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High-performance liquid chromatography and pharmacokinetics of aceclofenac in rats

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Abstract

A simple and sensitive high-performance liquid chromatographic (HPLC) method was developed for quantification of aceclofenac in rat plasma. Ibuprofen was used as an internal standard (IS). The present method used protein precipitation for extraction of aceclofenac from rat plasma. Separation was carried out on reversed-phase C_{18} column (250 mm × 4.6 mm, 5 μ) and the column effluent was monitored by UV detector at 282 nm. The mobile phase used was methanol-triethylamine (pH 7.0; 0.3% v/v in Milli-Q water) (60:40%, v/v) at a flow rate of 1.0 mL min^{-1}. This method was linear over the range of 50.0–3500.0 ng mL^{-1} with regression coefficient greater than 0.99. The mean recovery of aceclofenac and IS were 84.62 ± 3.23 and 89.19 ± 1.57%, respectively and the method was found to be precise, accurate, and specific during the study. The method was successfully applied for pharmacokinetic study of aceclofenac in rats.

Keywords: High-performance liquid chromatography; Aceclofenac; Pharmacokinetics

1. Introduction

Aceclofenac ([2-(2′, 6′-dichlorophenylamino) phenyl] acetoxyacetic acid) is a phenyl acetic acid derivative that shows analgesic properties and good tolerability profile in a variety of painful conditions [1,2]. It is used in the treatment of rheumatic disorders and soft tissue injuries. Aceclofenac inhibits the cyclooxygenase enzyme and thus exerts its anti-inflammatory activity by inhibition of prostaglandin synthesis. This effect seems to be correlated to the appearance of acute protocolitis associated with nonsteroidal anti-inflammatory drug therapy [3–5].

Few analytical methods have been reported for estimation of aceclofenac in human plasma. However, no literature is available for the estimation of aceclofenac in rat plasma. The plasma of two different species differs in their vascular components. Hence, United States Food and Drug Administration (US FDA) initiated partial validation to overcome the matrix effects [6]. Plasma protein binding differs from species to species; therefore separate studies in rat plasma will provide relevant information. High-performance liquid chromatographic (HPLC) method was reported using column-switching technique for the simultaneous estimation of aceclofenac and diclofenac in human plasma [7]. This method has a limit of detection (LOD) 10.0 ng mL^{-1} with linearity range of 50.0–10,000.0 ng mL^{-1}. The method employed three different columns with special instrumentation for the column switching, which may not be performed using conventional isocratic HPLC system. The other reported method involved free zone capillary electrophoresis [8]. The LOD of above method was 30.0 ng mL^{-1} and linearity was in the range of 250.0–4000.0 ng mL^{-1}. The capillary electrophoresis technique is gaining popularity nowadays, however, this technique is not widely used when compared with HPLC. The reported methods for the determination of aceclofenac were sensitive and specific, but require sophisticated instruments. Reversed phase HPLC methods for the estimation of aceclofenac in plasma by using the conventional liquid–liquid extraction method were also reported in the literature. These methods were found to be time consuming, expensive, and complicated due to use of the liquid–liquid extraction [9–12]. The comparison of the published methods with that of the present method is given in Table 1. The reported methods used human plasma as the matrix for pharmacokinetic studies in humans. However, during the process of drug development, the adverse effect such as gastric ulceration has to be

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Table 1
Comparison between published methods of aceclofenac to that of present method

Author	Title	Method, linearity range, LOD	Extraction procedure	Disadvantage compare to present method
Lee et al. [8]	Simultaneous determination of aceclofenac and diclofenac in human plasma by narrowbore HPLC using column-switching.	50.0–10,000.0 ng mL^{-1} LOD; 10.0 ng mL^{-1} isocratic method	By column-switching method	Costly technique. The technique is not widely used in laboratories. Three columns are required to carry out the method. Method was intended for the estimation of aceclofenac in human plasma.
Zinellu et al. [9]	Separation of aceclofenac and diclofenac in human plasma by free zone capillary electrophoresis using *N*-methyl-D-glucamine as an effective electrolyte additive.	25.0–4000.0 ng mL^{-1} LOD; 30.0 ng mL^{-1}	By acid protein precipitation	Derivatization of the analyte makes the method cumbersome in extraction steps. The technique is not widely followed.
Jin et al. [10]	Determination of aceclofenac in human plasma by reversed-phase high performance liquid chromatography.	50.0 ng mL^{-1} to 40000.0 ng mL^{-1}	Liquid–liquid extraction	Longer runtime makes this tedious for the preclinical study. In this method aceclofenac is extracted by using liquid–liquid extraction, which can cause extraction recovery to be inconsistent. This method is only applicable for the estimation of drug in the human plasma.
Hinz et al. [11]	Simultaneous determination of aceclofenac and three of its metabolites in the human plasma by high-performance liquid chromatography.	10.0–10000.0 ng mL^{-1} gradient method	Liquid–liquid extraction	Longer runtime makes the cited method nonfeasible for the analysis of preclinical samples. Extraction method is time consuming and recovery is not consistent due to liquid–liquid extraction. This method is intended for the estimation of aceclofenac in human plasma.
Liu et al. [12] (abstract only available)	High performance liquid chromatographic assay for aceclofenac in plasma and its pharmacokinetics in dogs.	50.0–51200.0 ng mL^{-1} LOD; 10.0 ng mL^{-1}		The cited method is intended for the determination of aceclofenac in dogs. The matrix effects can cause the interference in the quantitation of drug in other species.

Table 2
Comparison between different types of extraction methods [13]

Solid phase extraction	Liquid–liquid extraction (LLE)	Protein precipitation
Extraction technique based on the selective partition of one or more components between two phases, one of which a solid sorbent and other is liquid.	Selective partitioning of the compound of interest into one of two immiscible (or partially miscible) phases occurs by the proper choice of extraction solvent.	Plasma proteins which are present those are precipitated by addition of a water miscible precipitating agent.
Extraction of analyte from matrix is same as like HPLC column.		
One of the disadvantages of the most popular reversed phase silica based materials is the presence of residual silanol groups.	Large solvent consumption is there for extraction of drug.	Some components of plasma, which are soluble in diluting solvent that bound to stationary phase permanently that will affect the column performance.
Costly as compared to other technique.	Extraction of analyte is by equilibrium.	Extraction is by nonequilibrium process.
Extraction is via nonequilibrium process.	Liquid–liquid extraction is time-consuming process as compared to other extraction process. LLE require an evaporation step prior to analysis to remove excess of organic solvent. When one needs to assay for several analytes, it may be difficult to find proper solvent/conditions for all analytes, requiring more than one extraction per sample. In LLE some times there chance of formation of emulsions in two immiscible phases. There is also possibility of contamination of sample due to solvent.	

studied in the animal models. The preclinical studies are the preliminary studies before the drug is tested in humans.

Although several published methods are available for pharmacokinetic evaluation of diclofenac, very few reports are available for aceclofenac in animal models. The present work was aimed at developing a sensitive HPLC for determination of aceclofenac in rat plasma. The advantages of present method include small sample volume, single step optimized extraction procedure using inexpensive chemicals, and short run time. The optimization of extraction procedure was carried out by comparing protein precipitation, solid phase extraction, and liquid–liquid extraction for recovery and interference. Protein precipitation was selected because it had obvious advantages such as shorter processing time, lesser organic solvent consumption, fewer steps, and good plasma sample clean up. The comparison of protein precipitation, solid phase extraction, and liquid–liquid extraction methods are summarized in Table 2. Acetonitrile was found to be the most suitable organic precipitant in the present study. The pharmacokinetic study of aceclofenac in rats has been taken up after validation of the proposed method.

2. Experimental

2.1. Materials and reagents

Aceclofenac (99.9%) and ibuprofen (99.6%) (Figs. 1(a) and (b)) reference standards were procured from Karnataka Antibiotics Pvt Ltd. (Bangalore, Karnataka, India) and Sun Pharmaceuticals Ltd. (Baroda, Gujarat, India), respectively. Acetonitrile (HPLC grade) and methanol (HPLC grade) were purchased from Qualigen Ltd. (Mumbai, Maharashtra, India), triethylamine (HPLC grade) and orthophosphoric acid (HPLC grade) were purchased from Merck (Mumbai, Maharashtra, India). Disodium ethylenediamine tetra-acetic acid (EDTA) vacutainers (BD Franklin Lakes, NJ, USA) were used for collecting blood samples from animals. Milli-Q water purification system supplied by Millipore (Bangalore, Karnataka, India) was used for the preparation of the aqueous mobile phase.

2.2. Equipment

A gradient high-performance liquid chromatograph from Shimadzu (Nakagyo-Ku, Kyoto, Japan) HPLC Class VP series with two LC-10AT VP pumps, SPD-10AVP variable wavelength programmable UV–vis detector, SCL-10AVP system controller and Shimadzu Class VP version 6.12 SP2 data station system was used. Hypersil BDS C_{18} (250 mm × 4.6 mm, 5 μ) (Runcom, UK) was used for the present analysis.

2.3. Preparation of the calibration standards and quality control (QC) samples

The stock solutions of aceclofenac and ibuprofen were prepared in methanol at a concentration of 1.0 mg mL^{-1} each. The working solutions of 100.0 and 40.0 μg mL^{-1} were prepared by appropriately diluting the stock solutions of aceclofenac and ibuprofen. Aceclofenac working solution was used to prepare the spiking stock solutions for construction of eight-point calibration curve (50.0–3500.0 ng mL^{-1}) and QC samples at three different levels (125.0, 1200.0, 2400.0 ng mL^{-1}). All the stock

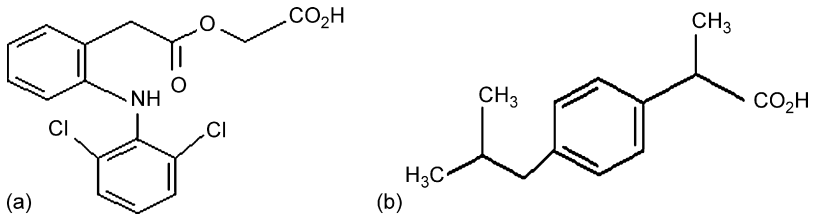

Fig. 1. Structure of aceclofenac (a) and ibuprofen (b).

solutions were refrigerated (2–8 °C) when not in use. Calibration standards and QC samples were prepared in bulk by spiking 25.0 μL of respective spiking stock solutions to 475.0 μL of control rat plasma and then aliquoted. These were stored at −70 °C until analysis.

2.4. Sample preparation for analysis

Aliquot (20.0 μL) of the plasma containing aceclofenac were pipetted into microtubes and 5.0 μL of internal standard (40.0 μg mL^{-1} ibuprofen) in acetonitrile was added and vortexed to mix. 1.0 mL of acetonitrile was added for precipitation of protein in plasma, vortexed for 1 min and centrifuged at 3500 × *g* for 5 min. Eight hundred microliters aliquot of the supernatant was transferred into a glass tube and evaporated under a stream of nitrogen in a Turbo-Vap evaporator (Zymark, Hopkinton, MA, USA) at 40 °C. The evaporated residue was reconstituted with 1.0 mL of reconstitution solution (Methanol: Milli-Q water 20:80%, v/v): 20.0 μL of the reconstituted samples were injected to the HPLC system for analysis. All the procedures were performed at room temperature.

2.5. Chromatographic conditions

The samples were chromatographed on a Hypersil BDS C_{18} (250 mm × 4.6 mm, 5 μ) column with a flow rate of 1.0 mL min^{-1}. The mobile phase used was methanol-triethylamine (60:40%, v/v). Triethylamine solution used was 0.3% v/v solution in Milli-Q water with pH being adjusted to 7.0 with 2M orthophosphoric acid solution. The injection volume was 20.0 μL. The UV–vis detector was set at 282 nm.

2.6. Validation

The method has been validated for selectivity, sensitivity, recovery, linearity, precision, accuracy, and stability.

Selectivity is the ability of the analytical method to differentiate and quantify the analyte in the presence of other expected components in the sample. This test was performed by analyzing the blank rat plasma samples from six different animals to test for interference at the retention time of drug and IS.

Sensitivity was determined by analyzing control rat plasma in replicates ($n = 5$) spiked with the analyte at the lowest level of the calibration standard that is 50.0 ng mL^{-1}. Accuracy and precision of the QC samples were calculated using the calibration curve. The model for the calibration curve of aceclofenac used the peak area ratio of aceclofenac to ibuprofen (y) and the aceclofenac concentration (C), as given in the following equation $y = \text{slope} \times C + (y \text{ intercept})$. Aceclofenac concentrations were estimated from y using the formula: $C = [y - (y \text{ intercept})]/\text{slope}$. Intraday precision was evaluated by analyzing the spiked controls six times over one day in random order, while interday precision was evaluated from the analysis of control one each in six different days. Assay precision (coefficient of variation, CV) was assessed by expressing the standard deviation of the measurements as a percentage of the average value. The accuracy was estimated for each spiked control by comparing the nominal concentration with the assayed concentration. The lower limit of quantitation (LLOQ) was the lowest nonzero concentration level, which could be accurately (relative error <20%) and reproducibly (CV <20%) determined.

The recovery of aceclofenac and IS were determined. The recovery of aceclofenac was determined for QC sample (at concentration of 125.0, 1200.0, 2400.0 ng mL^{-1}) and for IS was determined at a concentration of 200.0 ng mL^{-1}. Three replicates of each QC sample were extracted by the above mentioned sample preparation and injected into the HPLC system. The extraction recovery at each concentration was calculated using the following equation:

$$\text{Recovery} = \frac{\text{Peak area after extraction}}{\text{Peak area after direct injection}} \times 100$$

The stability of aceclofenac in solution as well as plasma matrix was evaluated. The stock solution stability was evaluated at room temperature for 6 h and at 2–8 °C for 15 days and these were compared with freshly prepared stock solution. The stability of spiked rat plasma stored at room temperature (bench-top stability) was evaluated for 6 h and compared with freshly prepared extracted samples. The freeze–thaw stability was conducted by comparing the stability samples that had been frozen and thawed three times with freshly prepared QC samples. The long term stability was conducted by analyzing low quality control (LQC), medium quality control (MQC), high quality control (HQC) samples stored at −70 °C for 30 days and compared with freshly prepared QC samples. All stability evaluations were based on back-calculations from the calibration curves.

2.7. Pharmacokinetic evaluation

Thirty young, male Albino rats (divided into five groups) (body weight 250–300 g) were obtained from the central animal house, Manipal academy of higher education, Manipal, Karnataka, India and the experimental protocol for the animal studies was approved by the Institutional Animal Ethical Com-

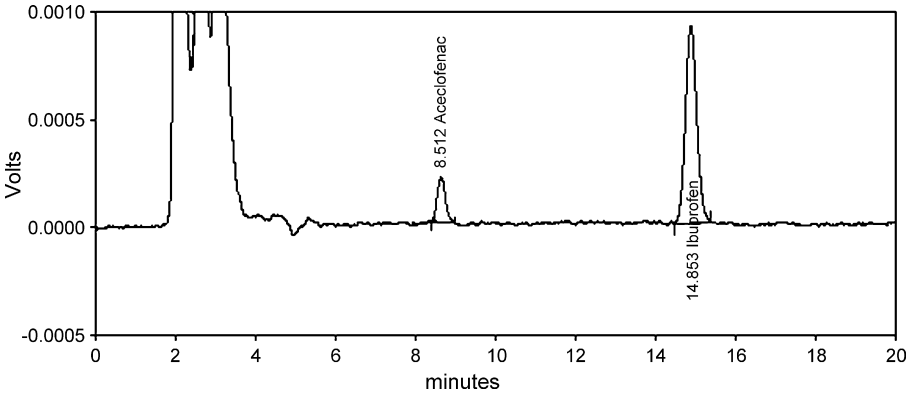

Fig. 2. Representative chromatogram of extracted plasma containing 50.0 ng mL of aceclofenac.

mittee. Animals were maintained under controlled temperature ($25 \pm 2\,^{\circ}C$) and humidity ($50 \pm 5\%$ RH) condition in polypropylene cages filled with sterile paddy husk. Animals were fasted 12 h before dosing. On the day of experiment, animals were dosed at $9\,mg\,kg^{-1}$ of aceclofenac reference standard orally. No other food was allowed until 10 h after dose administration while water intake was free. The blood samples were collected from retro-orbital plexus periodically at 0, 0.08, 0.17, 0.25, 1.0, 5.0, 8.0, and 10.0 h into the sterile disodium ethylenediamine tetra-acetic acid (EDTA) vacutainers. Plasma samples were obtained following centrifugation of blood at $1500 \times g$ for 10 min at 4 °C and kept frozen at −70 °C until analysis.

3. Result and discussion

Liquid–liquid extraction was attempted using various organic solvents like diethyl ether, chloroform, ethyl acetate, dichloromethane, petroleum ether, etc. as the aceclofenac is hydrophobic in nature. Also, combination of these solvents at different ratios had been tried with different precipitating agents. Since the drug was poorly soluble in the above said organic solvents, it resulted in poor extraction efficiency. Protein precipitating agents like acids and alkalis were used at different pH ranges to extract the drug efficiently.

Solid phase extraction can be carried out to achieve the higher extraction efficiency. The extraction efficiency is reproducible when compared to protein precipitation methods and also devoid of interferences. The sensitivity of solid phase extraction is higher but it is found to be expensive. Hence, the present study employed protein precipitation for extraction of the drug from rat plasma.

3.1. Selectivity

No interfering endogenous compound peak was observed at the retention time of analytes. Under chromatographic conditions described in Section 2.5, the retention times of aceclofenac and ibuprofen were 8.6 and 14.9 min, respectively. Representative chromatograms of LLOQ and one study sample containing aceclofenac are shown in Figs. 2 and 3, respectively.

3.2. Sensitivity (lower limit of quantitation)

The sensitivity of the experiment was carried out at LLOQ level. The average percentage deviation from the nominal concentration was less than 12.0% and the precision was within 1.4% relative standard deviation (R.S.D.).

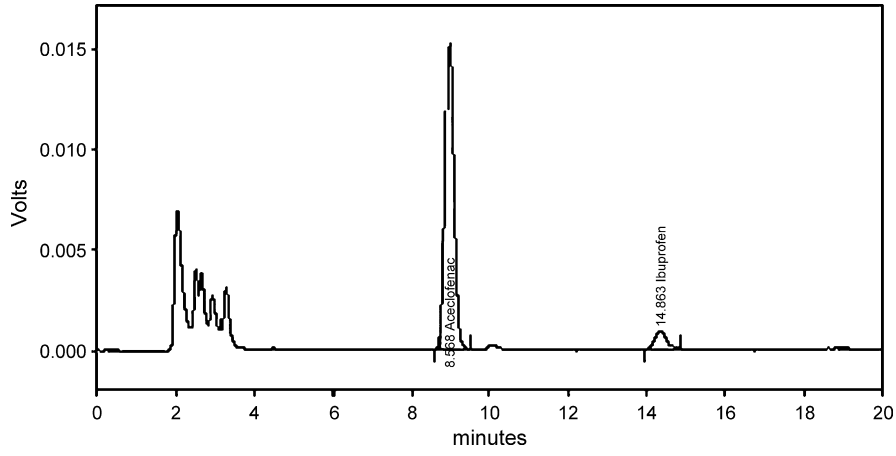

Fig. 3. Representative chromatogram of extracted aceclofenac from animal samples.

Table 3
Interrun accuracy and precision of plasma calibration standards for aceclofenac

Standard concentration (ng mL^{-1})	Average calculated concentration (ng mL^{-1})	R.S.D. (%)	R.E. (%)
50.00	45.28	5.41	9.44
100.00	91.87	5.02	8.13
250.00	255.35	2.44	−2.14
500.00	530.07	1.17	−6.01
1000.00	1051.03	1.52	−5.10
1500.00	1611.89	2.64	−7.46
2500.00	2385.93	0.54	4.56
3500.00	3490.72	1.36	0.27

3.3. Linearity

The calibration curves were linear over the range of 50.0–3500.0 ng mL^{-1}. The correlation coefficient was >0.9974. The mean (±S.D.) slope of the calibration curves for aceclofenac was 0.00524 (±0.0000548). The mean intercept of calibration curves for aceclofenac was 0.0183 (±0.00376). Calibration curve data of aceclofenac are listed in Table 3.

3.4. Precision and accuracy

Both intraday and interday accuracy and precision of the method were determined by analysis of the control rat plasma spiked with aceclofenac at LLOQ, LQC, MQC, and HQC. All QCs concentrations were calculated using the calibration curve. The accuracy and precision of the method were described as a percentage bias and the percentage R.S.D., respectively.

The interday bias was ≤4.34% and the interday precision was ≤6.5% at all QCs. The intraday deviation from the nominal concentration ≤6.66% and the intraday precision was ≤5.9% at all QCs. The result of accuracy and precision are shown in Table 4.

3.5. Recovery

The mean absolute recovery (±S.D.) of aceclofenac at LQC, MQC, and HQC was 84.62 ± 3.25%. The recovery of ibuprofen was found to be 89.19 ± 1.55%. The results of recovery studies are shown in Table 5.

Table 4
Intraday and interday accuracy and precision of aceclofenac in rat plasma

Added concentration (ng mL^{-1})	Average calculated concentration (ng mL^{-1})	R.S.D. (%)	R.E. (%)
Interday (n = 5)			
50.00	52.17 ± 3.21	4.75	−4.34
125.00	127.03 ± 2.13	6.5	−1.62
1200.00	1165.0 ± 1.56	5.53	2.91
2400.00	2382.6 ± 1.08	2.27	0.72
Intraday (n = 3)			
50.00	46.67 ± 4.56	5.9	6.66
125.00	119.97 ± 2.77	3.28	4.02
1200.00	1183.5 ± 1.33	3.96	1.37
2400.00	2341.8 ± 0.89	1.92	2.42

3.6. Stability

Analysis of the stock solution was performed at 3500.0 ng mL^{-1}. After storage for 15 days at 2–8 °C and at room temperature for 6 h, more than 98% of aceclofenac remained unchanged, based on peak areas in comparison with freshly prepared solution of aceclofenac (3500.0 ng mL^{-1}) This suggests that the aceclofenac in standard solution is stable for at least 15 days when stored at 2–8 °C and for 6 h at room temperature.

Bench top stability of aceclofenac in plasma was investigated at LQC and HQC levels. This revealed that the aceclofenac in plasma was stable for at least 6 h at room temperature with average percentage of ≥98.21 and ≥99.56%, respectively. It was confirmed that repeated freezing and thawing (three cycles) of plasma samples spiked with aceclofenac at LQC and HQC level did not affect the stability of aceclofenac as the average concentration of ≥98.96 and ≥98.57%, respectively, was obtained. Long-term stability of the aceclofenac in plasma at −70 °C was also performed after 30 days of storage at LQC, MQC, and HQC levels, which showed mean percentage concentration of ≥97.38, 98.76, and 97.87%, respectively. The results of the stability studies are shown in Table 6. The above results indicated that the aceclofenac was stable in the studied conditions.

4. Application

The plasma concentrations versus time profiles of aceclofenac after a single oral dose of 9 mg kg^{-1} in rats are shown in Fig. 4. The C_{max} and T_{max} values were obtained from plasma concentration vs. time curves, with interpolation. The area under the plasma concentration versus time curve (AUC_{0-10h}, $AUC_{0-\infty}$) and absorption constant (K_a) were estimated by using PK solutions 2.0^{TM} Noncompartmental pharmacokinetics data analysis software. The pharmacokinetic parameters in rats were estimated based on the mean concentration versus time curve. Data from PK analysis are summarized in Table 7.

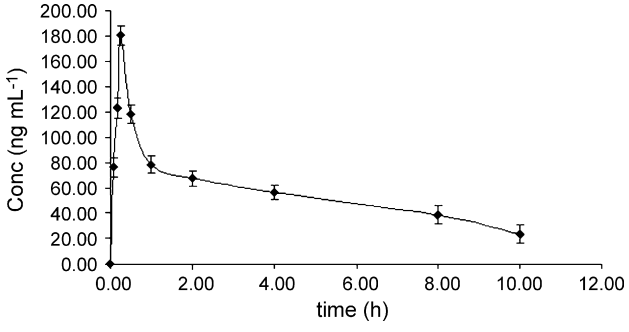

Fig. 4. Mean plasma concentrations vs. time curve for aceclofenac.

Table 5
Recovery data for aceclofenac and IS

Plasma concentration of aceclofenac (ng mL−^{1})	Recovery of extraction (%) (mean ± S.D., $n = 3$)	Plasma Concentration of IS (ng mL^{-1})	Recovery of extraction (%)
125.00	80.94 ± 5.63		87.51
1200.00	85.80 ± 3.22		90.56
2400.00	87.11 ± 1.47	200.00	89.49
Mean ± S.D.	84.62 ± 3.25		89.19 ± 1.55
CV (%)	3.84		1.74

Table 6
Stability sample results for aceclofenac ($n = 5$)

Stability	Spiked concentration (ng mL^{-1})	Average calculated comparison sample concentration (ng mL^{-1})	Average calculated stability sample concentration (ng mL^{-1})	Average percentage (%)
Bench top[a]	125.00	119.77 ± 2.53	117.63 ± 1.98	98.21
	2400.00	2374.20 ± 1.51	2363.75 ± 1.01	99.56
Freeze and thaw[b]	125.00	120.50 ± 3.21	119.24 ± 4.71	98.96
	2400.00	2394.46 ± 2.11	2360.22 ± 1.09	98.57
Long term[c]	125.00	121.15 ± 1.87	117.98 ± 2.65	97.38
	1200.00	1211.20 ± 2.55	1196.18 ± 3.08	98.76
	2400.00	2377.66 ± 1.11	2327.02 ± 2.03	97.87

[a] After 6 h at room temperature.
[b] After three freeze and thaw cycles at −70 °C.
[c] At −70 °C for 30 days.

Table 7
Pharmacokinetic parameters of aceclofenac

S.R. no.	Parameters	Values
1	$AUC_{(0-10\,h)}$ (ng h mL^{-1})	244.33 ± 3.06
2	$AUC_{(0-\infty)}$ (ng h mL^{-1})	256.00 ± 3.46
3	C_{max} (ng mL^{-1})	180.25 ± 4.50
4	T_{max} (h)	0.250 ± 0.012
5	K_a (h^{-1})	0.289 ± 0.076

5. Conclusion

A simple and sensitive method for the determination of aceclofenac, a novel analgesic antipyretic, in rat plasma by HPLC was developed and validated. The method consisted of sample preparation by protein precipitation, followed by chromatographic separation and UV detection. No interfering peaks were observed at the elution times of aceclofenac and IS. Adequate specificity, precision, and accuracy of the proposed method were demonstrated over the concentration range of 50.0–3500.0 ng mL^{-1}. The method was accurate, reproducible, specific, and applicable to the evaluation of pharmacokinetic profiles of aceclofenac in rats. The developed HPLC method was found to be suitable for the analysis of aceclofenac in rat plasma. This method was applied for the pharmacokinetic study of aceclofenac in rats.

References

[1] R.N. Brogden, L.R. Wiseman, Drugs 52 (1996) 113.
[2] M. Dooley, C.M. Spencer, C.J. Dunn, Drugs 61 (2001) 1351.
[3] J. Martel-Pelletier, J.M. Cloutier, J.P. Pelletier, Clin. Drug Invest. 14 (1997) 226.
[4] R. Yamazaki, S. Kawai, T. Matsuzaki, N. Kaneda, S. Hashimoto, T. Yokokura, R. Okamoto, T. Koshino, Y. Mizushima, Eur. J. Pharmacol. 28 (1999) 676.
[5] E. Legrand, Expert. Opin. Pharmacother. 5 (2004) 1347.
[6] Guidance for Industry: Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Rockville, MD, 2001.
[7] V. Rimbau, M.F. Fernandez, I. Guirao, A. Torralba, E. Izquierdo, Farmaco [Prat] 43 (1988) 19.
[8] H.S. Lee, C.K. Jeong, S.J. Choi, S.B. Kim, D.H. Sohn, J. Pharm. Biomed. Anal. 23 (2000) 775.
[9] A. Zinellu, C. Carru, S. Sotgia, E. Porqueddu, P. Enrico, L. Deiana, Eur. J. Pharm. Sci. 24 (2005) 375.
[10] Y. Jin, H. Chen, S. Gu, F. Zeng, Se Pu 22 (2004) 252.
[11] B. Hinz, D. Auge, T. Rau, S. Rietbrock, K. Brune, U. Werner, Biomed. Chromatogr. 17 (2003) 268.
[12] X.Q. Liu, X.J. Chen, L.H. Zhao, J.H. Peng, Yao Xue Xue Bao 32 (1997) 546.
[13] S. Mitra, Sample preparation techniques in analytical chemistry Chemical Analysis, A Series of Monographs on Analytical Chemistry and its Applications, vol. 165, Wiley-Interscience, A John Wiley and sons, Inc. Publication, 2003, p. 183.