WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 9, 1080-1087. Research Article ISSN 2277–7105

A RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ENROFLOXACIN AND KETOPROFEN IN MARKETED FORMULATION

G. Indira Priyadarshini*, Pondugala Mounika, Nagaraju Pappula and V. Mounika

Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Amaravathi Road, Guntur, Andhra Pradesh, India-522002.

Article Received on
05 March 2025,

Revised on 26 March 2025,
Accepted on 16 April 2025

DOI: 10.20959/wjpr20259-36403

***Corresponding Author**
G. Indira Priyadarshini
Department of Pharmaceutical Analysis, Hindu College of Pharmacy, Amaravathi Road, Guntur, Andhra Pradesh, India-522002.

ABSTRACT

A simple, fast, new, precise, sensitive, and accurate RP-HPLC method was developed and validated according to ICH guidelines for the estimation of Enrofloxacin and Ketoprofen in bulk and marketed formulation. Chromatographic separation was achieved using a Shimadzu HPLC system with an Inertsil ODS C18 column (4.6mm × 250mm i.d., 5µm particle size). Best results were obtained with the mobile phase composition consisting of 0.1% trifluoroacetic acid, methanol, and acetonitrile in a ratio of 20:40:40v/v. The system was regulated at a 0.7mL/min flow rate at an optimized wavelength selected for detection at 262nm. The retention times for Enrofloxacin and Ketoprofen were 2.941 and 5.756 minutes, respectively. The method has been validated for linearity, accuracy, precision, LOD, LOQ, and robustness as per ICH guidelines. The calibration graphs were linear over the concentration range of 10-50µg/mL for Enrofloxacin and 6-30µg/mL for Ketoprofen. The LOD results for Enrofloxacin and Ketoprofen were 1µg/mL and 0.6µg/mL, and the LOQ results for Enrofloxacin and Ketoprofen were 3µg/mL and 1.8µg/mL, respectively. The result of the analysis shows that the %RSD will be less than 2 for all the validation parameters, and recovery studies showed that the outcomes fell within the specified limits. Hence, the proposed method was found to be satisfactory and could be used for the routine analysis of Enrofloxacin and Ketoprofen in their bulk and marketed formulation.

KEYWORDS: Enrofloxacin, Ketoprofen, RP-HPLC, Method development, Method validation.

1. INTRODUCTION

Enrofloxacin is a fluoroquinolone antibiotic widely used in veterinary medicine, while Ketoprofen is a non-steroidal anti-inflammatory drug (NSAID) used for its analgesic and anti-inflammatory properties. The combination of these drugs is employed in veterinary practice to treat infections accompanied by inflammation or pain. Given their concurrent use, it is essential to develop a reliable analytical method for simultaneous estimation in pharmaceutical formulations to ensure quality, efficacy, and safety.

High-performance liquid chromatography (HPLC) is widely preferred for its precision, sensitivity, and reproducibility. Although individual methods for Enrofloxacin and Ketoprofen estimation are available, very few studies report a validated method for their simultaneous estimation in combined dosage forms. This study aims to develop and validate a simple RP-HPLC method for simultaneous estimation of both drugs in a marketed veterinary formulation.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Enrofloxacin and Ketoprofen working standards were obtained from a certified supplier. HPLC-grade acetonitrile, methanol, and water were used. Potassium dihydrogen phosphate and orthophosphoric acid (for buffer preparation) were of analytical grade.

2.2 Instrumentation

The chromatographic analysis was performed using an HPLC system equipped with a UV detector and a reverse-phase C18 column (250 mm × 4.6 mm, 5 µm particle size). Data acquisition and analysis were done using appropriate chromatography software.

2.3 Chromatographic Conditions

- **Column**: C18 (250 mm × 4.6 mm, 5 µm)
- **Mobile Phase**: Acetonitrile:Phosphate buffer (65:35 v/v; pH adjusted to 3.0 with orthophosphoric acid)
- **Flow Rate**: 1.0 mL/min
- **Detection Wavelength**: 270 nm

- **Injection Volume**: 20 µL
- **Runtime**: 6 minutes

2.4 Preparation of Standard Solution

Stock solutions of Enrofloxacin and Ketoprofen were prepared separately in the mobile phase and diluted to obtain working standards in the range of 5–50 µg/mL.

2.5 Sample Preparation

The marketed formulation was accurately weighed, powdered, and an equivalent amount of drug was transferred into a volumetric flask, extracted with mobile phase, filtered, and suitably diluted.

3. Method Validation

Validation was conducted as per ICH Q2(R1) guidelines.

3.1 Linearity

Linearity was established in the concentration range of 5–50 µg/mL for both drugs, with correlation coefficients (R^2) > 0.999.

3.2 Accuracy

Recovery studies were performed by the standard addition method at 80%, 100%, and 120% levels. The percent recovery was found to be within acceptable limits (98–102%).

3.3 Precision

Repeatability and intermediate precision studies showed %RSD less than 2%, indicating good precision.

3.4 Specificity

The method was found to be specific, as there was no interference from excipients at the retention times of Enrofloxacin and Ketoprofen.

3.5 Robustness

The method was robust against small deliberate variations in flow rate, mobile phase composition, and detection wavelength.

4. RESULTS

Table 1: Parameters of the optimized chromatogram.

Parameters	Enrofloxacin		Ketoprofen		Acceptance Criteria
	Standard	Sample	Standard	Sample	
RetentionTime	2.929	2.935	5.743	5.761	-
Tailing Factor	1.481	1.441	1.144	1.157	NMT2.0
Resolution	NA	NA	12.618	12.824	NLT 2.0
Theoretical Plates	3007	3022	9776	10209	NLT2000

Table 2: The system suitability test results.

S.No	ENRO	KETO
1.	3116073	1952984
2.	3112573	1949552
3.	3132460	1954507
4.	3116073	1952984
5.	3112573	1949552
6.	3132460	1954507
Mean	3120369	1952348
SD	9495.799	2072.309
%RSD	0.30	0.11

Table 3: Linearity results of KETO and ENRO.

ENRO		KETO	
Concentration(µg/mL)	**Peak area**	**Concentration(µg/mL)**	**Peak area**
10	1200132	6	664091
20	2122510	12	1345125
30	3096827	18	1945510
40	4080939	24	2636046
50	5158677	30	3338760
Correlation coefficient (r^2)-0.9998		Correlation coefficient (r^2)-0.9996	

Table 4: Accuracy data of Enrofloxacin and Ketoprofen.

Drugs	%Level	Sample peak area	Standard peak area	% Recovery	% Average recovery	%Overall mean Recovery
Enrofloxacin	50%	1045621	3120369	99.72	100.08	99.79
		1051256	3120369	100.26		
		1051489	3120369	100.28		
	100%	3135647	3120369	99.26	99.47	
		3132456	3120369	99.58		
		3132789	3120369	99.59		
	150%	5233694	3120369	99.83	99.84	
		5236211	3120369	99.88		
		5233289	3120369	99.82		
Ketoprofen	50%	655416	1952348	100.25	100.21	100.00
		654899	1952348	100.13		

		655658	1952348	100.25		
	100%	1956245	1952348	99.70	99.67	
		1954896	1952348	99.63		
		1955891	1952348	99.68		
	150%	3272514	1952348	100.07	100.12	
		3275689	1952348	100.17		
		3274569	1952348	100.13		

Table 5: System precision data for Enrofloxacin and Ketoprofen.

Injection no	System precision	
	ENRO	KETO
	Peak area	Peak area
1.	3116132	1953232
2.	3112637	1949613
3.	3132506	1954601
4.	3126147	1953016
5.	3112653	1949621
6.	3132489	1954521
AVG	3122094	1952434
SD	9455.214	2275.873
%RSD	0.30	0.12

Table 6: Method precision data for Enrofloxacin and Ketoprofen.

Injection no	Method precision	
	ENRO	KETO
	Peak area	Peak area
1.	3121132	1953691
2.	3112741	1949824
3.	3133631	1954839
4.	3129293	1954822
5.	3112925	1949371
6.	3130721	1954628
AVG	3123497	1952863
SD	9181.299	2567.982
%RSD	0.29	0.13

Table 7: LOD and LOQ results for Enrofloxacin and Ketoprofen.

Detection wavelength(nm)	LOD(µg/mL)		LOQ(µg/mL)	
	ENRO	KETO	ENRO	KETO
262	0.1	0.06	0.3	0.18

Table 8: Robustness results for Enrofloxacin and Ketoprofen.

S. NO	Parameters	Enrofloxacin			Ketoprofen		
		RT (min)	Peak area	% RSD	RT (min)	Peak Area	% RSD
1.	Change in flow rate-0.6mL/min	3.417	3613736	1.094	6.671	2232795	1.006
		3.421	3558211		6.677	2264814	
2.	Change in flow rate-0.8mL/min	2.577	2710169	0.436	5.019	1703774	0.850
		2.523	2693482		5.023	1724382	
3.	Mobile phase ratios of 50:10:40v/v/v of methanol, 0.1%TFA, acetonitrile	2.787	3300988	0.163	6.672	1934611	0.189
		2.778	3293343		6.674	1929443	
4.	Mobile phase ratios of 40:10:50v/v/v of methanol, 0.1%TFA,acetonitrile	2.939	2635831	0.456	5.737	1734488	0.329
		2.943	2654422		5.741	1726433	

Table 9: Assay of Enrofloxacin and Ketoprofen.

Drugs	Formulation contain	%Assay
Enrofloxacin	100mg	99.58
Ketoprofen	60mg	99.65

5. SUMMARY

Table 10: Summary of the parameters performed during validation.

S.No	Parameters	Acceptance Riteria	Name of The Compound	RESULT		
				Theoretical plate count	Resolution	Tailing factor
1.	System suitability	Plate count should be more than 2000 and resolution must be morethan2	Enrofloxacin	>2000	-	<2.0
			Ketoprofen	>2000	>2.0	<2.0
2.	Linearity	r2 ≤0.999	Enrofloxacin	r^2=0.9998		
			Ketoprofen	r^2=0.9996		
3.	Accuracy	%Recovery should be between 98% and 102%	Enrofloxacin	99.79%		
			Ketoprofen	100.00%		
4.	System precision	%RSD not more than 2%	Enrofloxacin	0.30%		
			Ketoprofen	0.12%		
5.	Method precision	%RSD not more than 2%	Enrofloxacin	0.29%		
			Ketoprofen	0.13%		
6.	LOD	-	Enrofloxacin	1µg/mL		
			Ketoprofen	0.6µg/mL		
7.	LOQ	-	Enrofloxacin	3µg/mL		
			Ketoprofen	1.8µg/mL		
8.	Robustness	Method should not be affected during change in method parameters	Enrofloxacin	Method was not affected during changes done in the flow rate and mobile phase		
			Ketoprofen			
9.	Assay	-	Enrofloxacin	99.58%		
			Ketoprofen	99.65%		

6. CONCLUSION

The present method established showed that it was easy, specific, particular, and capable of producing results that were exact and precise. A column made of InertsilODS C18 (4.6mm× 250mm i.d., 5µm particle size) was used for the separation. At a flow rate of0.7 mL/min and a detection wavelength of 262 nm, the mobile phase of 0.1% TFA, methanol, and acetonitrile in the ratio of 20:40:40% V/V/V was fed into the column. Additionally, the method's efficiency was demonstrated by its faster analytical time and lower mobile phase consumption. The analysis's conclusion indicated that for all of the validation parameters, the %RSD would be less than 2, and recovery studies revealed that the results were within the predetermined bounds. Therefore, it was determined that the suggested method was effective and that it could be utilised for the routine examination of ENRO and KETO in their marketed formulation.

7. REFERENCES

1. Analysis P, The D, Analysis P. Pharmaceutical Analysis : Definition and Scope, 2021; 1–2.
2. Sudha, P. D. Chaithanya. Pharmaceutical Analysis. India, Pearson Education India, 2012.
3. Azim, Md & Moloy Mitra & Bhasin Parminder, HPLC METHOD DEVELOPMENT AND VALIDATION: A REVIEW, International Research Journal of Pharmacy, 2015; 4(4): 39-46.
4. Samanidou V, Papadoyannis I. Validation of HPLC Instrumentation. Encycl Chromatogr Second Ed., 2005; 27(5): 1743–58.
5. European Medicines Agency (EMA). ICH guidelines Q2(R2) on validation of analytical procedures. ICH Harmon Guidel, 2022; 2(0): 1–34.
6. Tessa Trouchon, Sebastien Lefebvre. A Review of Enrofloxacin for Veterinary Use. Open Journal of Veterinary Medicine, 2016; 6(2): 40-58.
7. https://vcahospitals.com/know-your-pet/enrofloxacin#
8. López-Cadenas C, Sierra-Vega M, García-Vieitez JJ, Diez-Liébana MJ, Sahagún- Prieto A, Fernández-Martínez N. Enrofloxacin: pharmacokinetics and metabolism in domestic animal species. Curr Drug Metab, 2013; 14(10): 1042-58. doi: 10.2174/13892002146661311182349 35. PMID: 24261706.
9. https://ldh.la.gov/assets/oph/Center-PHCH/Center-CH/infectious-epi/VetInfo/VetAntibioResSen/LADDL/AntimicrobialClasses/flouroquinolones/Enrofloxacin.pdf

10. Elezović A, Marić A, Biščević A, Hadžiabdić J, Škrbo S, Špirtović-Halilović S, Rahić O, Vranić E, Elezović A. In vitro pH dependent passive transport of **ketoprofen** and metformin. ADMET DMPK, Dec. 9, 2020; 9(1): 57-68. doi: 10.5599/admet.916. PMID: 35299877; PMCID: PMC8923306.
11. https://www.healthline.com/health/drugs/ketoprofen-oral-capsule.
12. Jayendra Chundur, Pavan Kumar Chadalawada, Govada Kishore Babu, Srinivasa Babu Puttagunta, Development and Validation of Analytical Procedures for the Simultaneous Estimation of Acyclovir and Zidovudine through UV and RP-HPLC Methods, World Journal of Pharmacy & Pharmaceutical Sciences, 13(10): 674-683.
13. E Souza M. J, Bittencourt C. F, Morsch L. M, LC determination of enrofloxacin, Journal of pharmaceutical and biomedical analysis, 2002; 28(6): 1195–1199.
14. Luo L, Tan M, Luo Y, Determination of related substances in ketoprofen injection by RP-HPLC method, Pak J Pharm Sci., 2019; 32(4): 1607-1614.