RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF DIACERIN AND ACECLOFENAC IN BULK AND IT'S PHARMACEUTICAL DOSAGE

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Submitted on: 30.07.2025; **Revised on:** 07.08.2025; **Accepted on:** 12.08.2025

ABSTRACT

A sensitive high-performance liquid chromatographic (HPLC) method were developed and validated for the estimation of Aceclofenac (ACE) & Diacerein (DIA) in tablet dosage form. The chromatographic separation was achieved by RP- HPLC using a mixture of Acetonitrile: Water (50:50) pH 5.5 as the mobile phase, a C18 column & at 272 nm with flow rate 1ml/min. The linear and reproducible calibration curve over the range was 10–100 µg/ml for ACE & 5-50 µg/ml. for DIA. The retention time of ACE & DIA was found to be 8.264 & 5.913 respectively. These methods were tested and validated for various parameters according to ICH guidelines. The proposed methods were successfully applied for the determination of Aceclofenac (ACE) & Diacerein (DIA) in pharmaceutical formulations. The results demonstrated that the procedure is accurate, precise and reproducible (relative standard deviation <2%), while being simple, cheap and less time consuming. The method showed adequate precision with a relative standard deviation (RSD) smaller than 3%. The accuracy was analyzed by adding a standard drug and good recovery values were obtained for all drug concentration used. The analytical procedure is reliable and offers not only advantages in terms of speed but also meets the regulatory requirements for specificity, Linearity, LOD, LOQ, Precision, accuracy

KEYWORDS

Aceclofenac, Diacerein, High-Performance Liquid Chromatography

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Indian Research Journal of Pharmacy and Science; 43(2025); 3311-3319
Journal Home Page: https://www.irjps.in

1. INTRODUCTION

Aceclofenac is a nonsteroidal anti-inflammatory drug (NSAID) analog of diclofenac. with marked anti-inflammatory and analgesic properties used to treat osteoarthritis, rheumatoid arthritis and ankylosing spondylitis. It is reported to have a higher anti-inflammatory action or at least comparable effects than conventional NSAIDs in double-blind studies. Aceclofenac potently inhibits the cyclo-oxygenase enzyme (COX) that is involved in the synthesis of prostaglandins, which are inflammatory mediators that cause pain, swelling, inflammation, and fever. Aceclofenac belongs to BCS Class II as it possesses poor aqueous solubility [2]. It displays high permeability to penetrate into synovial joints where in patients with osteoarthritis and related conditions, the loss of articular cartilage in the area causes joint pain, tenderness, stiffness, crepitus, and local inflammation. Aceclofenac ($C_{16}H_{13}Cl_2NO_4$), chemically [(2-{2, 6-dichlorophenyl) amino} phenylacetooxyacetic acid]. Aceclofenac works by inhibiting the action of cyclooxygenase (COX) that is involved in the production of prostaglandins (PG) which is accountable for pain, swelling, inflammation and fever. [1,2,3]

Diacerein is a slow-onset anthraquinone IL-1 inhibitor used in the treatment of degenerative joint diseases like osteoarthritis. Diacerein is a prodrug which is metabolized to rhein. It is currently approved in France for the treatment of osteoarthritis although the use of diacerein is restricted due to the side effects including severe diarrhea. Diacerein is under investigation for the treatment of Insulin Resistance, Diabetes Mellitus (Type 2), and Diabetes-Related Complications. [4,5]

HPLC has gained valuable position in the field of analysis due to ease of performance, specificity, sensitivity and the analysis of sample of complex nature. This technique is commonly used for the quantitative estimation of the drugs from their formulation as well as for studying their metabolites of drugs and their estimation in their biological fluids. This method offers advantages of estimating the constituents for the multicomponent system without prior separation and even nano quantities can be estimated. This technique was employed in the present investigation for simultaneous estimation of Aceclofenac (ACE) & Diacerein (DIA) in tablet dosage form. Careful evaluation of various parameters influencing analysis is an important aspect for the development of analytical method. In order to establish RP-HPLC method the following parameters were studied.

2. EXPERIMENTAL

2.1 Materials

Aceclofenac (ACE) & Diacerein (DIA) was supplied by Mg lab India and was used without further purification. Sodium hydroxide was purchased from Molychem (Mumbai). Hydrochloric acid and hydrogen peroxide was procured from LOBA Chemie Pvt. Ltd. (Mumbai). HPLC grade methanol and acetonitrile was purchased from S. D. Fine-chem Ltd. (Mumbai) whereas HPLC grade water was purchased from Merck Ltd. All other chemicals were of analytical reagent grade. The marketed formulation ie Dycerin A (manufactured by Glenmark Pharmaceutical Ltd India.) were purchased from Local market.

2.2 Chemical structure :

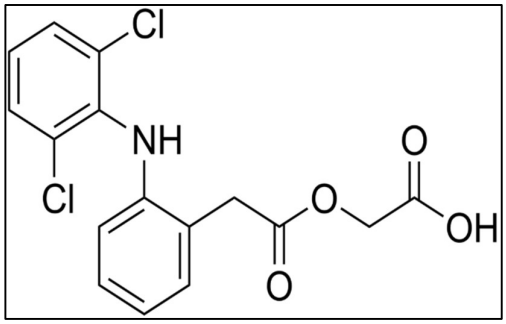

Figure 1: Structure of Aceclofenac

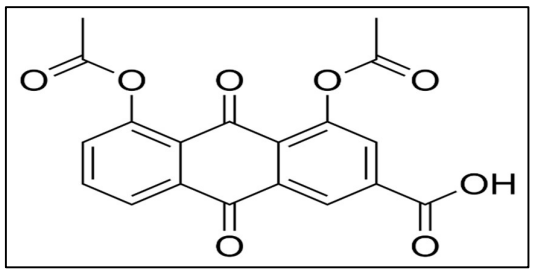

Figure 2: Structure of Diacerein

2.3 Instrumentation

The HPLC system consisting of Thermo Separation Quaternary Gradient HPLC pump Spectra System P4000 with UV detector of Spectra System, manual rheodyne injection system, the software was a Data ace software version 6.1. The chromatographic separation was performed using Intersil C_{18} (250mm × 4.6 mm i.d., 5mm particle size) Separation was achieved using a mobile phase consisting of Acetonitrile: Water (50:50) pH 5.5 as the mobile phase, a C18 column & at 272 nm with flow rate 1ml/min.The column was maintained at ambient temperature with injection volume of 20 µl. The mobile phase was filtered through 0.45 µm Chrom Tech Nylon-66 filter and degassed in ultrasonic bath prior to use. A blank chromatogram was recorded before the studies. Quantization of result was performed using peak area counts.

2.4 Standard preparation

- **ACE standard solution:** Accurately weighed 5 mg of ACE was dissolved in methanol and volume was made up to 25 ml mark (200 mg/ml). The stock standard solution was diluted further with methanol to get final concentration of about 100 mg/ml of ACE.
- **DIA standard solution:** Accurately weighed 5 mg of DIA was dissolved in methanol and volume was made up to 25 ml mark (200mg/ml). The stock standard solution was diluted further with methanol to get final concentration of about 50 mg/ml of DIA. As like above procedure, the standard solution are also prepare in mobile phase.

2.5 System suitability test:

System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standard solutions.

2.6 Application of proposed method for estimation of ACE and DIA Laboratory mixture

Three different laboratory mixtures of ACE and DIA were prepared by appropriately weighing the quantities of drug samples so as to get the concentration of 100 µg/ml of ACE and 50 µg/ml of DIA. The peak area of standard laboratory mixture and sample laboratory mixture was compared to obtaining the concentration.

2.7 Application of proposed method for estimation of ACE and DIA in formulation:

The twenty tablets were weighed, and then average weight was determined and finely grounded. The weight of the powdered tablet equivalent to 100 mg of ACE and 50 mg of DIA were transferred into a 100 mL standard volumetric flask. Added 50 mL of solvent sonicated for 10 min and diluted to 100 mL with the same solvent and then filtered through Whatmann filter paper No: 41. Equal volume (20µL) of standard and sample solution were injected separately after equilibrium of stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. The content of ACE and DIA was calculated by comparing a sample peak with that of standard.

2.8 Method Validation

- **Specificity (Selectivity)**

Specificity was measured as ability of the proposed method to obtain well separated peak for ACE & DIA without any interference from component of matrix. The values obtained were very close to that

in standard laboratory mixture indicates no interference from the component of matrix.

- **Accuracy and precision**

It was ascertained on the basis of recovery studies performed by standard addition method. The results of recovery studies and statistical data are recorded in Table No. 3 Precision of an analytical method is expressed as S.D or R.S.D of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method.

- **Ruggedness:**

The studies of ruggedness were carried out under two different conditions-

a) Days

b) Analyst.

a) Interday (Different days):

Same procedure was performed as under marketed formulation analysis on different days. The % label claim was calculated. Data obtained for day 1, day 2, and day 3 is shown in Table No. 4

b) Different analyst:

The sample solution was prepared by two different analysts and same procedure was followed as described earlier. The % label claim was calculated as done in marketed formulation estimation.

3 RESULT & DISCUSSION:

3.1 Preparation of Calibration Curve: -

The mobile phase was allowed to equilibrate with the stationary phase until steady baseline was obtained. The series of concentration from 10-100µg/ml & 5-50 µg/ml of both drug solutions were injected and peak area was recorded. The graph plotted as the concentration of the drug Vs peak area depicted in Figure 03& 04

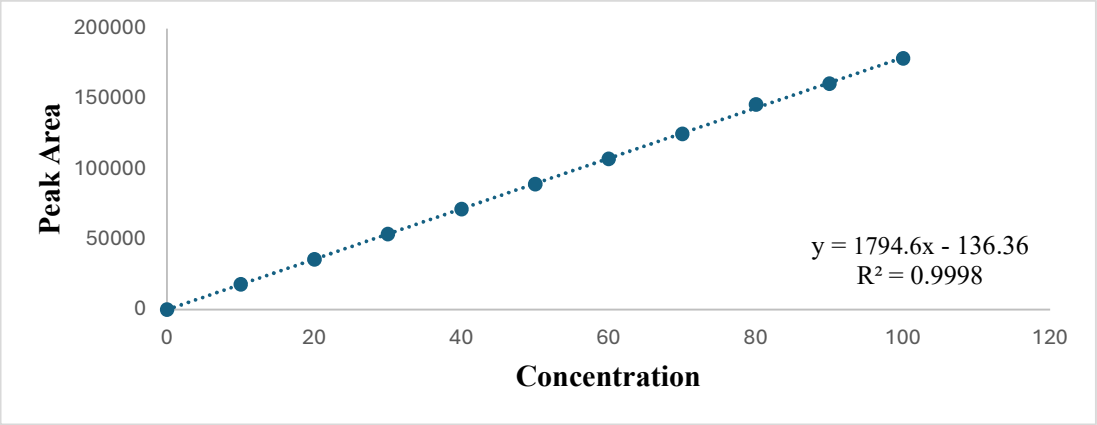

Figure 3: Standard calibration curve of ACE

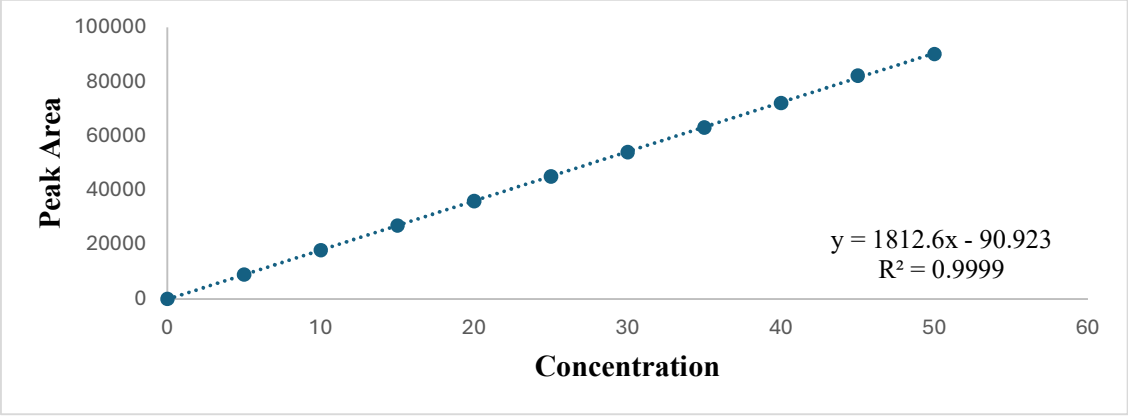

Figure 4: Standard calibration curve for DIA

3.2 System suitability test:

Filtered mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. A 20 µL std. drug solution was injected which was made in five replicates and the system suitability parameters were recorded as shown in Table 01

Table 01.: Summary of system suitability test results

Sl. no.	Parameter	ACE	DIA
1.	Peak area	178642.1	90247.82
2.	Retention time (min)	8.2646	5.913
3.	Asymmetry	1.845	2.145
4.	Efficiency	116554.3	61917.11

*Results are mean of five replicates

3.3 Application of proposed method for estimation of ACE & DIA in laboratory sample & Marketed formulation

The proposed methods applied for the estimation of the drugs in standard laboratory mixture has yielded very encouraging results and thus it was extended for the estimation of drug in marketed tablet formulation. Recovery studies were performed by adding a known amount of standard drug to preanalysed sample and contents were reanalyzed by proposed methods. The peak area of standard laboratory Sample and Test laboratory sample was compared to obtain concentration. The % Label claim of marketed formulation was calculated

Table 2: Summary of laboratory mixture and marketed formulation analysis by RP-HPLC Method

Sl. no.	Sample	Statistical data	% Estimation		% Recovery	
			ACE	DIA	ACE	DIA
1.	Standard Laboratory mixture	Mean	100.13	100.10	-	-
		S.D.	0.208	0.436	-	-
		C.V.	0.002	0.004	-	-
2.	Dycerin A	Mean	100.23	100.03	100.54	100.06
		S.D.	0.814	0.551	0.541	0.557
		C.V.	0.008	0.006	0.005	0.006

3.4 Validation

Validation is normally done to assure the reliability of the proposed method and was performed as per the ICH guidelines for the following criteria.

- **Accuracy**

Accuracy of method is ascertained by recovery studies performed at different levels of concentrations. Mean % recovery were found to be within 98-102%.

- **Precision**

The methods were found to be precise with ±S.D. of 0.001 and 0.004 for the estimation of ACE and DIA respectively. Results are shown in table 3.

Table 3: Summary of validation parameters for the proposed method

Validation Parameters	ACE	DIA
Linearity µg mL-1	10-100	5-50
Accuracy mean	100.54	100.06
Precision (% RSD)	0.001	0.004

- **Ruggedness**

The studies of ruggedness were carried out under two different conditions-

Different days and different analysts. The % label claim was calculated. Data obtained for day 1, day 2, and day 3 is shown in Table No. 4

- **Specificity:**

Specificity was measured as ability of the proposed method to obtain well separated peak for ACE and DIA without any interference from component of matrix.

Mean retention time for –

ACE – 8.264

DIA – 5.913

The values obtained were very close to that in standard laboratory mixture indicates no interference from the component of matrix.

Typical chromatograms are shown in Figure 05

Table 4: Summary of Ruggedness by RP-HPLC method

Parameter	Statistical data	% Estimation by RP-HPLC method	
		ACE	DIA
Interday	Mean	99.80	100.03
	S.D.	0.265	0.551
	C.V.	0.003	0.006
Intraday	Mean	99.80	100.03
	S.D.	0.265	0.551
	C.V.	0.003	0.006
Different analyst	Mean	100.36	100.78
	S.D.	0.795613	0.712039
	C.V.	0.007928	0.007065

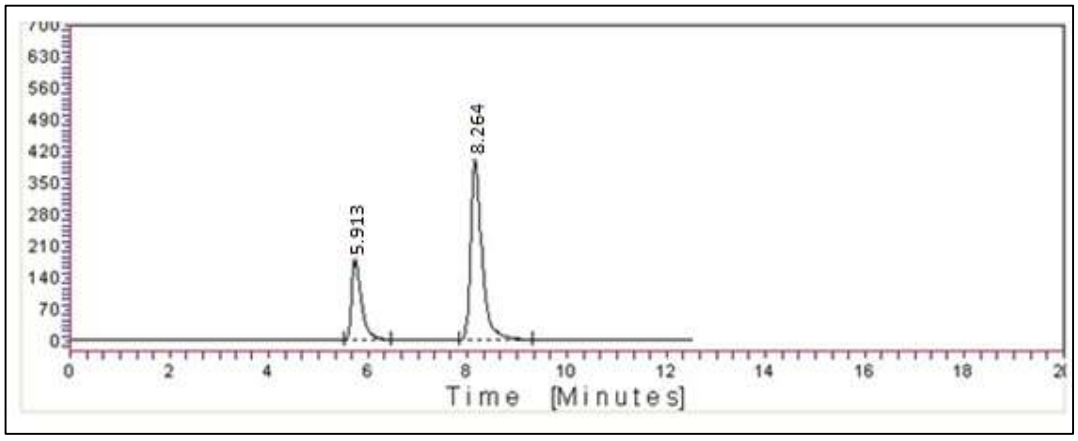

Figure 5: Chromatogram obtained by formulation of ACE & DIA

- **Linearity and range:**

According to USP tablet powder equivalent to 80, 90, 100, 110, 120 % of label claim was taken and dissolved & diluted appropriately with mobile phase to obtain a concentration in the range of 80%-120% of the test concentration. The chromatograms of the resulting solutions were recorded. The plot showing linearity and range study for ACE & DIA is shown in the Figure 06 and 07.

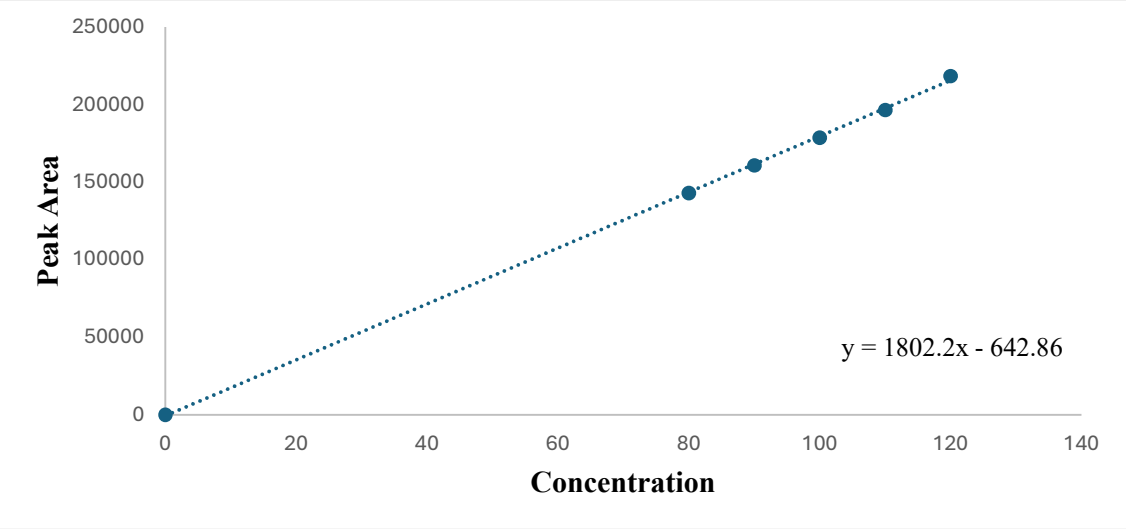

Figure 6: Plot of linearity and range study for ACE

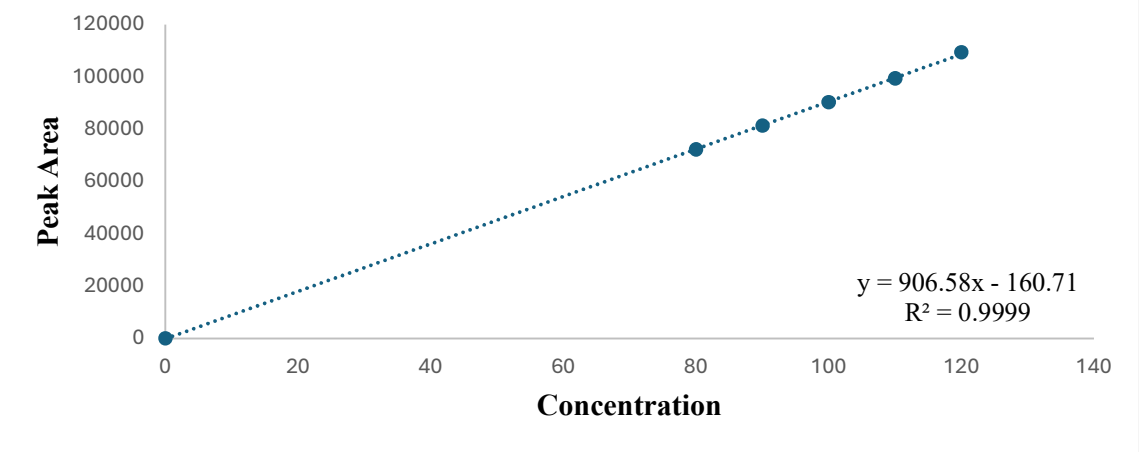

Figure 7: Plot of linearity and range study for DIA

4. CONCLUSION

A simple, specific, accurate and precise RP- HPLC method has been developed and validated as per ICH guideline for simultaneous estimation of Aceclofenac (ACE) & Diacerein (DIA) in their combined dosage form. Validation parameters like Linearity, Accuracy, Precision, Robustness, System suitability, Specificity were tested. Observation of all these parameters leads to the point that developed RP-HPLC method is linear, accurate, precise, specific and robust. It can be successfully adopted for routine quality control analysis of Aceclofenac (ACE) & Diacerein (DIA) in Combined dosage form without any interference from common excipients and impurity. This method can now transfer to utilize for routine laboratory analysis and assay of Aceclofenac (ACE) & Diacerein (DIA) in their combined dosage form

5. REFERENCES

1. Raza K, Kumar M, Kumar P, Malik R, Sharma G, Kaur M, Katare OP. Topical delivery of aceclofenac: challenges and promises of novel drug delivery systems. Biomed Res Int. 2014;2014:406731. doi:10.1155/2014/406731
2. Legrand E. Aceclofenac in the management of inflammatory pain. Expert Opin Pharmacother. 2004;5(6):1347-57.
3. Pareek A, Chandurkar N. Comparison of gastrointestinal safety and tolerability of aceclofenac with diclofenac: a multicenter, randomized, double-blind study in patients with knee osteoarthritis. Curr Med Res Opin. 2013;29(7):849-59. doi:10.1185/03007995.2013.795139
4. Tamura T, Kosaka N, Ishiwa J, Sato T, Nagase H, Ito A. Rhein, an active metabolite of diacerein, down-regulates the production of pro-matrix metalloproteinases and up-regulates tissue inhibitor of metalloproteinase-1 in cultured rabbit articular chondrocytes. Osteoarthritis Cartilage. 2001;9(3):257-63.
5. World Health Organization. Diacerein: Restricted Use BASG Product Information: Verboril (diacerein) oral capsules.
6. Sethi PD. HPLC: Quantitative analysis of pharmaceutical formulation. New Delhi: CBS Publishers and Distributors; Preface, p.101.
7. Beckett AH, Stenlake JB. Practical pharmaceutical chemistry. 4th ed. Vol. 2. New Delhi: CBS Publishers and Distributors; 1997. p.1-85.
8. Sudheer M, Rao ABN, Theja DHH, Prakash MS. Development of stability indicating RP-HPLC method for simultaneous determination of azithromycin and ambroxol HCl (SR) in the tablet formulation. Der Pharm Lett. 2012;4(3):803-10.
9. International Conference on Harmonisation (ICH). Stability Testing of New Drug Substances and Products. IFPMA, Geneva; 2003.
10. Indian Pharmacopoeia. Vol. III. Ghaziabad: Indian Pharmacopoeia Commission; 2014. p.2774.
11. Soni IJ, Panchal HJ. Development and validation of dual wavelength UV spectrophotometric method for simultaneous estimation of cilnidipine and olmesartan medoxomil in tablet dosage form. Indian J Pharm Biol Res. 2014;2:76-81.
12. Jayaprakash R, Natesan S. Optimization of stability indicating RP-HPLC method for estimation of anti-cancer drug sorafenib tosylate in pure and pharmaceutical dosage form. Int J Pharm Anal Res. 2017;6(1):141-52.
13. International Council for Harmonisation (ICH). Q1A(R2): Stability Testing of New Drug Substances and Products. Geneva; 2003. Available from: http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html
14. Heinz WJ, Kahle K, Beyersdorf A, Schirmer D, Lenker U, Keller D, et al. High-performance liquid chromatographic method for the determination of sorafenib in human serum and peritoneal fluid. Cancer Chemother Pharmacol. 2011;68:239-45.
15. Kalaichelvi R, Jayachandran E. Quantitative estimation of sorafenib tosylate in its pure form and in tablet formulation by RP-HPLC method. J Chem. 2013;2013:1-3.
16. Damle MC, Kale S. Development and validation of stability-indicating HPTLC method for estimation of sorafenib tosylate. Eur J Biomed Pharm Sci. 2017;4(6):432-8.
17. International Council for Harmonisation (ICH). Q2(R1): Validation of Analytical Procedures: Text and Methodology. Geneva; 2005. Available from: http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html
18. Dong MW. Modern HPLC for practicing scientist. Hoboken: John Wiley & Sons; 2006. p.194, 217.
19. Jeffery GH, Basset J, Mendham J, Denney RC. Vogel's textbook of quantitative analysis. 5th ed. Harlow: Longman Scientific & Technical; 1999. p.10-11.
20. Skoog DA, West DM, Holler FJ. Analytical chemistry – An introduction. 6th ed. Philadelphia: Saunders College Publishing; 1994. p.3.
21. Sharma BK. Instrumental methods of chemical analysis. 23rd ed. Meerut: Goel Publishing House; 2004. p.7-8.
22. Beckett AH, Stenlake JB. Practical pharmaceutical chemistry. 4th ed. Vol. 2. New Delhi: CBS Publishers and Distributors; 1997. p.1-85.
23. Christianah MA, Pui-Kai L. Analytical profile of drug substances. In: Florey K, editor. New York: Academic Press; p.124-41.
24. Beckett AH, Stenlake JB. Practical pharmaceutical chemistry. 4th ed. Part II. New Delhi: CBS Publishers and Distributors; p.278-82.

25. Ahuja S, Scypinski S. Handbook of modern pharmaceutical analysis. Vol. 3. San Diego: Academic Press; 2001. p.349.
26. Christen GD. Analytical chemistry. 5th ed. New York: John Wiley & Sons; 2001. p.505.
27. Illet M, Ripper J. Selectivity optimization in HPLC. In: Brown PR, Grushka E, editors. Advances in chromatography. Vol. 39. New York: Marcel Dekker; 1998. p.264-5.
28. Sethi PD. PLC – Quantitative analysis of pharmaceutical formulations. New Delhi: CBS Publishers and Distributors; 2001. p.11.
29. Sethi PD. HPLC – Quantitative analysis of pharmaceutical formulations. New Delhi: CBS Publishers and Distributors; 2000. p.1-5.

CONFLICT OF INTEREST REPORTED: NIL; **SOURCE OF FUNDING: NONE**