ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 1, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

SEPARATION AND DETERMINATION OF THE R-ISOMER OF KETOPROFEN IN A BULK DRUG SUBSTANCE BY NORMAL PHASE LIQUID CHROMATOGRAPHY

APPASAHEB B LAWANDE*

Department of Chemistry, Shri Jagdish Prasad Jhabarmal Tibrewala University, Jhunjhunu - 333 001, Rajasthan, India.
Email ab.lawande@rediffmail.com

Received: 23 September 2016, Revised and Accepted: 15 October 2016

ABSTRACT

Objective: The objective is defined to develop and validate simple, rapid, precise, and accurate for separation and determination of R-isomer impurity of ketoprofen bulk drug material by the normal phase high-performance liquid chromatographic method as per the International Conference of Harmonization Guideline (ICH) guidelines.

Methods: The R-isomer and S-isomer were baseline resolved on a Chiralcel OJ-H, 150 mm × 4.6 mm, and 5 µm stationary phase column. Mobile phase system containing n-hexane:isopropyl alcohol:glacial acetic acid (50:50:0.1 v/v.). The detector wavelength has been selected 254 nm and column oven temperature 25°C. The chromatographic resolutions between R-isomer and S-isomer were more than two. The developed method was extensively validated according to ICH guidelines.

Results: Ketoprofen linearity was found for R-isomer over the concentration range of 600-6000 ng/ml, with the linear regression (Correlation coefficient R=0.999) and proved to be robust. The limit of detection and limit of quantification of ketoprofen R-isomer were found to be 200 and 600 ng/ml. The percentage recovery of R-isomer has been ranged from 97.5 to 102.0 in bulk drug material samples of ketoprofen. The proposed method was found to be suitable and accurate for the quantitative determination of R-isomer of ketoprofen in bulk drug sample.

Conclusion: A simple, rapid, precise, and accurate normal phase liquid chromatography method has been developed and validated to determine R-isomer impurity of ketoprofen in bulk drugs material as per ICH.

Keywords: Ketoprofen, High-performance liquid chromatography, Known impurity, Normal phase, Chiralcel OJ-H, Validation.

 DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i1.15318

INTRODUCTION

Ketoprofen is a nonsteroidal anti-inflammatory drug and used to treat pain, inflammation caused by arthritis [1]. Ketoprofen bulk drug is used in the manufacturing of capsules and tablets. It works by reducing hormones that cause inflammation and pain in the body [2]. Racemic ketoprofen is chemically designated as (RS)-2-(3-Benzophenyl)-propionic acid [3]. Its molecular formula is $C_{16}H_{14}O_3$ and its molecular weight is 254.3 g/mol. The chemical structure shows in Fig. 1.

Many of the methods have been reported for qualitative and quantitative analysis of ketoprofen. In the literature, there is no method for the rapid separation of R-isomer and S-isomer of ketoprofen in bulk drugs using normal phase high-performance liquid chromatography (HPLC).

This report describes a normal phase liquid chromatography method for the rapid separation of R-isomer and S-isomer of ketoprofen. The developed HPLC method has been validated for quantitative determination of R-isomer of ketoprofen by referring the International Conference of Harmonization Guideline (ICH) [4-10].

METHODS

Material and standards

Racemic mixture of ketoprofen, R-isomer of ketoprofen, and S-isomer of ketoprofen was donated by Lavender Laboratories Private Limited, Pune, Maharashtra, India.

Chemicals and reagents

1. n-Hexane: HPLC grade purchased from Merck
2. Isopropyl alcohol: HPLC grade purchased from Merck
3. Glacial acetic acid: AR grade purchased from Merck.

Instruments, apparatus, and equipment specification

1. HPLC system: Shimadzu 2010CHT with ultraviolet (UV)-visible detector
2. Chromatographic software: LC solution
3. Analytical balance: Electronic balance type 200 Shimadzu Corporation, Japan
4. Stationary phase: Chiralcel OJ-H, 150 mm × 4.6 mm, 5 µm, column.

Analytical method

Chromatographic conditions

- Instrument: HPLC with UV detector
- Column: Chiralcel OJ-H, 150 mm × 4.6 mm, 5 µm
- Wavelength: 254 nm
- Flow rate: 1.0 mL/minute
- Injection volume: 5 µl
- Column oven temperature: 25°C
- Run time: 10.0 minutes
- Mode: Isocratic.

Mobile phase preparation

Mobile phase

n-Hexane:isopropyl alcohol:glacial acetic acid (50:50:0.1) v/v, filter it through 0.25 µ filter paper. Degas before remove dissolved gases if any, before use.

Diluent preparation

n-Hexane:isopropyl alcohol:glacial acetic acid (50:50:0.1) v/v.

Preparation of racemic mixture of ketoprofen (system suitability solution)
Weigh and transfer accurately 10 mg of racemic ketoprofen working standard to a 100 ml volumetric flask. Add 70 ml of diluent and sonicate for 1 minute to dissolve. Dilute to volume with diluent and mix.

Preparation of test solution (working solution)
Weigh and transfer accurately 20 mg of ketoprofen sample to a 50 ml volumetric flask. Add 30 ml of diluent and sonicate for 1 minute to dissolve. Dilute to volume with diluent and mix.

Procedure
1. Inject diluent blank to check for interference by system related peaks
2. Inject the system suitability solution preparation to check for resolution of R-isomer and S-isomer of ketoprofen. Determine the resolution of system suitability solution
3. Inject test solution. Record the chromatogram and calculate the percentage of R-isomer by area normalization method.

Validation of the method

Method reproducibility

The developed and validated method has been used for determination of R-isomer of ketoprofen by measuring repeatability for instrument and method precision. Method reproducibility was carried out by measuring repeatability and intermediate precision (between - days precision) for retention times and peak areas of R-isomer and S-isomer of ketoprofen.

To determine the repeatability of the method by replicate injections (n=6) of a 0.4 mg/ml solution containing R-isomer spiked in S-isomer (0.5%) of ketoprofen was carried out. The intermediate precision was performed on six successive injections at different days.

A limit of detection (LOD), defined as the lowest concentration of analyte that can be detected on above the baseline signal. The detected signal is need not required to quantified and is estimated as three times the signal to noise ratio.

Limit of quantification (LOQ), defined as the lowest concentration of analyte that can be quantified. The quantification performed with suitable precision and accuracy, the signal was estimated as 10 times the signal to noise ratio. Achieved LOD and LOQ are by injecting a series of dilute solutions of R-isomer of ketoprofen.

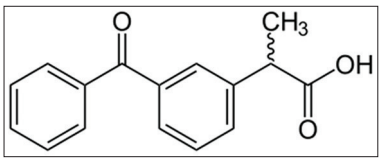

Fig. 1: Chemical structure: Ketoprofen

The precision study of the developed method for R-isomer at LOQ was checked by analyzing six test solutions of R-isomer prepared at LOQ level and calculating the relative standard deviation (RSD) in percentage for peak area response.

Linearity of R-isomer

The linearity of detector response was assessed on preparing six calibration sample solutions of R-isomer of ketoprofen by covering from 600 (LOQ) to 3000 ng/ml (600, 1200, 1800, 2400, 3000, and 6000 ng/ml), solution prepared in mobile phase from R-isomer stock solution.

The curve of regression was obtained by plotting peak area against concentration, used the least squares method. Linearity was performed for 3 consecutive days on the same stock solution in the same concentration range. The percentage RSD of the slope and Y-intercept of the calibration curve was calculated.

Quantification of R-isomer in bulk sample

The ketoprofen bulk sample, provided by Lavender Laboratories Private Limited, showed the presence of 0.50% of R-isomer. Addition of standard and recovery experiments was conducted for determination accuracy of the present method for the quantification of R-isomer of ketoprofen in bulk drug samples.

The study was carried out in triplicate at 0.25%, 0.50%, and 0.75% of the ketoprofen target analyte concentration. The recovery of R-isomer was calculated.

Robustness

The method robustness is ability to small change in parameter of analytical method by deliberately changed such as column temperature, mobile phase composition, and flow rate. To determine robustness of the method, altered experimental conditions purposely and evaluated resolution between R-isomer and S-isomer of ketoprofen on recorded chromatogram.

The flow rate of mobile phase was 1.0 ml/min. To study the effect on resolution of flow rate of isomers, it was changed from 0.8 m to 1.2 ml/min by 0.2 units. The effects of change in percent isopropyl alcohol on resolution were studied by varying from −1 to +1%, whereas the other mobile phase components were held constant.

The effect of column oven temperature on resolution was changed at 20°C and 30°C instead of 25°C, whereas the other parameters such as mobile phase components and flow rate were held constant as stated.

Solution stability and mobile phase stability
Stability of ketoprofen in solution at analyte concentration was studied by keeping the solutions in tightly capped volumetric flask at ambient

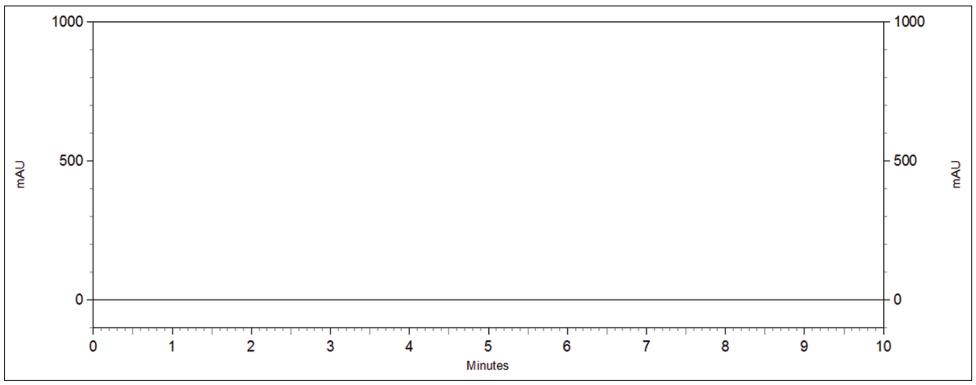

Fig. 2: Typical chromatogram of blank

temperature on a laboratory bench for 2 days. Content of R-isomer of ketoprofen was checked for every 6 hrs interval up to the study period.

Mobile phase stability was carried out by evaluating the content of R-isomer in ketoprofen sample solutions prepared freshly at 6 hrs interval for 2 days. Same mobile phase was used during the study period.

RESULT AND DISCUSSION

Method development

The target of the work is a separation and determination of R-isomer and S-isomer of ketoprofen using normal phase HPLC within a very short run time. The ketoprofen enantiomeric analysis is time-consuming using normal phase. The concentration of racemic mixture solution was 0.1 mg/ml prepared in mobile phase.

To develop a simple, rugged, precise, and suitable LC method for the separation of ketoprofen isomer, different mobile phases and stationary phases were employed. In an experiment has been attempted to separate the isomers of ketoprofen, various experiments were conducted, to select the best stationary and mobile phases that would give suitable resolution and selectivity for the two isomers from racemic mixture. There is no method in literature using mobile phase composition of n-hexane:isopropyl alcohol:glacial acetic acid (50:50:0.1) v/v using Chiralcel OJ-H, 150 mm × 4.6 mm, 5 µm.

The chromatographic separation was achieved on a Chiralcel OJ-H (150 mm × 4.6 mm, 5 micron) column using a mobile phase system containing n-hexane:isopropyl alcohol:glacial acetic acid (50:50:0.1 v/v).

The flow rate of the mobile phase was 1.0 ml/min. At 25°C column oven temperature, the peak shape of ketoprofen was found symmetrical.

In the developed and validated optimized method, No interference observed by injecting a diluent solution as a blank shown in Fig. 2. The typical retention times of R-isomer and S-isomer of ketoprofen were about 2.8 and 3.5 minutes, respectively. The isomeric separation of ketoprofen is shown in Fig. 3. The system suitability test results are tabulated as below presented in Table 1.

Validation results of the method

The percentage % RSD was better than 0.7% for the retention times of the isomers, 0.6% for ketoprofen peak area, and 3.0% for R-isomer peak area presented in Table 2. In the intermediate precision study, results show that values of % RSD were in the same manner obtained for repeatability and presented in Table 2.

The LOD and LOQ concentrations were determined to be 200 and 600 ng/ml for R-isomer of ketoprofen when a signal-to-noise ratio of 3 for detection and 10 for quantitation were used as the criteria. The method precision for R-isomer of ketoprofen at LOQ was <4% RSD presented in Table 2.

Good linearity was observed for R-isomer of ketoprofen over the concentration range of 600-6000 ng/ml. Linearity was checked for R-isomer of ketoprofen over the same concentration range for 3 consecutive days.

The recovery experiments and standard addition were conducted for R-isomer of ketoprofen in bulk samples in triplicate at 0.25%, 0.50%, and 0.75% of analyte concentration. The percentage recovery was ranged from 97.5 to 102.0 presented in Table 3.

An HPLC chromatogram of spiked R-isomer at 0.5 % level in ketoprofen sample is shown in Fig. 4. The chromatographic resolution of R-isomer and S-isomer of ketoprofen peaks was used to evaluate the method robustness under modified conditions. The resolution between R-isomer and S-isomer of ketoprofen was >2.0, under all separation tested conditions and presented in Table 4.

No significant change in the R-isomer content was observed in ketoprofen sample during experiments of solution stability and mobile phase

Table 1: System suitability parameters and report

S.No.	Parameter	Results
1	Retention time of R-isomer	2.8 minutes
2	Retention time of S-isomer	3.5 minutes
3	Resolution (USP)	2.6
4	Number of theoretical plates of R-isomer*	5210
5	Number of theoretical plates of S-isomer*	4850
6	Tailing factor (USP) of R-isomer	1.1
7	Tailing factor (USP) of S-isomer	1.2

*Calculated on USP tangent method

Table 2: Validation results of the developed normal phase method

S.No.	Validation parameter	Results
	Repeatability (n=6, %RSD)	
1	Retention time of R-isomer	0.6
2	Retention time of S-isomer	0.7
3	Peak area of R-isomer	2.9
4	Peak area of S-isomer	0.6
	Intermediate precision (n=18, %RSD)	
1	Retention time of R-isomer	0.7
2	Retention time of S-isomer	0.6
3	Peak area of R-isomer	3.0
4	Peak area of S-isomer	0.6
	LOD-LOQ (R-isomer)	
1	LOD (ng/ml)	200
2	LOQ (ng/ml)	600
3	Precision at LOQ (% RSD)	3.3
	Linearity (R-isomer)	
1	Calibration range (ng/ml)	600-6000
2	Calibration points	6
3	Correlation coefficient	0.999

LOD: Limit of detection, LOQ: Limit of quantification, RSD: Relative standard deviation

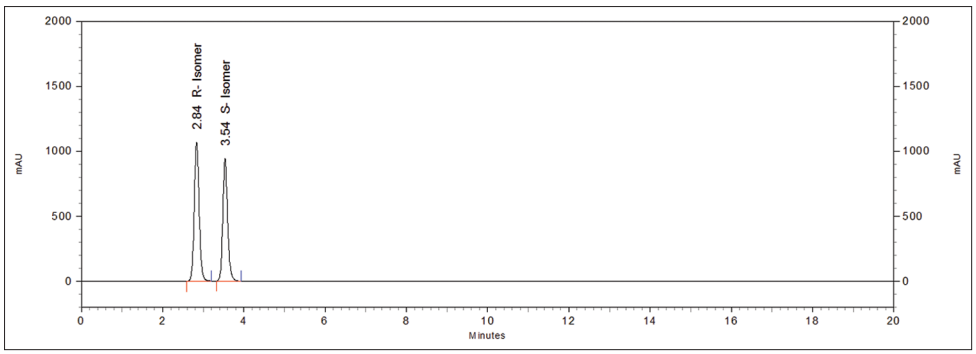

Fig. 3: Typical chromatogram of system suitability standard solution

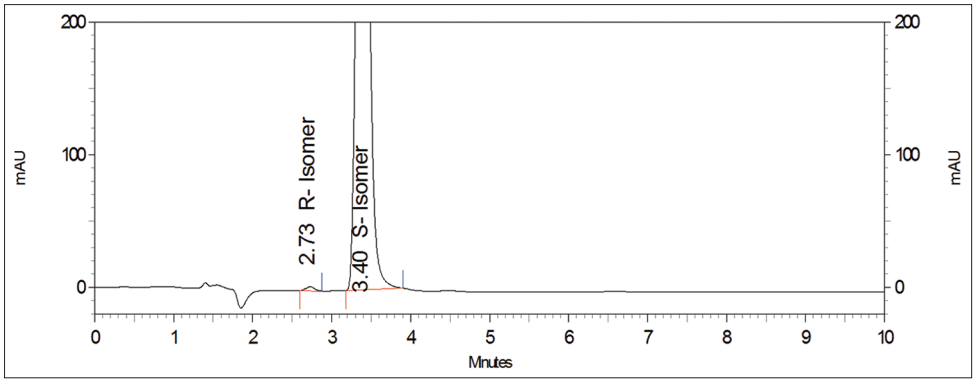

Fig. 4: Typical chromatogram of test solution

Table 3: Recovery results of R-isomer of ketoprofen in bulk drugs

Added (ng) (n=3)	Recovered (ng)	Percentage recovery	Percentage RSD
630	614	97.5	3.3
1255	1280	102.0	2.9
1892	1885	99.6	1.9

RSD: Relative standard deviation

Table 4: Method robustness

S.No.	Parameters	USP resolution between R-isomer and S-isomer
	Flow rate (ml/min)	
1	0.8	2.3
2	1.0	2.1
3	1.2	2.0
	Column temperature (°C)	
1	20	2.3
2	25	2.2
3	30	2.1
	Ethanol percentage in mobile phase	
1	49	2.5
2	50	2.2
3	51	2.2

stability. Hence, ketoprofen sample solution and mobile phase stable for at least 48 hrs.

CONCLUSION

Separation of R-isomer and S-isomer of ketoprofen is within 5 minutes and shorter run time method. A simple, rapid, precise, specific, and accurate normal phase liquid chromatography method was described for the separation of ketoprofen isomer. The method was completely validated as per ICH guidelines and showing satisfactory data for all the tested method validation parameters. The developed method can be used for the quantitative determination R-isomer of ketoprofen in bulk materials.

ACKNOWLEDGMENTS

We would like to thank management of Lavender Laboratories Pvt. Ltd, Pune, India, for providing the required facilities for completion of this work.

REFERENCES

1. Björkman S. Determination of the enantiomers of ketoprofen in blood plasma by ion-pair extraction and high-performance liquid chromatography of leucinamide derivatives. J Chromatogr 1987;414(2):465-71.
2. Guo Z, Wang H, Zhang Y. Chiral separation of ketoprofen on an achiral C8 column by HPLC using norvancomycin as chiral mobile phase additives. J Pharm Biomed Anal 2006;41(1):310-4.
3. Blanco M, Coello J, Iturriaga H, Maspoch S, Perez-Maseda C. Chiral and non-chiral determination of ketoprofen in pharmaceuticals by capillary zone electrophoresis. J Chromatogr A 1998;799(1-2):301-7.
4. International Conference on Harmonization Guidance for Industry in: Q2A Text on Validation of Analytical Methods. Switzerland: IFPMIA: 1994. p. 4.
5. International Conference on Harmonization Guidance for Industry in: Q2B Text on Validation of Analytical Methods. Switzerland, IFPMIA: 1996. p. 1-8.
6. Jadhav HP, Pathare DB. Separation and determination of the S-isomer of (10-camphorsulfonyl) oxaziridine in a bulk drugs substance by normal phase liquid chromatography. Int J Pharm Pharm Sci 2015;7(7):66-9.
7. Mali AD. Simultaneous determination of carvedilol and hydrochlorothiazide in pharmaceutical dosage form by first order derivative UV spectrophotometry. Int J Pharm Pharm Sci 2015;7(9):371-4.
8. Jadhav HP. Determination of R-isomer impurity of pantoprazole sodium in a bulk drug substance by normal phase chiral liquid chromatography method. Int J Pharm Pharm Sci 2015;7(8):45-8.
9. Raul SK, Aravelli AB. RP-HPLC method development and validation for the simultaneous estimation of atorvatanin and ezetimibe in pharmaceutical dosage form. Asian J Pharm Clin Res 2015;8(2):178-81.
10. USFDA. Reviewers Guidance: Validation of Chromatographic Methods. Washington DC: Food and Drug, Centre for Drug Evaluation and Research; 1994. p. 1-29.