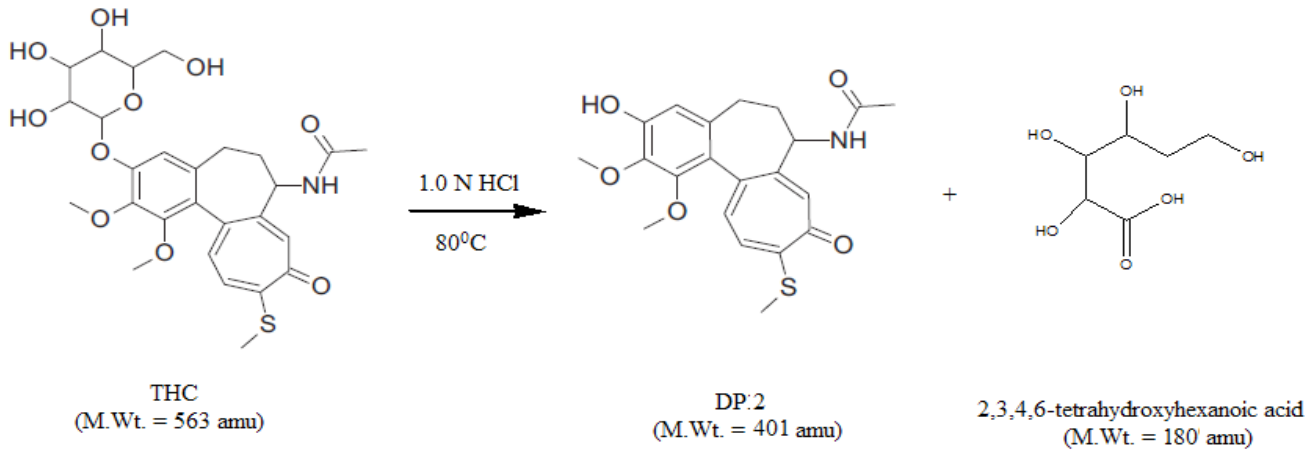


Figure 6: Chemical reaction pathway for acidic hydrolysis of THC in 1.0N HCl

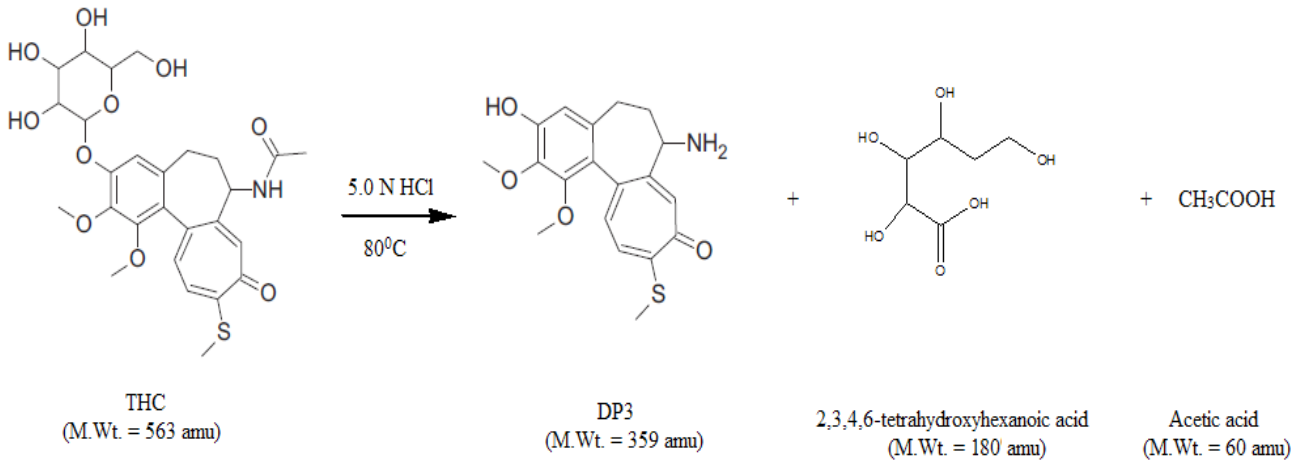


Figure 7: Chemical reaction pathway for acidic hydrolysis of THC in 5.0 N HCl

degradation data at different temperatures and HCl strengths using the Design expert software (Factor A–Temperature: -1 →40°C, 0→50°C, 1→60°C, Factor B -H_2O_2 strength: -1 →0.5%, 0→1.0%, 1→1.5%). The degradation rate constant, half-life and shelf-life of acidic degradation of THC were predicted in different strength of H_2O_2 at 25, 30 and 37°C using design expert-9 software (Figure 4).

Characterisation of degradation products of THC

IR Spectroscopy: In comparison with IR spectrum of standard THC, all peaks in IR spectrum of degradation product DP1 was found to be same except sharp phenolic O-H stretching at 3450 cm^{-1} and phenolic C-O stretching 1207 cm^{-1}. In comparison with IR spectrum of standard THC, IR spectrum of degradation product DP2 showed two sharp peaks at 3313.29 cm^{-1} and 3253.36 cm^{-1} for symmetrical and asymmetrical N-H stretching of primary amino group respectively. The peak of C=O stretching of secondary amide at 1645.61 cm^{-1} of IR spectrum of standard THC was not observed in IR spectrum of degradation product DP2 while it was observed in IR spectrum of degradation product DP1 at 1631.39 cm^{-1}.

NMR Spectroscopy: In comparison with NMR spectrum of standard THC, signals from the protons of glucoside O-H (at 4.64-5.35 ppm) and protons of glucoside C-H (at 3.15-3.83 ppm) were not observed in NMR spectrum of degradation product DP1 and DP2. In NMR spectrum of degradation product DP1, sharp peak of phenolic O-H (at 9.59 ppm) was observed.In comparison with NMR spectrum of THC, signals from the protons of glucoside OH (at 4.64-5.35 ppm), protons of glucoside C-H (at 3.15-3.83 ppm), protons of N-H amide (at 8.64-8.66 ppm) and protons of C-H of amide group (at 1.80-1.87 ppm) were not observed in NMR spectrum of degradation product DP2. In NMR spectrum of degradation product DP2, signal of primary amine N-H protons was observed at 2.11 ppm.

Mass Spectrometry: In the mass spectrum of standard THC, molecular ion peak of THC was observed as $[M+H]^+$ at 564 that indicate the molecular weight of THC was found to be 563 amu. In the mass spectrum of degradation product DP1, molecular ion peak of degradation product was observed as $[M+H]^+$ at 402 that indicate the molecular weight of degradation product DP1 was found to be 401 amu and confirming absence of sugar moiety ($M.W_t$.=162 amu) in chemical structure of THC. In the mass spectrum of degradation product DP2, molecular ion peak of degradation product was observed as $[M+H]^+$ at 360 that indicate the molecular weight of degradation product DP2 was found to be 359 amu and confirming absence of sugar moiety ($M.W_t$=162 amu) and methyl ketone ($M.W_t$=43 amu) in chemical structure of THC.

From the data of TLC, melting points, IR spectroscopy, NMR spectroscopy and mass spectroscopy, the chemical formula of degradation product DP1 is $C_{21}H_{23}O_5SN$ and the chemicalname of both degradation product is N-[1,2-dimethoxy-3-hydroxy-10-methylsulphanyl-9-oxo-5,6,7,9-tetrahydro-benzo[a]heptalen-7-yl]-acetamide. The chemical structure of degradation product is shown in Figure 5-a. These spectral data also allowed to assign the chemical formula of degradation product DP2 is $C_{19}H_{21}O_4SN$ and chemicalname is [1, 2-dimethoxy-10-methylsulphanyl- 9-oxo-3-(3, 4, 5-trihydroxy-6-hydroxymethyl-tetrahydropyran-2-yloxy)-5, 6, 7, 9-tetrahydro-benzo-[a] heptalen-7-yl]-amine and chemical structure is shown in Figure 5-b.

From the results of IR spectroscopy, NMR spectroscopy and mass spectroscopy, the possible chemical reaction pathways for the acidic hydrolysis were found as described in Figure 6 and 7.

CONCLUSION

The developed method was found to be specific, precise, accurate, sensitive andstability indicating for estimation of thiocolchicoside. In comparison with reported RP-HPTLC method, the developed method is faster and economical because it includes use of normal silica plate as stationary phase and saturation time for mobile phase is half. The developed HPTLC method is able to separate more no. of degradation products in all stress conditions as compare to developed RP-HPTLC method. So, developed HPTLC method is more efficient stability indicating method for estimation of thiocolchicoside in its pharmaceutical dosage forms. The developed method was applied for assay of pharmaceutical dosage forms of thiocolchicoside and assay results found were in good agreement with labeled claim of dosage forms. The developed method was also applied degradation kinetic study of thiocolchicoside in acidic medium.

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CONFLICT OF INTEREST

The developed method was found to be specific, precise,

accurate, sensitive and stability indicating for estimation of thiocolchicoside. The developed HPTLC method is more efficient stability indicating method for estimation of thiocolchicoside in its pharmaceutical dosage forms.

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