Arabian Journal of Chemistry (2015) **8**, 118–128

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Validated stability indicating RP-HPLC method for simultaneous determination and in vitro dissolution studies of thiocolchicoside and diclofenac potassium from tablet dosage form

Suraj D. Jadhav *, **S.R. Butle, Sachin D. Patil, P.K. Jagtap**

School of Pharmacy, Swami Ramanand Teerth Maharathwada University, Vishnupuri, Nanded 431 606, MS, India

Received 3 November 2010; accepted 16 January 2011
Available online 21 January 2011

KEYWORDS

Thiocolchicoside;
Diclofenac potassium;
Sink condition;
Photodiode array detector;
Dissolution

Abstract A simple, rapid, and robust stability indicating RP-HPLC method has been developed and validated to measure thiocolchicoside (TH) and diclofenac potassium (DP) at single wavelength (258 nm) in order to assess assay and in vitro drug release profile of drug from tablet formulation. A gradient elution of samples performed on Zorbax SB CN 250 mm × 4.6 mm, 5 μm column with buffered mobile phase consisting solvent A (5 mM sodium dihydrogen phosphate, pH 2.5) and solvent B (methanol) delivered at flow rate 1.0 mL/min. For dissolution study, the sink condition has been established from quantitative solubility of TH and DP API in different dissolution medium recommended by USP for immediate release formulation and the optimized dissolution condition was: pH 6.8 deaerated potassium dihydrogen phosphate buffers, paddle rotation speed 50 rpm and vessel volume 900 mL. Discriminating release of TH and DP achieved more than 96% of labeled amount over 45 min and drug dissolution was concluded after 60 min. The HPLC method and dissolution test condition were validated to meet requirement for regulatory filling and this validation inferred from specificity, precision, accuracy, linearity and robustness. In addition filter suitability, standard and sample solution stability was demonstrated.All results were acceptable and this confirmed that the method is suitable for its intended use in routine quality control and assay of drugs.

1. Introduction

Thiocolchicoside (TH) is (s)-N-[3-(BD-glucopyranoxyloxy)-5,6,7,9-tetrahydro-1,2-dimethoxy-10-(methylthio)9-oxobenzo-[a]heptalen-7yl]acetamide, sulfur derivative of cochicoside (Fig. 1a) and possesses non-sedating muscle relaxant action (Merck Index, 1998; Indian Pharmacopoeia, 2010), while diclofenac potassium (DP) is 2-[(2,6-dichlorophenyl)amino]

* Corresponding author. Tel.: +91 9096254036.
E-mail address: surajjadhav111@gmail.com (S.D. Jadhav).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2011.01.018
1878-5352 © 2011 Production and hosting by Elsevier B.V. on behalf of King Saud University.

phenyl acetic acid (Fig. 1b) which possesses anti-inflammatory and analgesic properties (Indian Pharmacopoeia, 2010; British Pharmacopoeia, 2004; United State Pharmacopoeia, 2005). The combination of TH and DP have synergetic action and is prescribed for symptomatic relief of low back pain, post operative pain, and rheumatic arthritis osteoarthritis, musculoskeletal injuries and chronic pain associated with cancer (Janbroers, 1987).

Literature survey revealed that several analytical methods have been described for analysis of TH as single component or in combinations with other drugs, Viz-spectrophotometric methods (Sutherland et al., 2002; Lu et al., 2006), TLC (El-Ragehy et al., 2003), HPLC (Ondra et al., 1995; Rosso and Zuccaro, 1998; Tracqui et al., 1996) and radioimmunoassay (Sandouk et al., 1995). Similarly literature of DP revealed several spectrometric methods (Agatonovic-kustrin et al., 1997; Botello and Caballero, 1995; Matin et al., 2005; Sparidans et al., 2008) as well as chromatographic methods (Vora et al., 2007; Kaphalia et al., 2006; Lee et al., 2000; Klimes et al., 2001) for determination of DP as single drug or in combination with other drugs.

However, the exhaustive literature survey revealed that none of the most recognized pharmacopoeias or any journals includes these drugs in combination for the simultaneous determination of TH and DP and the information regarding the stability of the drugs is not available. So it is felt essential to develop a liquid chromatographic procedure which will serve a reliable, accurate and stability indicating HPLC method for the simultaneous estimation and in vitro dissolution studies of TH and DP in tablet dosage form.

The main purpose of an oral solid pharmaceutical dosage form is to make available a certain and defined amount of active substance to human body through the gastrointestinal system (Morrison and Campbell, 1965). The pharmaceutical industry and the regulatory agencies focus on the evaluation of the release kinetics from dosage forms, and this study is generally performed on official or nonofficial dissolution devices (Jashnani et al., 1993). The in vitro dissolution profiles obtained from dissolution rate studies have also been used in an attempt to characterize the in vivo behavior of drugs with little success (Rowe and Carless, 1981; Anchisi et al., 1998; Satiropoulus et al., 1981). The significance of stability testing is to provide evidence on how the quality of a
drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, enables recommendation of storage conditions, retest periods, and shelf lives to be established.

The principal aspects of drug products that play an important role in shelf life determination of tablet formulation are assay and dissolution of active drug and degradants generated during the stability study. The assay of drug product in stability test sample needs to be determined using stability indicating method, as recommended by the International Conference on Harmonization (ICH) guidelines (ICH, 2000) and USP 29 (United State Pharmacopoeia, 2005).

The main purpose of this investigation is to develop and validate simple, precise, sensitive and accurate stability indicating reversed phase high-performance liquid chromatographic methods for assay and in vitro dissolution studies.

2. Experimental

2.1. Chemicals and reagents

All reagents and solvent were of analytical and HPLC grade and included hydrochloric acid, monobasic sodium phosphate, monobasic potassium phosphate, sodium acetate trihydrate, orthophosphoric acid and methanol were purchased from Merck Ltd., Mumbai, India. Wockhardt Ltd., Aurangabad, India, kindly supplies TH and DP API, tablet and placebo of tablet. High purity deionized water was obtained from Millipore, Milli-Q (Bedford, MA, USA) purification system. The 0.45-μm nylon filters were purchased from Advanced Micro Devices Pvt. Ltd., Chandigarh, India.

2.2. Instrumentations

HPLC system (Waters Milford, USA) equipped with in built auto-sampler and quaternary gradient pump with an on-line degasser was used. The column compartment having temperature control, photodiode array (PDA) detector (2996) and dual wavelength detector (2487) was employed throughout the analysis for detection. Chromatographic data was acquired using Empower software-2. All dissolution experiments were carried out using a dissolution instrument Electro lab TDT-08L (Electrolab, India) attached to auto-sampler.

2.3. Chromatographic condition

Zorbax SB CN 250 mm × 4.6 mm, 5 μm (Agilent technology, USA) column was used as stationary phase maintained at ambient temperature. Gradient elution with the mobile phase involved a variable composition of solvent A (5 mM sodium dihydrogen phosphate, pH 2.5) and solvent B (methanol). The mobile phase was pumped through the column with flow

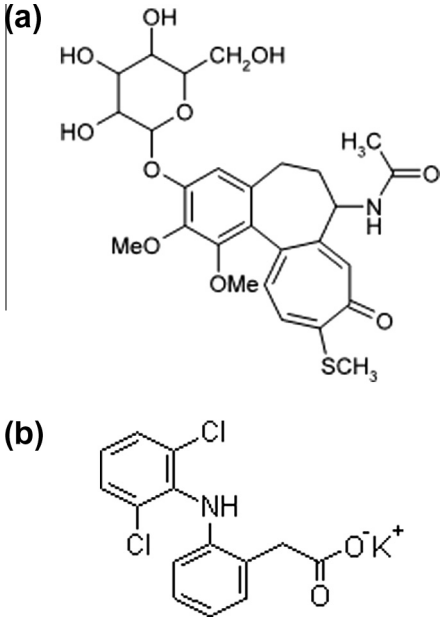

Figure 1 Chemical structure of (a) thiocolchicoside (TH) and (b) diclofenac potassium (DP).

Table 1 Mobile phase program for gradient elution.

Time (min)	Flow (mL min^{-1})	Solvent A (%)	Solvent B (%)
0	1.0	60	40
1	1.0	60	40
10	1.0	20	80
11	1.0	60	40
15	1.0	60	40

rate of 1 mL min^{-1} (Table 1). Injection volume 20 μL was used in all experiments.

The optimum wavelength selected was 258 nm, which represents the wavelength of maximum response for both TH and DP. The stressed samples were analyzed using a PDA detector covering the range of 200–400 nm.

2.4. Preparation of standard solution and calibration graph

2.4.1. For assay method

Mixed Standard stock solution of 80 μg mL^{-1} of TH and 50 μg mL^{-1} of DP was prepared by dissolving about 80 mg of TH reference standard and about 50 mg DP reference standard in 30 mL of diluent which was a mixture of water:methanol (50:50, v/v) in 100 mL volumetric flask and stirred in an ultrasonic bath for 10 min. The volume was completed with the same diluent. Further diluted 5 mL of mixed standard stock to 50 mL with the same diluent.

To study the linearity range of each component, the serial dilutions of mixed standard stock were made in the range of 40.48–121.43 μg mL^{-1} of TH and 24.91–74.72 μg mL^{-1} of DP (i.e. 50–150% of test concentration). A graph was plotted as concentration of drugs versus peak area response. It was found to be linear for both the analytes. The system suitability test was performed from five replicate injections of mixed standard solution.

2.4.2. For in vitro dissolution studies

Standard stock of TH (8.8 μg mL^{-1}) was prepared by dissolving about 88 mg of reference standard of TH in 100 mL of diluent which was respective dissolution media, this 5 mL solution to 50 mL was diluted with same diluent. Standard solution of DP (55 μg mL^{-1}) was prepared by dissolving about 55 mg reference standard of DP into 100 mL of same diluent. The mixed standard was prepared by mixing 5 mL of standard stock of TH and 5 mL of DP standard stock solution.

To study linearity of each component, the serial dilution of TH and DP stocks were made in the range of 0.4–13.19 μg mL^{-1} of TH and 2.7–82.50 μg mL^{-1} of DP (i.e. 1–150% test concentration). A graph was plotted as concentration of drugs versus peak area response. It was found to be linear for both the analytes. The system suitability test was performed from five replicate injections of mixed standard solution.

2.5. Preparation of sample solution

2.5.1. For assay method

Twenty tablets each containing 8 mg of TH and 50 mg DP were weighed, averaged and finely powdered. A portion of powder equivalent to the weight of one tablet was accurately weighed into 100 mL A-grade volumetric flasks and 70 mL diluent was added. The volumetric flasks were sonicated for 20 min to effect complete dissolution of the TH and DP in the solutions, and then made up to volume with diluent. The solution was filtered through 0.45 μm nylon filter.

TH sample (80 μg mL^{-1}) – aliquot portion of filtrate use as such.

DP sample (50 μg mL^{-1}) – further dilute aliquot portion of the filtrate to 50 mL with diluent.

Twenty microliters of test solution was injected and chromatogram was recorded for the same and amount of drug calculated.

2.5.2. For in vitro dissolution studies

A calibrated dissolution tester was used with paddle (USP-II) at 50 rpm and bath temperature maintained at 37 ± 5 °C. Nine hundred milliliters of freshly prepared and degassed pH 6.8 phosphate buffers was used as dissolution medium. Six tablets were evaluated. Dissolution samples were collected at 5, 10, 15, 30, 45, 60 min. At each time point, a 10 mL sample was removed from each vessel using an auto-sampler and filtered through a 0.45 μm nylon filter into labeled glass tubes and analyzed by HPLC.

2.6. Forced degradation sample solution for specificity

Multiple stressed samples were prepared as indicated below. They were chromatographed along with a non-stressed as control sample.

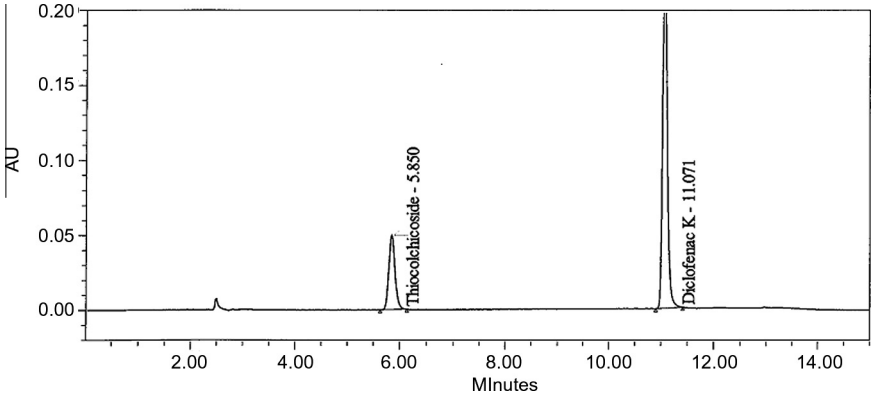

Figure 2 A typical chromatogram of a tablet sample solution containing 80 μg mL^{-1} of thiocolchicoside and 50 μg mL^{-1} of diclofenac potassium.

Table 2 Summary of stationary phases used to optimize the method.

Stationary phase	Dimension	Observation				
		R_t (min)		Capacity factor (K')		Separation factor (α)
		TH	DP	TH	DP	
Hypresil C18	150 × 4.6 mm, 5 μm	1.2	[a]	0.07	–	–
	250 × 4.6 mm, 5 μm	2.1	[a]	0.14	–	–
Kromasil C18	250 × 4.6 mm, 5 μm	2.4	[a]	0.30	–	–
Hypresil C8	250 × 4.6 mm, 5 μm	2.5	23.8	0.35	11.86	33.8
Hypresil phenyl	250 × 4.6 mm, 5 μm	2.6	14.7	0.41	6.95	17.1
Zorbax SB CN	250 × 4.6 mm, 5 μm	5.8	11.0	2.14	4.95	2.3

[a] Peak not eluted up to 60 min.

2.6.1. Hydrolytic conditions: acid, base induced degradation
Solutions containing 80 μL of TH and 50 μL of DP were treated with different conc. of HCl and NaOH which was 0.1 N, 0.5 N and 1 N for 30 min at 80 °C. The solutions were neutralized as needed.

2.6.2. Oxidative condition: hydrogen peroxide-induced degradation
Sample was treated with 5 mL of 5% and 10% w/v H_2O_2 in dark under the condition 80 °C for 30 min.

2.6.3. Thermal degradation
To investigate the stability of the drugs under thermal stress conditions, sample was spread in a thin layer on a petri plate and subjected to the conditions indicated at 60 °C for 12 h.

2.6.4. Photolytic degradation
Photolytic studies were performed after exposition of sample in solid state and diluted in purified water, subjected in a photo stability chamber up to 22 h.

Similarly the placebo of tablet was treated as same like above in each condition separately and tested. All these stressed samples and placebos were periodically analyzed by HPLC for the appearance of degradant impurity, calculated against main drug component.

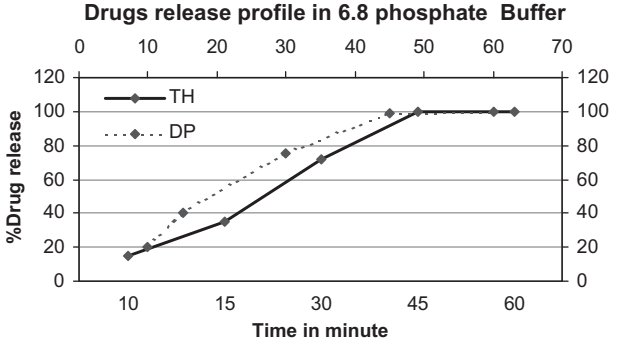

Figure 3 Drug release of TH and DP.

Table 4 System suitability parameters.

Parameters	TH		DP	
	Assay	Dissolution	Assay	Dissolution
Theoretical plates	69,992	12,169	24,219	68,601
USP tailing (T_f)	1.31	1.05	1.18	1.06
Resolution	–	–	11.87	11.23
Capacity factor	2.14	2.34	4.92	5.04
% RSD	0.12	1.84	0.16	1.36

3. Result and discussion

3.1. Optimization of chromatographic condition

It was clear from the molecular structure of TH and DP (Fig. 1) that TH is weak base of pK_a value 10 and very polar in nature while, DP is weak acid of pK_a value 4 and less polar than TH.

The main aim of this study is to analyze the polar drugs with sufficient resolution in reasonable analysis time. To obtain a good chromatographic condition, different stationary phases were tested considering:

(a) The feature of stationary phase (RP-C_8 and RP-C_{18}, Phenyl and Cyano column).
(b) The particle size of the column (3 μm and 5 μm).

Considering that TH and DP are having different pK_a, we tested following mobile phases with gradient elution:

Table 3 Quantitative solubility and sink condition verification of drugs.

Properties	pH 4.5 Acetate buffer dissolution media		pH 6.8 Phosphate buffer dissolution media	
	TH	DP	TH	DP
mg mL^{-1} dissolved	11.44	0.0024	16.40	16.35
mg 900 mL^{-1} dissolved	10,296	2.16	14,760	14,715
Sink condition requirement (mg 900 mL^{-1})	24	150	24	150
Conclusion	Satisfy	Fail	Satisfy	Satisfy

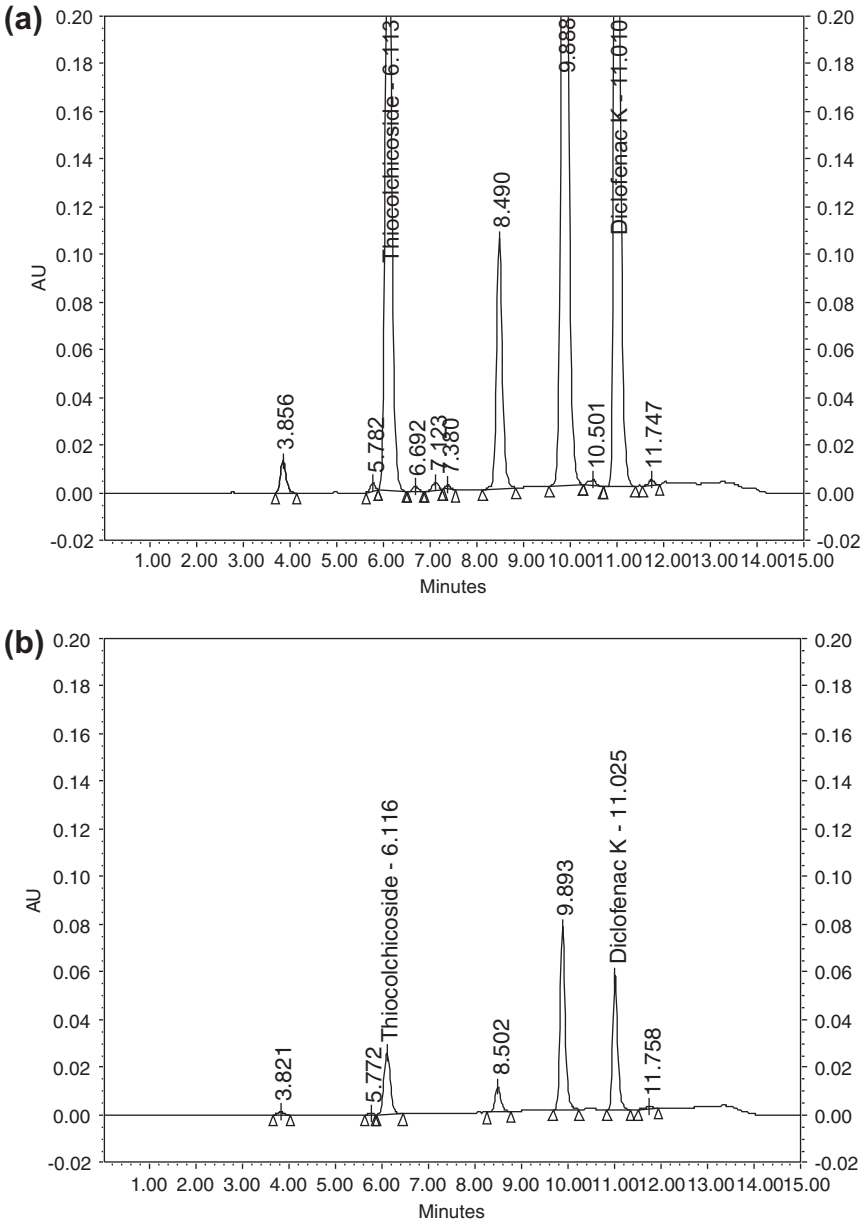

Figure 4 Typical HPLC chromatogram of 1 N HCl stressed sample of TH (a) and DP (b).

(a) KH_2PO_4, K_2HPO_4, 0.3 mL L^{-1} orthophosporic acid as a buffer (pH 2.5, 3.5 and 6.5) in combination with methanol.
(b) NaH_2PO_4 Na_2HPO_4 and 0.3 mL L^{-1} orthophosphoric acid as a buffer (pH 2.5, 3.5 and 6.5) in combination with methanol.
(c) Water:methanol:acetonitrile:triethylamine (75:15:10:01) and 1-pentane sulfonic acid of pH 2.5 and 3.5.

3.1.1. Selection of stationary phase

As both drugs were polar in nature they can readily ionize. Hence we started the development activity with different stationary phases starting from non-polar to polar, i.e. C_{18}, C_8, Phenyl, and Cyano of various manufacturers using different mobile phases. Columns with C_8 and C_{18} stationary phases were tried for better peak shape and resolution, but TH was eluting at about void volume due to the highly polar nature indicating that it is not retaining on the column surface but eluting with mobile phase. Hence to retain the peaks the polar stationary phase needs to be used. The poor resolution between TH and DP and distorted peak shape on phenyl column implies that this column is also not suitable for this application. Hence column with Cyano stationary phase was chosen to improve the peak shape and resolution among the peaks. The peak shape and system suit parameters among all components were improved with Zorbax SB CN (250 mm × 4.6 mm, 5 μm) column (Fig. 2 and Table 2). But the stationary phase

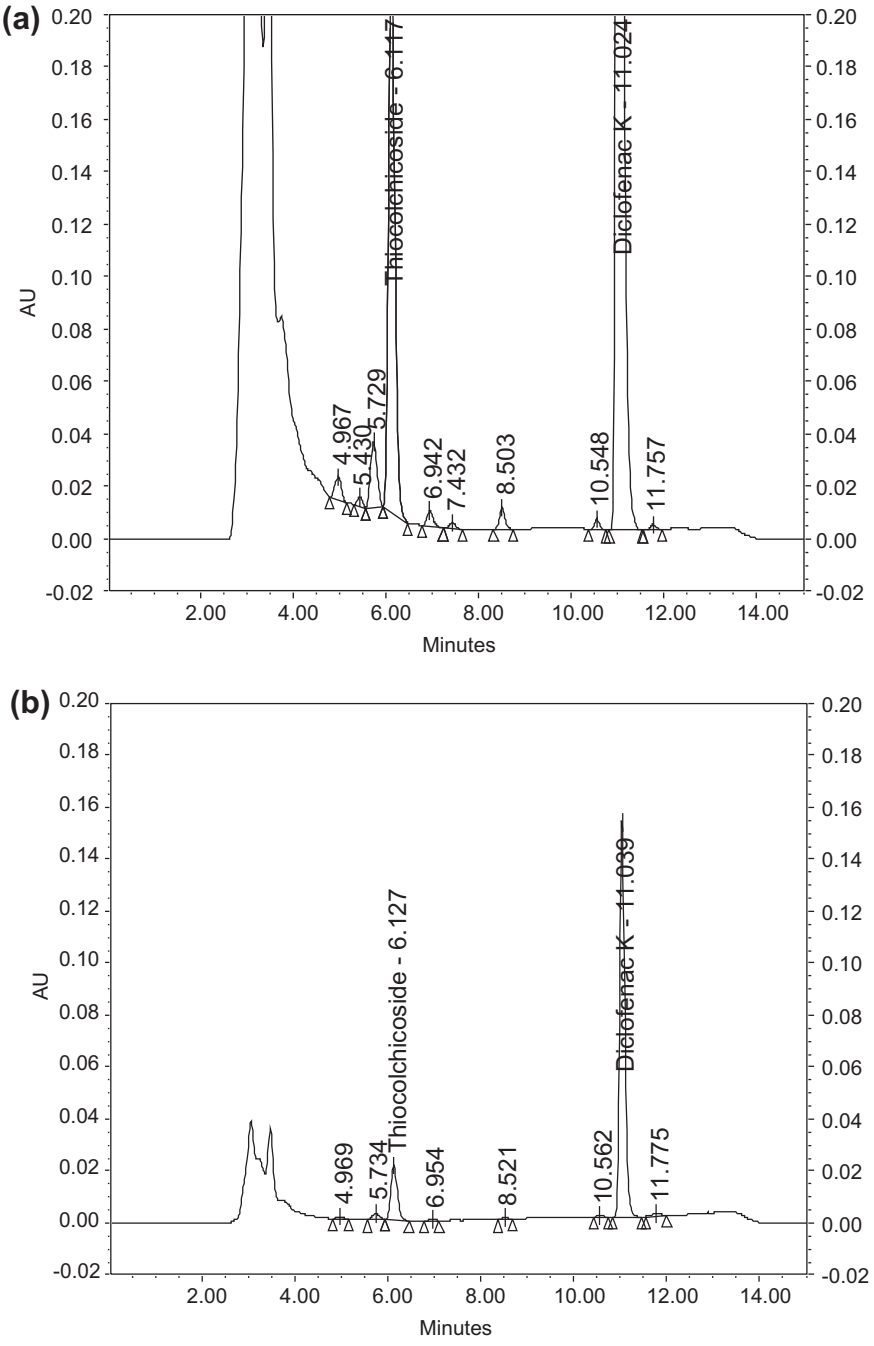

Figure 5 Typical HPLC chromatogram of 1 N NaOH stressed sample of TH (a) and DP (b).

is not only the parameter, which can give better chromatography. Mobile phase pH and organic modifiers also play very important role which leads to the best chromatographic condition.

3.1.2. Selection of mobile phase

Highly polar substances, especially those which take ionized form in water, have peak tailing and bad peak symmetry when separated by applying the RP-HPLC method, which affects resolution, sensitivity and reproducibility. Hydrogen bonding and ion exchange interaction can occur between the chromatographic support and the basic compounds. It can be prevented by addition of modifier to the mobile phase such as acid, base and ion pairing agents. More accepted approach is to adjusted pH value of the mobile phase (Swadesh, 1997).

The elution of TH and DP was done using ion pair agent 1-pentane sulfonic acid in mobile phase containing water and water:acetonitrile:triethylamine (75:15:10 v/v/v) but elution of TH occurred at void volume, while DP does not elute up to 60 min on different stationary phases given in Table 2. The buffer KH_2PO_4, K_2HPO_4, Na_2HPO_4, NaH_2PO_4 and

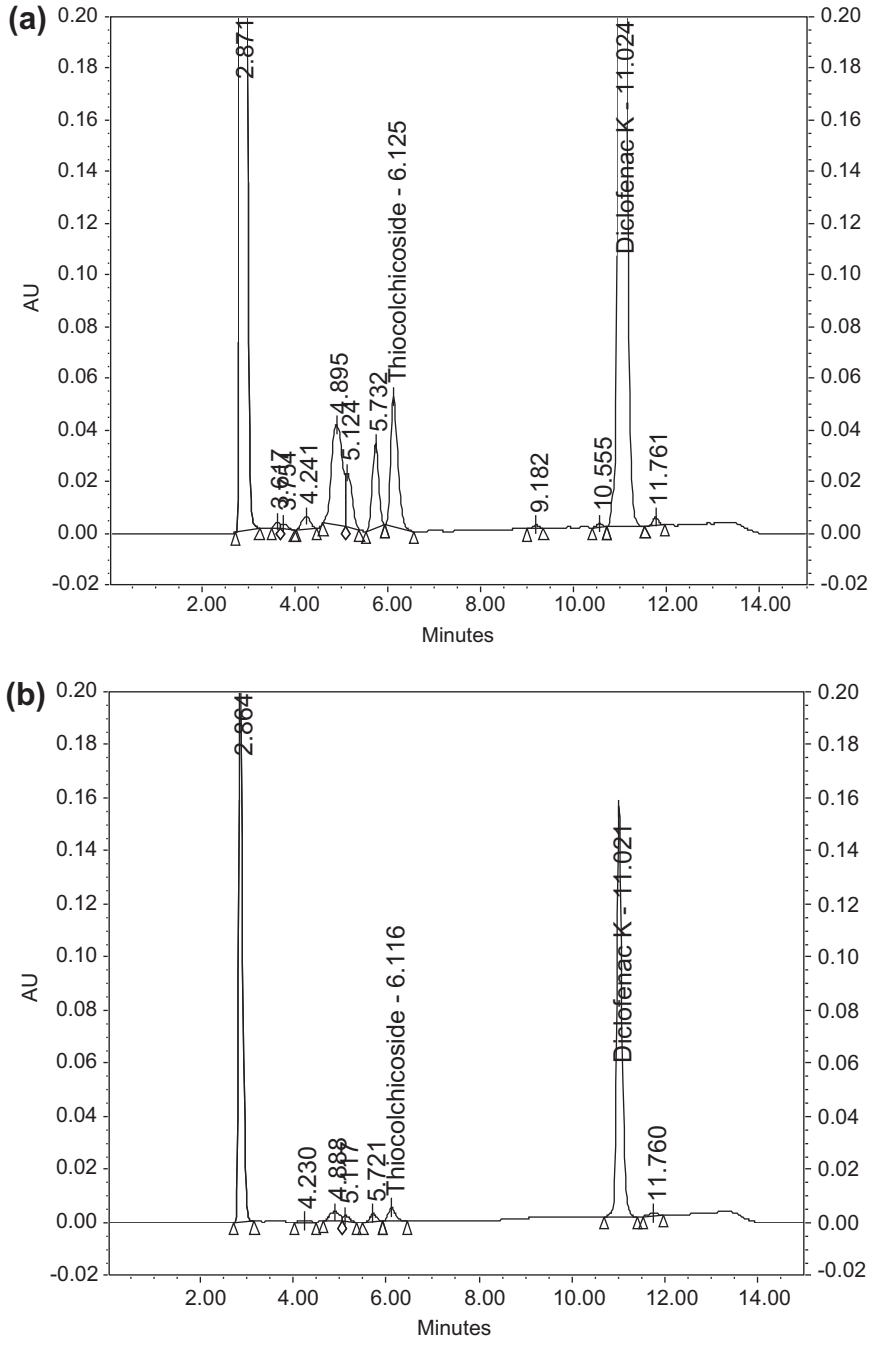

Figure 6 Typical HPLC chromatogram of 10% H_2O_2 stressed sample of TH (a) and DP (b).

sodium acetate were tested. The good performance and better separation was achieved in NaH_2PO_4 buffer and methanol using Cyano stationary phase (Fig. 2). We optimized the elution by setting gradient program at flow rate 1.0 mL min^{-1} (Table 1).

3.1.3. Influence of pH and organic modifier

The molecular structure of both components implies that TH is having amide group and DP is having carboxylic acid functional group, which makes separation pH sensitive. For the method to be robust, pH of mobile phase must not be at pK_a of compounds. The basic pH deteriorates the silica in HPLC column. We tried different mobile phase buffers pH ranging from 2.5 to 5 and monitored the resolution and performance of column. The best separation was achieved with pH 2.5. DP was not eluted on polar (Cyano) stationary phase as it retains due to polar likes polar theory. To elute within our satisfactory run time, organic modifier composition needs to be increased. The satisfactory elutions were observed at gradient program mentioned in Table 1.

3.2. Optimization of dissolution test condition

3.2.1. Solubility determination and sink condition verification

Drug solubility and solution state stability need to be determined before selecting the dissolution medium. The dissolution characteristics of an oral formulation should be evaluated in the physiologic pH range of 1.2–6.8 (1.2–7.5 for modified-release formulations). USP <711>. Solubility data were used as the basis for the selection of a dissolution medium for TH and DP. Drugs solubility was determined at 37 °C in 0.1 N HCl, pH 4.5 acetate buffer and pH 6.8 phosphate buffers.

DP is practically insoluble in 0.1 N HCl. The quantitative solubility of drugs was determined in pH 4.5 acetate buffer and pH 6.8 phosphate buffer. Based on solubility results for TH and DP in each dissolution medium tested (Table 3) and considering a test volume of 900 mL per vessel, *sink* conditions were verified. The term *sink* conditions is defined as the volume of medium at least greater than three times of that required to form a saturated solution of drug substance (United State Pharmacopoeia, xxxx; Tracqui et al., 1996). A pH 6.8 phosphate buffer was used as dissolution media for TH and DP tablet (Table 3).

3.2.2. Development of the dissolution test condition

From pre-selected, pH 6.8 phosphate buffer media, dissolution testing was performed on tablet ($n = 6$) in compliance with USP <711> using paddle (USP-II). A paddle speed of 50 rpm was selected to minimize coning. The media volume used 900 mL. The medium which was vacuumed and degassed under house vacuum was maintained at 37 ± 0.5 °C. The 1-L glass dissolution vessels were covered to minimize evaporation. Samples were drawn at 5, 10, 20, 30, 45, 60 min. The tablet was immediate releasing, the earlier time points provided more discriminating ability (Fig. 3). Sampling was performed using auto sampler of 10 mL aliquots. This solution was immediately filtered using a syringe of 0.45 µm nylon filters. The first 2–3 mL of sample was discarded prior to collecting the sample for analysis.

3.3. Method validation

The optimized RP-HPLC method for assay method and in vitro dissolution studies validated according to ICH guidelines (ICH, 2000, 2005) with respect to specificity, accuracy, precision (repeatability and intermediate precision), linearity, range and robustness. System suitability features were also assessed.

3.3.1. System suitability test

The system suitability test performed according to USP 29 (United State Pharmacopoeia, xxxx) and BP 2007 (British Pharmacopoeia, 2004) indications. The observed RSD values at 1% level of analyte concentration were well within the usually accepted values (⩽2%). Theoretical plates, USP tailing factor (T_f) and USP resolution (R_s) between TH and DP were determined for both assay and dissolution. The results are all within acceptable limits summarized in Table 4.

3.3.2. Specificity

The specificity of Assay HPLC method carried out by the peak purity indices for the analytes in stressed solutions were determined with PDA detector (Young and Gorenstein, 1994) under optimized chromatographic conditions found to be better (purity angle < purity threshold) indicating that no additional peaks were co-eluting with the analytes and evidencing the ability of the method to assess unequivocally the analyte of interest in the presence of potential interference. Baseline resolution was achieved for all investigated compounds. The FDA guidelines indicated that well separated peaks, with resolution, $R_s > 2$ between the peak of interest and the closest

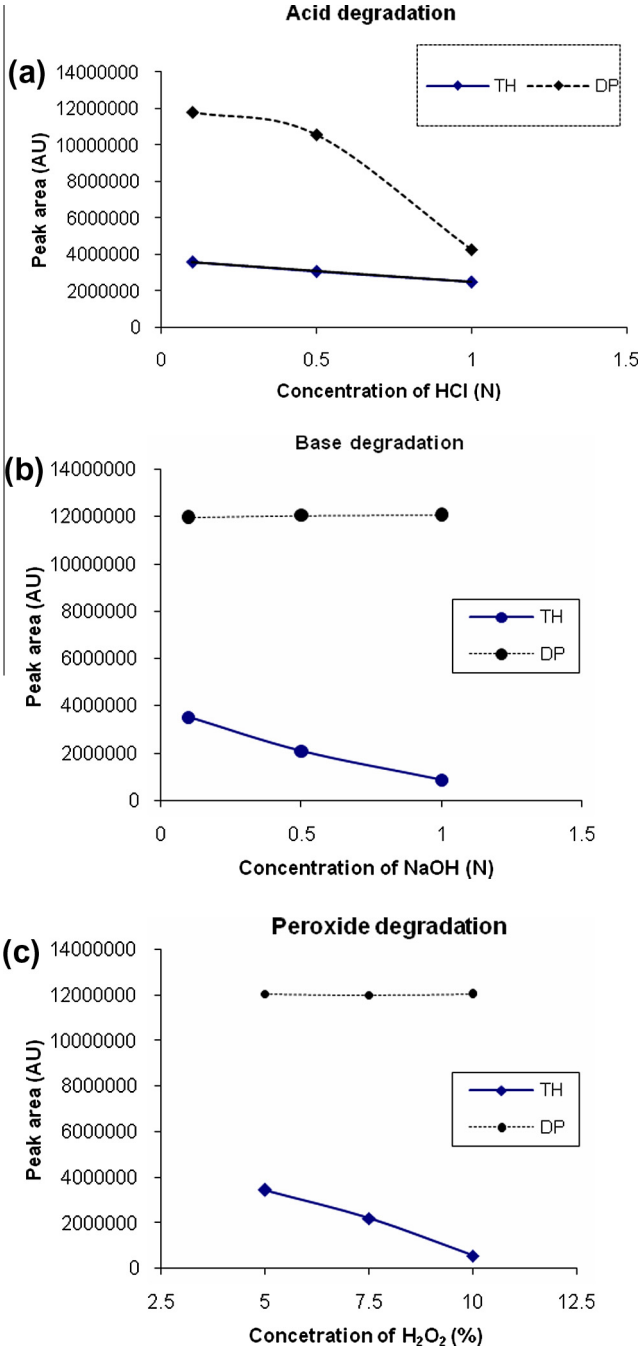

Figure 7 Degradation trends of TH and DP in acidic (a), basic (b) and peroxide (c) stressed condition.

Table 5 Results of recovery analysis of TH and DP.

Compound	Wt. spiked (mg)		Wt. recovered (mg)		Recovery (%)		RSD (%)	
	AM	DM	AM	DM	AM	DM	AM	DM
TH	39.4	4.40	39.5	4.34	100.25	98.64	0.29	0.97
	78.9	8.80	79.1	8.75	100.25	99.43	0.24	0.34
	118.3	13.19	118.5	13.09	100.17	99.24	0.04	0.50
DP	25.15	27.67	25.10	27.79	99.80	100.43	1.31	0.95
	50.32	55.39	50.00	54.97	99.36	99.24	0.31	0.76
	75.49	83.10	74.98	84.33	99.32	101.48	0.99	0.30

RSD: Relative standard deviation; Wt: weight, AM: assay method; DM: dissolution method.

Table 6 Linearity study parameters.

Parameter	Assay		Dissolution	
	TH	DP	TH	DP
Linearity range (μg/mL)	40.48–121.43	24.91–74.72	0.44–13.19	2.75–82.50
Slope	45,089	24,272	44,933	26,144
Intercept	−313.860	−141.555	−1066.831	2354.605
Correlation coefficient (r)	0.999	1.000	1.000	0.998

eluting peak, are reliable for the quantification (FDA, 1994). All stressed samples peak of TH and DP meet this specification. Major degradation impurity formed by both drug in hydrolytic stressed condition at 1 N HCl and NaOH and in oxidative condition at 10% H_2O_2, visibly confirmed in Figs. 4–6.

Dissolution method specificity was examined by analyzing a solution of a placebo containing the excipients mixture for the TH and DP tablet dissolved in the dissolution medium. Absence of interference was demonstrated.

3.3.2.1. Degradation kinetics of drugs in stressed condition. Sample solutions were treated with HCl, NaOH of different normality and H_2O_2 at different concentration. The chromatogram obtained revealed that the peak area for TH was degraded in all stressed conditions while DP peak area degraded only in acidic condition. The percentage degradation of each compound was calculated with respect to the controlled sample. The plot of respective component % degradation against concentration of HCl, NaOH and percentage of H_2O_2 indicated an apparent first order degradation of TH in all conditions and DP only in HCl conditions (Fig. 7). In thermal and photolytic conditions, the solution was stable.

3.3.3. Accuracy

Accuracy of the assay method as well as dissolution method was calculated by recovery studies at three concentrations of 50%, 100%, and 150% levels by standard addition method (Table 5). The mean percentage recoveries obtained for TH were 100.25% for assay and 99.43% for dissolution, while 99.81%, 99.29% for DP assay and dissolution, respectively.

3.3.4. Linearity and range

The linearity of the HPLC detector response for TH and DF was determined for their assay method and dissolution method by analyzing series of different concentrations of each compound.

The plots of area under the curve (AUC) of the peak responses of the analytes against their corresponding concentrations, they exhibit linear responses with $r > 0.999$ for both methods. All the validation parameter such as a correlation coefficient, concentration ranges are summarized in Table 6.

3.3.5. Robustness

In order to demonstrate the robustness of the assay method, system suitability parameters were evaluated in the study as shown in Table 7 and include pH, flow rate, column temperature. The parameters and criteria used to define robustness were retention time (>4.5 and $\leqslant 6.5$ min for TH and >10.8 and $\leqslant 12.8$ min for DP), peak symmetry ($\leqslant 2.0$) and efficiency (>2000).

The most important factors that affect peak asymmetry are % of organic, flow rate and temperature. The method, however, is robust with respect to peak asymmetry; this value

Table 7 Factors of robustness study.

	Low	Nominal	High
For HPLC method			
pH	2.3	2.5	2.7
Flow rate (mL/min)	0.8	1.0	1.2
Temperature (°C)	20	25	30
For dissolution test			
Paddle	Teflon coated (TC)	Teflon coated (TC)	Solid teflon (ST)
Sampling device	A	B	B
Degassing procedure	Overnight house vacuum (HV)	15 min degas (15 min)	Overnight open and stirring (OS)

was consistent <1.2 across all value of these parameters. Method suitability, therefore, will not be adversely affected with respect to peak asymmetry.

Flow rate is the parameter that significantly affects peak efficiency. The method, however, is robust with respect to peak efficiency; this value was consistent >4000 across all value of these parameters. Method suitability, therefore, will not be adversely affected with respect to peak efficiency. The mobile pH, in the study 1.5 and 3.5 has negligible affect on suitability criteria.

To evaluate robustness of the dissolution test, the variable selected mention in Table 7 includes paddle type, sampling device and degassing procedure. The variables were chosen as they were deemed the most significant factors that can potentially affect the rate of dissolution. The degassing procedure parameter involves three conditions. The media was either left spinning in an open flask overnight, left vacuum degassing overnight or vacuum degassing for 15 min as per the method. The first two options chosen to maximize and minimize, respectively, the amount of dissolved oxygen in the media. The all variable not significantly affect on % drug release.

4. Conclusion

A stability indicating RP-HPLC method has been reported for simultaneous estimation and in vitro dissolution studies of thiocolchicoside (TH) and diclofenac potassium (DP) in tablet dosage form. The proposed method gives good resolution of TH, DP and degradant form in degradation study. The degradation kinetics studies of drugs was also carried out and TH degrade with first order in acid, base and peroxide stressed condition, while first order degradation of DP only in acidic condition.

The proposed method successfully applied for studying in vitro dissolution of TH and DP from tablet dosage form. The discriminating dissolution medium was selected by studying quantitative solubility both drugs and confirmation of sink condition in each media as per USP dissolution guidelines. The validation of proposed method was done as per ICH guidelines and proved that method to be simple, precise, reliable and robust.

References

Agatonovic-kustrin, S., Zivanovic, Lj., Zecevic, M., Radulovic, D., 1997. Spectrometric study of diclofenac–Fe (III) complex. J. Pharm. Biomed. Anal. 16, 147–153.

Anchisi, C., Baroli, B.M., Fadda, A.M., Manconi, M., Sinico, C., Valenti, D., 1998. Dissolution properties of Flurazepam capsules. Farmaco 53, 662–666.

Botello, J.C., Caballero, G., 1995. Spectrometric determination of diclofenac sodium with methylene blue. Talanta 42, 105–108.

British Pharmacopoeia, 2004, vol. I, International ed. HMSO Publication, London, p. 469.

El-Ragehy, N.A., Ellaithy, M.M., El-Ghobashy, M.A., 2003. Determination of thiocolchicoside in its binary mixtures (thiocolchicoside-glafenine and thiocolchicoside-floctafenine) by TLC-densitometry. II Farmaco 58, 463–468.

FDA, 1994. Reviewer Guidance: Validation of Chromatographic Methods, Center for Drug Evaluation Research (CDER), Washington, USA.

ICH, 2005. International Conference on Harmonization: Draft Revised Guidance on Q1A (R) Stability Resting of New Drug Substances and Products. Federal Register 65 (2000) 21446.

ICH, 2005. International Conference on Harmonization (ICH) Q2 (R1): Validation of Analytical Procedures—Test and Methodology. Geneva, Switzerland, 2005.

Indian Pharmacopoeia, 2010, vol. III, 6th ed. Government of India, The Controller of Publications, New Delhi, p. 2213.

Janbroers, M., 1987. Review of the toxicology, pharmacodynamics and pharmacokinetics of thiocolchicoside, a GABA-agonist muscle relaxant with antinflammatory and analgesic action. Acta Ther. 13, 221–225.

Jashnani, R.N., Byron, P.R., Dalby, R.N., 1993. Validation of an improved wood's rotating disk dissolution apparatus. J. Pharm. Sci. 82, 670–671.

Kaphalia, L., Kaphalia, B.S., Kumar, S., Kanza, M.F., Treinen-Moslen, M., 2006. Efficient high performance liquid chromatograph/ultraviolet method for determination of diclofenac and 4′-hydroxydiclofenac in rat serum. J. Chromatogr. B 830, 231–237.

Klimes, J., Sochor, J., Dolezal, P., Korner, J., 2001. HPLC evaluation of diclofenac in transdermal therapeutic preparations. Int. J. Pharm. 217, 153–160.

Lee, H.S., Jeong, C.K., Choi, S.J., Kim, S.B., Lee, M.H., Ko, G.I., Sohn, D.H., 2000. Simultaneous determination of aceclofenac and diclofenac in human plasma by narrowbore HPLC using column-switching. J. Pharma. Biomed. Anal. 23, 775–781.

Lu, Q., Copper, C.L., Collins, G.E., 2006. Ultraviolet absorbance detection of colchicine and related alkaloids on a capillary electrophoresis microchip. Anal. Chim. Acta 572, 205–211.

Matin, A., Farajzadeh, M.A., Jouyban, A., 2005. A simple spectrophotometric method for determination of sodium diclofenac in pharmaceutical formulations. Farmaco 60, 855–858.

The Merck Index, 1998. The Merck Index, 12th ed., Merck and Co. Inc., 1998.

Morrison, A.B., Campbell, J.A., 1965. Tablet disintegration and physiological availability of drugs. J. Pharm. Sci. 54, 1–8.

Ondra, P., valka, I., Vicar, J., Sutlupinar, N., Simanek, V., 1995. Chromatographic determination of constituents of the genus Colchicum (Liliaceae). J. Chromatogr. A 704, 351.

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

Rowe, J.S., Carless, J.E., 1981. Comparison at the in vitro dissolution behavior of various indomethacin formulations with their in vivo bioavailability. J. Pharm. Pharmacol. 33, 561–565.

Sandouk, P., Chappey, O., D'Yvair, M.B., Scerrmann, J.M., 1995. Pharmacokinetics of thiocolchicoside in humans using a specific radioimmunoassay. Ther. Drug Monit. 17, 544–548.

Satiropoulus, B., Deutsch, T., Plakogiannis, F.M., 1981. Comparative bioavailability of three commercial acetaminophen tablets. J. Pharm. Sci. 70, 422–425.

Sparidans, R.W., Lagas, J.S., Schinkel, A.H., Schellens, J.H.M., Beijnen, J.H., 2008. Liquid chromatography-tandem mass spectrometric assay for diclofenac and three primary metabolites in mouse plasma. J. Chromatogr. B 872, 77–82.

Sutherland, F.C.W., Smit, M.J., Herbst, L., Els, J., Hundt, H.K.L., Swart, K.J., Hundt, A.F., 2002. Highly specific and sensitive liquid chromatography–tandem mass spectrometry method for the determination of 3-desmethylthiocolchicine in human plasma as analyte for the assessment of bioequivalence after oral administration of thiocolchicoside. J. Chromatogr. A 949, 71–77.

Swadesh, J., 1997. HPLC Practical and Industrial Application. CRS Press, New York.

The United State pharmacopoeia, 2005, 29th ed. United States Pharmacopoeial Convention, Inc., Rockville, MD 206, p. 683.

Tracqui, A., Kintz, P., Ludes, B., Rouge, C., Douibi, H., Mangin, P., 1996. High-performance liquid chromatography coupled to ion spray mass spectrometry for the determination of colchicine at ppb levels in human biofluids. J. Chromatogr. B 675, 235.

Vora, A., Damle, M., Bhat, L., Godge, R., 2007. Simultaneous determination of diclofenac sodium and rabeprazole sodium in bulk and pharmaceutical dosage form by LC. Chromatographia 66, 941–943.

Young, M., Gorenstein, M.V., 1994. Tryptic mapping by reversed-phase HPLC with photodiode-array detection incorporating the spectral-contrast technique. LC GC 12, 832–838.