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Application of RP-HPLC Method for Simultaneous Estimation of Thiocolchicoside and Diclofenac in Commercially Available Capsules

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ABSTRACT

A simple, economic and precise RP-HPLC method has been developed and validated for simultaneous estimation of thiocolchicoside (THC) and diclofenac (DCF) both in bulk drug and in capsule formulation. Reversed-phase chromatography was performed on a C_{18} Phenomenex-Gemini column with mobile phase acetonitrile: water (70:30 % v/v, adjusted at pH 3.0) at a flow rate of 1.0 ml/min. Detection was performed at 258 nm and sharp peaks were obtained for THC and DCF at a retention time of 1.537 min and 4.010 min respectively. The method was validated for accuracy, precision, detection and quantification limits, and system suitability in accordance with ICH guidelines. Linear regression analysis data for the calibration plot showed there was a linear relationship between response and concentration for THC in the range 4 - 24 µg/ml with the correlation coefficient 0.9998 and the linear regression equation y = 20.64x + 26.08. Linearity was observed in the range of 25 - 150 µg/ml with the correlation coefficient 0.9998 and the linear regression equation y = 11.13x + 65.27 for DCF. The detection (LOD) and quantification (LOQ) limits for THC were found to be 1.052 and 3.187 µg/ml and for DCF it was found to be 1.475 and 4.470 µg/ml. Statistical analysis proved that the method was precise, reproducible and accurate for simultaneous estimation of THC and DCF. The wide linearity range, sensitivity, accuracy, short retention time, and simple mobile phase imply that the method is suitable for routine quantification of THC and DCF with high precision and accuracy.

Keywords: Thiocolchicoside, Diclofenac, Simultaneous estimation, Reverse Phase-HPLC

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INTRODUCTION

Chemically, Thiocolchicoside (THC) is 2-Demethoxy-2-glucosidoxythiocolchicine. THC is a semi synthetic derivative of naturally occurring compound colchicoside from the seeds of various species of *Colchicum autumnale* belonging to family *Liliaceae*. (Autumn crocus, meadow saffron, *Gloriosa superba*).[1] It is a muscle relaxant with anti-inflammatory and analgesic effects.[2] Diclofenac (DCF) is monosodium salt of 2-[(2, 6-dichlorophenyl) amino] benzene acetic acid or 2-(2, 6-dichloranilino) phenyl acetic acid.[3] It is benzene acetic acid derivative and belong to non-steroidal anti-inflammatory drug (NSAID) taken to reduce inflammation and as an analgesic reducing pain in conditions such as arthritis or acute injury.[4] Literature survey reveals that assay of DCF in bulk and dosage form is official in Indian Pharmacopoeia 2007, British Pharmacopoeia 2008 and United state Pharmacopoeia NF-27.[5-7] Several analytical methods reported for estimation of THC include spectrophotometry[8], RP-HPLC[9], LC-MS[10] and capillary electrophoresis[11] and that for DCF are spectrophotometry[12-14] HPLC[15-18], colorimetric assay[19], HPTLC[20-21] and supercritical fluid chromatography[22-24]. The analytical methods reported for estimation of THC in combination with other drugs are spectrophotometry[25] and LC[26]. For estimation of DCF with other drugs spectrophotometry[27], atomic absorption spectrophotometric[14, 27-28], RP-HPLC[29-32] are reported methods. Reverse Phase-HPLC (RP-HPLC) method for simultaneous estimation of THC and DCF is not reported yet. Hence, the present paper describes simple, accurate, specific and precise method for simultaneous estimation of THC and DCF in their combined dosage form. The proposed method is optimized and validated as per the ICH guidelines[33-35]. In the present work, a successful attempt has been made to estimate both these drugs simultaneously using RP-HPLC method.

MATERIALS AND METHODS

Apparatus

A Shimadzu's HPLC (LC-10AT) with UV-Visible Detector (SPD-10A) and 7725 Rheodyne Injector (Fixed Capacity Loop of 20 µl) was used. Hamilton 25 µl syringe was used for injection. Separation was performed on C_{18} Phenomenex-Gemini column (150 mm x 4.6 mm i.d., 5 µm particle size) maintained at 26 ± 2°C temperature.

Reagents and chemicals

Pure drug samples of THC (Potency 99.8%) and DCF (Potency 99.6%) were obtained from reputed pharmaceutical company. HPLC Grade Acetonitrile, Methanol and Analytical Grade

Ortho Phosphoric acid were procured from S. D. Fine Chemicals Ltd., Mumbai, India. Distilled water was used as solvent. Calibrated glass wares were used throughout the work. All other chemicals and reagents were of AR grade.

Marketed formulation

Combined capsule formulation Thioact D8 (Brand A) and Mobiwok – plus (Brand B) were procured from local market. Each capsule contains 8 mg Thiocolchicoside and 50 mg Diclofenac.

Preparation of standard stock solution

Accurately weighed quantity of THC (100 mg) and DCF (100 mg) was transferred to two separate 100 ml volumetric flasks, dissolved in distilled water and diluted to the mark with same (stock solutions: 1000 µg/ml of THC and 1000 µg/ml of DCF).

Preparation of working standard solution

100 µg/ml of THC solution was prepared by diluting 10 ml of stock solution with distilled water in 100 ml volumetric flask up to the mark. Similarly 200 µg/ml of DCF solution was prepared as working standard solution.

Method development

A) Chromatographic separation

Standard or sample solution was injected in column with 25 µl micro-syringe. The chromatogram was run for appropriate minutes. Varieties of mobile phases were investigated in the development of an HPLC method suitable for simultaneous estimation of THC and DCF in the bulk drug and in capsules. It consisted of different ratios of acetonitrile, methanol and water at pH range 3.0- 6.75. Finally acetonitrile: water (70:30 % v/v, adjusted at pH 3.0) was selected as mobile phase. The mobile phase was filtered through nylon 0.45 µm - 47 mm membrane filter and was degassed before use. Flow rate was adjusted to 1.0 ml/min and wavelength was set to 258 nm. The chromatogram was stopped after complete separation was achieved. Data of peak like area, height, retention time, resolution etc was recorded using clarity software (v2.3.0.197).

B) Calibration curve of THC and DCF

From the aliquots of working standard solution (100µg/ml) of THC 0.4, 0.8, 1.2, 1.6, 2.0 and 2.4 ml were transferred into a series of 10 ml volumetric flasks. Into the same series of 10 ml volumetric flasks 1.25, 2.5, 3.75, 5.0, 6.25 and 7.5 ml aliquots of working standard solution (200µg/ml) of DCF were transferred and volume was adjusted to the mark with mobile phase (acetonitrile: water, 70:30, v/v) to get THC concentrations of 4, 8, 12, 14, 16, 20 and 24 µg/ml and DCF concentrations of 25, 50, 75, 100, 125 and 150 µg/ml respectively. Solutions were

injected to system with stated chromatographic conditions as described in Table 1. The graph of area of peak obtained verses respective concentration was plotted. The mean area and its standard deviation were calculated.

Method validation

A) Linearity and range

Aliquots of standard solutions of THC and DCF in the range of 4-24 µg/ml and 25-150 µg/ml respectively were prepared from working standard solution. The prepared dilutions were injected in series, peak area was calculated for each dilution, and concentration was plotted against peak area. Data was analyzed through linear regression. The mean area with its standard deviation and % relative standard deviation of peak were calculated.

B) Precision

Precision was determined as repeatability, intraday precision and interday precision in accordance with ICH recommendations. Repeatability of sample was determined using three different concentrations of THC (12, 14, and 16 µg/ml) and DCF (75, 100, and 125 µg/ml) and injected three times to system. For both intraday precision and interday precision replicate analysis of standard solutions of THC (4-24 µg/ml) and DCF (25-150µg/ml) at six different concentrations were carried out for three times a day and on three different days respectively.

C) Accuracy (as % Recovery)

Accuracy may often be expressed as percentage recovery. The accuracy was determined by standard addition method. Previously analyzed samples (THC 8 µg/ml and DCF 50 µg/ml) were spiked separately with 50, 100 and 150% extra standard of each and the mixtures were analyzed by the proposed method. The experiment was performed in triplicate. The mean % recovery from peak areas was calculated.

D) Detection (LOD) and Quantification (LOQ) Limits

LOD and LOQ were calculated from five calibration curves of both drugs. Equations used were

$LOD = 3.3\ \sigma/S$ $LOQ = 10\ \sigma/S$

Where S is mean slope of five calibration lines and σ is standard deviation of y intercept of five calibration lines.

E) System suitability

As system suitability test was an integral part of chromatographic methods development and was used to verify that the system is adequate for the analysis to be performed. System suitability tests are based on the concept that the equipment, electronics, analytical operations and samples constitute an integral system that can be evaluated as a whole. System suitability testing provides

assurance that the method will provide accurate and precise data for its intended use. The system suitability parameters like Resolution (Rs), Column efficiency (N), Symmetry factor (S) and Tailing factor (Tf) were evaluated for both drug (THC and DCF). The suitability of the chromatographic system was demonstrated by comparing the obtained parameter values, with the acceptance criteria of the CDER guidance document.

Application of the proposed method

Applicability of the proposed method was tested by analyzing the commercially available capsule formulation of two different companies. Sample solution was prepared as same that of standard working solution using the powder from 20 capsules that was equivalent to 50mg DCF (or 8mg THC). The finally prepared solutions were analysed under proposed chromatographic condition. The amount of THC and DCF present in sample solution was determined by fitting the area response into the regression equation.

RESULTS AND DISCUSSION

Method optimizations

A) Selection of elution mode

Reverse phase chromatography was chosen because of its recommended use for ionic and moderate to non-polar compounds. Reverse phase chromatography is not only simple, convenient but also better perform is in terms of efficiency, stability and reproducibility. C_{18} column was selected because it is least polar compare to C_4 and C_8 columns. C_{18} column allows eluting polar compounds more quickly compare to non-polar compounds. In addition to this, UV detector is used, which allows easy detection of the compounds in UV transparent organic solvents. A 150 x 4.6 mm column of 5µm particles packing was preferred as a starting point for method development. Isocratic mode was chosen due to simplicity in application and robustness with respect to longer column stability. This configuration provides a large number of theoretical plate values for most separation.

B) Selection of wavelength and mobile phase

The detection was carried out in the UV region and wavelength selected for detection was 258 nm in mobile phase. The mobile phase should be sufficiently transparent at the wavelength of detection i.e. minimum absorbance. Different compositions of acetonitrile, methanol and water were tried for selection of the mobile phase.

Reason to select acetonitrile was that it is best initial choice of organic solvent for the mobile phase. Acetonitrile-water mixture can be used with UV detection at low wavelength (220- 240

nm) range. Acetonitrile-water mixture also has lower viscosity, resulting in higher number of plates and lower column back pressure than methanol-water mixture. Methanol was chosen because it is next best organic solvent after acetonitrile. Water was selected because it is best universal solvent. It has more viscosity than methanol and acetonitrile. In our studies various mobile phases with different ratios were used are depicted in Table 2.

The mobile phase consists of acetonitrile: water (70:30 v/v) at pH 3.00 (adjusted with 5% O-phosphoric acid) provided optimum polarity for proper migration, separation and resolution of Thiocolchicoside and Diclofenac. The optimized chromatographic conditions are mentioned in Table 1.

Table 1: Optimized chromatographic conditions

Parameters	Description
Column	C_{18} Phenomenex-Gemini (150mm x 4.6mm, 5 µm particle size)
Column temperature	26 ± 2°C
Mobile phase	Acetonitrile: Water (70:30 % v/v, adjusted at pH 3.0)
Detection	At 258 nm using UV-Visible Detector (SPD-10A)
Injection volume	20 µl
Flow rate	1.0 ml/min
Total run time	5 minutes

Table 2: Effect of different mobile phase on peak separation and resolution

pH	Mobile Phase	Ratio	Retention time (min.)		Remark
			THC	DCF	
6.75	Methanol : Water	70:30	2.10	3.59	Poor Peak Resolution
6.50	Methanol : Water	60:40	2.11	3.01	Long uneven peak broadening
6.5	Methanol: Water (0.2% acetic acid)	45:55	2.11	3.58	Broad Peak
6.00	Acetonitrile : Methanol : Water	70:10:20	2.10	3.59	Uneven Peak broadening
6.30	Acetonitrile : Methanol : Water (0.2% acetic acid)	70:10:20	2.11	3.58	Very broad Peak
3.00	Acetonitrile : Water	70:30	1.5	4.0	Good peak, separation and Resolution

Method validation

A) Linearity and range

The linearity range for Thiocolchicoside and Diclofenac was found to be 4-24 µg/ml and 25-150 µg/ml respectively which is depicted in Figure 1 and Figure 2. Correlation co-efficient for calibration curve of Thiocolchicoside and Diclofenac was found to be 0.999 and 0.999. Chromatogram of calibration curve for standard Thiocolchicoside and Diclofenac is depicted in Figure 3.

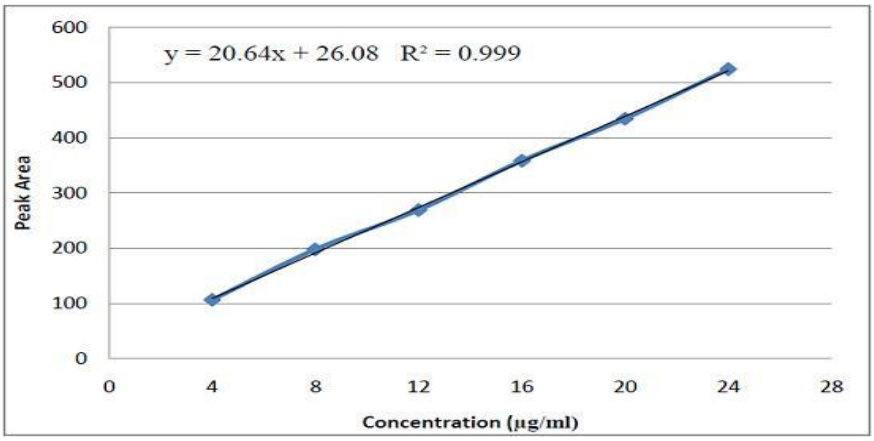

Figure1: Calibration curve for Thiocolchicoside

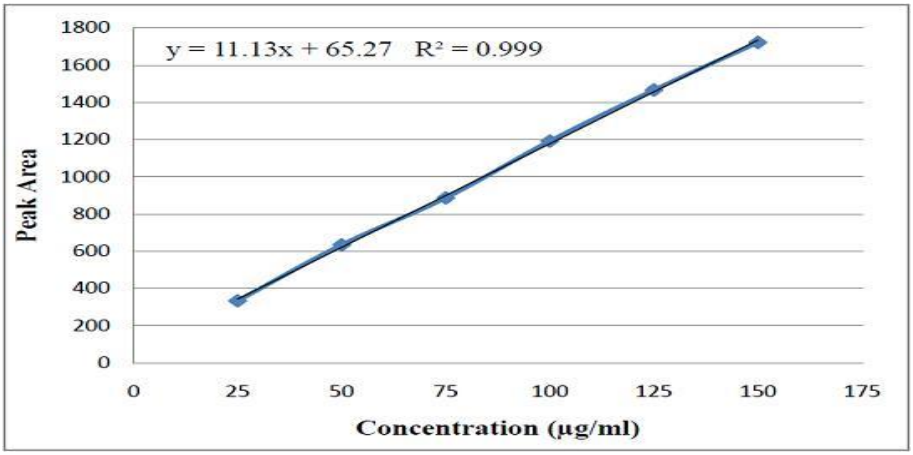

Figure 2: Calibration curve for Diclofenac

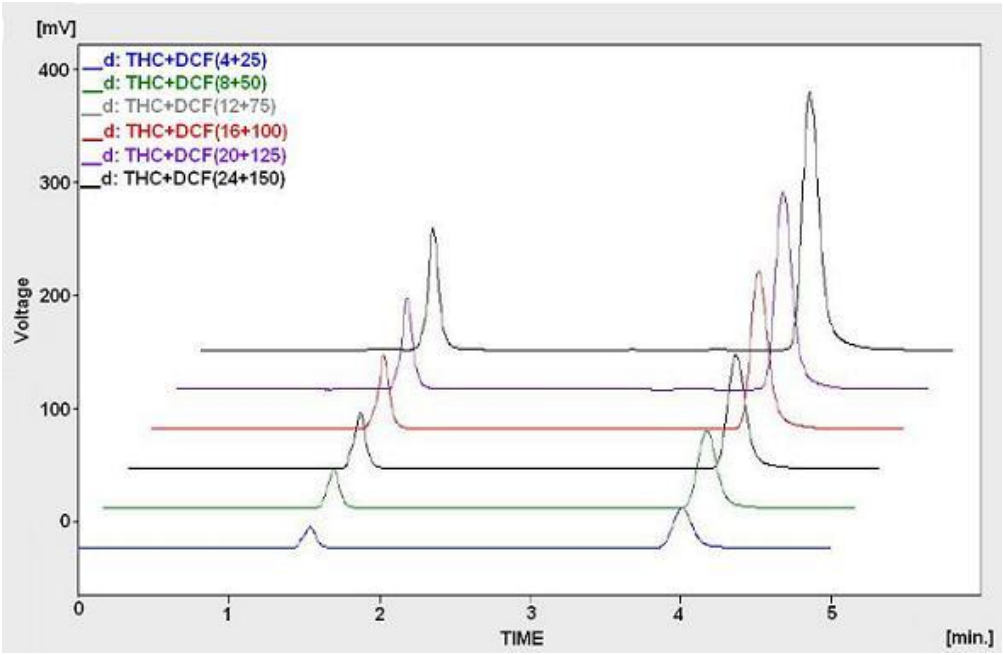

Figure 3: 3D view of chromatogram of calibration curve for Thiocolchicoside (4-24µg/ml) and Diclofenac (25-150µg/ml)

Table 3: Data for repeatability of Thiocolchicoside

Concentration (µg/ml)	Area	Mean area	S.D.	R.S.D.
12	269.174	266.7293	7.4520	1.7938
12	272.652			
12	258.362			
16	358.439	355.8943	12.396	0.0348
16	342.423			
16	366.821			
20	434.543	436.5903	9.2721	2.1237
20	428.513			
20	446.715			

S.D = Standard Deviation
R.S.D. = Relative Standard Deviation

Table 4: Data for repeatability of Diclofenac

Concentration (µg/ml)	Area	Mean area	S.D.	R.S.D.
75	886.62			
75	894.213	889.7513	3.9674	0.4459
75	888.421			
100	1191.977			
100	1186.324	1194.751	10.103	0.8456
100	1205.952			
125	1466.415			
125	1456.284	1467.041	11.083	0.7555
125	1478.425			

S.D = Standard Deviation, R.S.D. = Relative Standard Deviation

B) Precision

The results for repeatability, inter day precision and intraday precision are depicted in Table 3, Table 4, Table 5 and Table 6 which indicate that method is precise.

Table 5: Intraday precision data for Thiocolchicoside and Diclofenac

Thiocolchicoside			Diclofenac		
Conc. (µg/ml)	Area Mean ± S.D. (n=3)	R.S.D.	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	R.S.D.
4	105.343 ± 1.9636	1.8640	25	334.33 ± 5.0466	1.5095
8	198.033 ± 3.7786	1.9081	50	633.775 ± 6.8418	1.0795
12	267.084 ± 2.1394	0.8684	75	887.284 ± 6.9134	0.7792
16	358.22 ± 5.0339	1.4053	100	1194.344 ± 5.7879	0.4846
20	438.255 ± 6.4738	1.4772	125	1464.125 ± 4.6859	0.3200
24	528.018 ± 5.8394	1.1059	150	1723.281 ± 4.6072	0.2674

S.D = Standard Deviation
R.S.D. = Relative Standard Deviation

Table 6: Inter day precision data for Thiocolchicoside and Diclofenac

Thiocolchicoside			Diclofenac		
Conc. (µg/ml)	**Area Mean ± S.D. (n=3)**	**R.S.D.**	**Conc. (µg/ml)**	**Area Mean ± S.D. (n=3)**	**R.S.D.**
4	106.550 ± 1.9718	1.6816	25	335.371 ± 4.6551	1.3880
8	197.025 ± 4.4637	2.2655	50	635.210 ± 6.7151	1.0571
12	265.679 ± 5.7456	2.1626	75	887.554 ± 4.0240	0.4534
16	358.686 ± 6.4690	1.8035	100	1194.665 ± 5.3796	0.4503
20	436.746 ± 8.0095	1.8339	125	1457.728 ± 6.0660	0.4161
24	526.136 ± 7.1321	1.3556	150	1725.752 ± 6.2466	0.3620

C) Accuracy (% Recovery)

Accuracy of method was determined by standard addition method at three different concentrations of Thiocolchicoside and Diclofenac in the range of calibration. Results are shown in Table 7.

Table 7: Accuracy data for Thiocolchicoside and Diclofenac

Thiocolchicoside				Diclofenac			
Sample Conc. (µg)	**Conc. Added (µg/ml)**	**Total Conc. (µg/ml)**	**Mean % Recovery (n=3)**	**Sample Conc. (µg)**	**Conc. Added (µg/ml)**	**Total Conc. (µg/ml)**	**Mean % Recovery (n=3)**
8	4	12	99.97	50	25	75	99.96
8	8	16	100.31	50	50	100	99.62
8	12	20	99.90	50	75	125	100.39

D) Limit of detection (LOD) and quantification (LOQ)

The limit of detection for Thiocolchicoside and Diclofenac was found to be 1.052 and 1.475 µg/ml. The limit of quantification for Thiocolchicoside and Diclofenac was found to be 3.187 and 4.470 µg/ml.

E) System suitability

Resolution observed for two drug peaks THC and DCF was 4.87. For column efficiency (N) number of plates observed for Thiocolchicoside and Diclofenac were 2226 and 5324 respectively. Tailing factor obtained for Thiocolchicoside and Diclofenac were 1.071 and 1.045 respectively, which fulfills requirement of BP 2008 (0.8-1.5).

Application of the proposed method

The results for the analysis of marketed products are shown in Table 8.

Table 8: Analysis of marketed products

Brand	Label claim(mg)	% mean Recovery ± S.D.	
	THC:DCF	**THC***	**DCF***
Thioact D8	08:50	99.64 ± 0.2378	100.36 ± 0.3592
Mobiwok – plus	08:50	100.92 ± 0.2792	99.78 ± 0.1958

*Average of three experiments, S.D. = Standard deviation

CONCLUSION

The chromatographic separation was achieved on C_{18} column using acetonitrile: methanol (70:30 v/v) at pH 3.00 (adjusted with 5% O-phosphoric acid) as mobile phase at 258.0 nm. The proposed method gave accurate and precise results for determination of THC and DCF in marketed formulation (capsule) without prior separation and can easily be applied for routine analysis. Method validation has been demonstrated by variety of tests for linearity, accuracy, precision, LOD and LOQ. The proposed method was successfully applied to determination of these drugs in commercial capsules.

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REFERENCES

1. Janbroers JM. Review of the toxicology, pharmacodynamics and pharmacokinetics of thiocolchicoside, a GABA – agonist muscle relaxant with anti-infammatory and analgesic action. Acta Therapy 1987; 13: 221-250.
2. Ketenci A, Basat H, Esmaeilzadeh S. The efficacy of topical thiocolchicoside (Muscoril) in the treatment of acute cervical myofascial pain syndrome: a single-blind, randomized, prospective, phase IV clinical study. The Journal of the Turkish Society of Algology 2009; 21 (3): 95–103.
3. Salmann AR. The history of diclofenac. Am J Med 1986; 80(4B): 29-33.
4. "Data Sheet: Voltaren Rapid 25". *Information for Health Professionals*. Medsafe - New Zealand Medicines and Medical Devices Safety Authority. 2007-07-10. http://www.medsafe.govt.nz/profs/datasheet/v/voltarenrapidtab.htm. Retrieved 2009-03-17
5. Govt. of India. Ministry of Health and Family Welfare. Indian Pharmacopoeia Volume II. The Indian Pharmacopoeial Commission, Ghaziabad; 2007: 1020-1021.
6. British Pharmacopoeia Volume III, London, HMSO Publication; 2008: 2624-2625.
7. The United Pharmacopoeia XXIV and National Formulary 19, Asian Edition, U.S.Pharmacopeial convention; 2000: 546-547.
8. Gupta KR, Joshi RR. UV-Spectrophotometric determination of thiocolchicoside in capsule. Der Pharma Chemica 2010; 2(2): 384-391.

9. Rosso A, Stefano Z. Determination of alkaloids from the colchicine family by reversed-phase high-performance liquid chromatography. Journal of Chromatography A 1998; 825(1): 96-101.
10. Sutherland FCW, Smit MJ, Herbst L, Els J, Hundt HKL, Swart KJ and Hundt AF. 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. Journal of Chromatography A 2002; 949(1-2): 71-77.
11. Lui Q, Copper CL, Collins GE. Ultraviolet absorbance detection of colchicine and related alkaloids on a capillary electrophoresis microchip. Analytica Chimica Acta 2006; 572(2), 205-211.
12. Tubino M, DeSouza RL. Spectrophotometric Determination of diclofenac in pharmaceutical preparations. J Braz Chem Soc 2005; 16(5): 1068-1073.
13. Khaskheli AR, Kamran A, Sherazi STH, Afridi HI, Mahesar SA, Sirajuddin, Saeed M. Simpler and faster spectrophotometric determination of diclofenac sodium in serum and urine samples. Pak J Anal Environ Chem 2009; 10(1-2): 53-38.
14. Ghasemi J, Niazia A, Ghobadi S. Simultaneous spectrophotometric determination of benzyl alcohol and diclofenac in pharmaceutical formulations by chemometrics method. Journal of the Chinese Chemical Society 2005; 52: 1049-1054.
15. Thongchai W, Liawruangrath B, Thongpoon C, Machan T, Chiang M. High performance thin layer chromatographic method for the determination of diclofenac sodium in pharmaceutical formulations. J Pharm Sci 2006; 33(1): 123-128.
16. Hanysova L, Mokry M, Kastner P and Klimeschem J. HPLC evaluation of diclofenac in the various forms of therapeutic preparation. Chemical paper 2005; 59(2): 103-108.
17. Mutschler E, Hanses A, Spahn-Langguth H. A new rapid and sensitive high-performance liquid chromatographic assay for diclofenac in human plasma. Archiv der Pharmazie 2006; 328 (3): 257-260.
18. Vora A, Damle M, Bhat L, Godge R. Simultaneous determination of diclofenac sodium and rabeprazole sodium in bulk and pharmaceutical dosage form. Chromatographia 2007; 66: 941-943.
19. Olakunle SI, Adegoke OA, Oderinu BA, Olaniyi AA. Rapid colorimetric assay of diclofenac sodium tablets Using 4-carboxyl-2,6-dinitrobenzene diazonium ion (CDNBD). Pak J Pharm Sci 2006; 19(2): 134-141.

20. Lalaa LG, D'Melloa PM, Naikb SR. HPTLC determination of diclofenac sodium from serum. J Pharm Biomed Anal 2002; 29: 539-544.
21. Dighe VV, Sane RT, Menon SN, Tambe HN, Pillai S, Gokarn VN. Simultaneous determination of diclofenac sodium and paracetamol in a pharmaceutical preparation and in bulk drug powder by high-performance thin-layer chromatography. Journal of Planar Chromatography - Modern TLC 2006; 19(112): 443-448.
22. Sundaresan M, Patil ST, Bhoir IC, Bhagwat AM. Packed column supercritical fluid chromatographic separation and estimation of acetaminophen, diclofenac sodium and methocarbamol in pharmaceutical dosage forms. Talanta 1998; 47: 3-10.
23. Subramanian G, Musmade P, Agarwal S, Udupa N. Simultaneous RP HPLC estimation of tizanidine, diclofenac potassium and paracetamol in tablets. Indian J Pharm Sci 2004; 66(5): 694-696.
24. Jain KS, Mulgund SV, Phoujdar MS, Londhe SV, Mallade PS, Kulkarni TS, Deshpande AS. Stability Indicating HPLC method for simultaneous determination of mephenesin and diclofenac diethylamine. Indian J Pharm Sci 2009; 71(1): 35–40.
25. Wankhede SB, Zambare SS, and Chitlange SS. Estimation of thiocolchicoside and ketoprofen in pharmaceutical dosage form by spectrophotometric methods. J Pharm Res 2010; 3(4): 707-710.
26. Chitlange SS, Shinde PS, Pawbake GR, Wankhede SB. Simultaneous estimation of thiocolchicoside and aceclofenac inpharmaceutical dosage form by spectrophotometric and LC method. Der Pharmacia Lettre 2010; 2(2): 86-93.
27. Lohe RW, Suruse PB, Kale MK, Barethiya PR, Kasture AV, Lohe SW. Spectrophotometric methods for simultaneous estimation of rabeprazole and diclofenac from combined tablet dosage form. Asian J Research Chem 2008; 1(1):26-28.
28. Jawla S, Jain S. Atomic absorption spectrometric method for estimation of diclofenac sodium and mefenamic acid in pharmaceutical formulations. Int J Pharm Sci and Drug Res 2010; 2(1): 45-47.
29. Biswas A, Basu A. Simultaneous estimation of paracetamol, chlorzoxazone and diclofenac potassium in pharmaceutical formulation by a RP-HPLC method. Int J Pharm Bio Sci 2010; 1(2): 1-6.
30. Kasperek R. Determination of diclofenac sodium and papaverine hydrochloride in tablets by HPLC method. Acta Pol Pharm 2008; 65(4): 403-408.

31. Goyal A, Jain S. Simultaneous estimation of paracetamol, chlorzoxazone and diclofenac sodium in pharmaceutical formulation by a novel HPLC method. Acta Pharma Sciencia 2007; 49: 147-151.
32. Huesgen H, Hoerth P, Thielsch D. Development and validation of a method for simultaneous determination of paracetamol, diclofenac and ibuprofen using the Agilent 1120 Compact LC. Agilent Technologies, Waldbronn, Germany; 2007: 1-4.
33. Beckett AH and Stenlake JB. Practical Pharmaceutical Chemistry, 4th Edition, part-2; 2002: 293-296.
34. ICH, Q2 (R1): Validation of Analytical Procedures: Text and Methodology, Geneva, 2005.
35. Robert AN and Alfred HW. Pharmaceutical Process Validation. 3rd Edition, Marcel Dekker, New York; 2005: (129) 515-522.