IJPSR (2016), Vol. 7, Issue 5 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 08 December, 2015; received in revised form, 22 January, 2016; accepted, 07 February, 2016; published 01 May, 2016

DEVELOPMENT OF RP-HPLC METHOD FOR THE ESTIMATION OF *IN VITRO* AND *IN VIVO* SAMPLES OF KETOPROFEN IN BULK DRUG AND TRANSDERMAL DOSAGE FORM

Kavitha Kotthireddy * and B. Rama Devi

College of Engineering, Jawaharlal Nehru Technological University, Kukatpally, Hyderabad, Telangana State - 500085, India

Key words:

HPLC, ketoprofen, *in vitro*, *in vivo*, analytical method.

Correspondence to Author:
Kavitha Kotthireddy

Lecturer in Chemistry,
College of Engineering,
Jawaharlal Nehru Technological University, Kukatpally, Hyderabad, Telangana State -500085, India

Email: kavithakothireddy@gmail.com

ABSTRACT: A simple and accurate Reverse Phase High Performance Liquid Chromatography (RP-HPLC) method has been developed for the estimation of ketoprofen *in vitro* samples and *in vivo* samples from bulk drug and from administered transdermal dosage form, using Spherosorb S5 ODS of 10cm X 4.6 mm column, (5µm particle size). Mobile phase for in vitro sample analysis and *in vivo* plasma samples consists of 0.01 M sodium dihydrogen phosphate (pH adjusted to 6.5 with ortho phosphoric acid), methanol, and acetonitrile, 4:3:3 (v/v) respectively. Isocratic elution technique was followed. The flow rate was 0.5ml/min and the detection was monitored out by UV detector at 265nm. The retention time for ketoprofen was found to be 2.982 in *in vitro* sample and 3.025 min in *in vivo* sample. Naproxen was used as internal standard for *in vivo* sample analysis. The proposed method has permitted the quantification of ketoprofen over linearity in the range of 100-1000 ng/ml.

INTRODUCTION: Ketoprofen, is chemically (RS)2-(3-benzoylphenyl)-propionic acid, is one of the propionic acid class of non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic effects [1]. It acts by inhibiting the body's production of prostaglandin. Ketoprofen is a white or almost white crystalline powder having empirical formula $C_{16}H_{14}O_3$ with molecular weight of 254.3 and melting point 94˚ to 97˚C. It has pKa of 5.94. It is practically insoluble in water, freely soluble in alcohol, acetone, and dichlormethane [2]. Several analytical methods were reported for estimation of ketoprofen in pharmaceutical dosage forms [3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(5).2152-56

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (5).2152-56

Some techniques includes capillary zone electrophoresis [4], UV-spectrophotometry [5-8], high-performance liquid chromatography [9-12], flow injection technique with hemiluminiscence [13, 14], flow injection with UV-detection [15], polarography [16], micellar elecrokinetic chromatography [17], electrochemical methods [18, 19] and quantitative Fourier transformation infrared spectro photometry [21]. European Pharmacopoeia recommended acid-base titration for analysis of ketoprofen in substance, UV-spectrophotometry for its determination in capsules as well as liquid chromatography for assay in gel. All these methods clearly not described the procedure for the estimation of ketoprofen in biological samples.

Hence, the current research work was aimed to develop a specific, precise and accurate reverse phase HPLC method that could be applied in quality control for the determination of ketoprofen

in *in vitro* and as well as *in vivo* biological samples in pure drug and from transdermal dosage form

MATERIALS AND METHODS:

Chemicals and Reagents:

Ketoprofen was obtained as gift sample from Ranbaxy. HPLC grade methanol and acetonitrile were supplied from Merck (Germany). All other chemical reagents were of analytical grade.

Instrumentation and chromatographic conditions:

Chromatographic separation was carried out on HPLC system Shimadzu model LC-10 ATVp, a Shimadzu model SPD-6AV variable wavelength detector (Possessing deuterium lamp with a sensitivity of 0.005 AUFs and adjusted to an absorbency of 265nm), consisting of Spherosorb S5 ODS of 10cm X 4.6 mm column, (5µm particle size). The mobile phase, degassed under vacuum in an ultrasonic bath, consists of 0.01 M sodium dihydrogen phosphate (adjusted to pH 6.5 with orthophosphoric acid), methanol and acetonitrile, 4:4:3 (v/v), respectively. Isocratic elution technique was followed. The flow rate was 0.5ml/min and the detection was monitored out by UV detector at 265nm. The analysis was carried out at an ambient temperature and injection volume was 20 µl.

Preparation of Standard Solutions for *in vitro* sample analysis:

25 mg of ketoprofen was weighed accurately and dissolved in 25 ml of methanol. It was considered as primary standard stock solution whose concentration was 1000 µg/mL. From this primary standard stock solution, secondary stock solution was prepared whose concentration was 100µg/mL. From this secondary stock solution various concentrations such as 100, 200, 400, 600, 800 and 1000 ng/mL were prepared and used as *in vitro* standard samples for preparing calibration curve.

Preparation of Standard Solutions for *in vivo* sample analysis:

100 mL of fresh blood sample of sheep was collected from animal slaughter house for method development purpose. Plasma was separated from collected blood. To the 1 mL of separated plasma, 1mL of previously prepared standard ketoprofen solution of 100ng/mL was added. A suitable amount of the internal standard, Naproxen, 0.5 M phosphate buffer pH 2.0 (2 ml) and a mixture (4:1 v/v) of diethyl ether/chloroform (6 ml) was added to above solution. The obtained mixture was then taken in centrifuge tube and centrifuged for 15 min at 5000 rpm.

After being shaken for 15 min and centrifuging, the upper organic layer was removed, dried with anhydrous sodium sulphate, and then evaporated to dryness at 30°C under a stream of oxygen-free nitrogen. The residue was dissolved in 100 µl of mobile phase and injected on to the HPLC column. Same procedure was repeated with other concentrations like 200, 400, 600, 800 and 1000ng/mL.

Preparation of test sample solutions for *in vitro* sample analysis:

Fastum gel 2.5 %w/w was purchased from market. Weight equivalent to 10 mg of gel was taken and *in vitro* diffusion study was conducted using franz diffusion cell using cellophane membrane. Samples were collected at various intervals and analysed for diffused drug content by HPLC using calibration plot.

Preparation of test sample solutions for *in vivo* sample analysis:

The study was conducted with the prior approval of institutional animal ethical committee. Male albino rabbits were included in the study. Hair present on the rabbit abdominal skin was carefully removed and weight equivalent to 10 mg of gel was applied to the abdomen. Blood samples were withdrawn from ear marginal vein at various time intervals and analysed as per the procedure given in the previous section. Concentration in each sample was analysed by using standard calibration plot.

RESULTS AND DISCUSSION:

The **Fig. 1** showed typical chromatogram obtained from analysis of standard solution using the proposed method. **Fig. 1a** is obtained with *in vitro* sample and **Fig. 1b** is obtained with *in vivo* sample.

The retention time for ketoprofen was found to be 2.982 in *in vitro* sample and 3.025 min in *in vivo* sample.

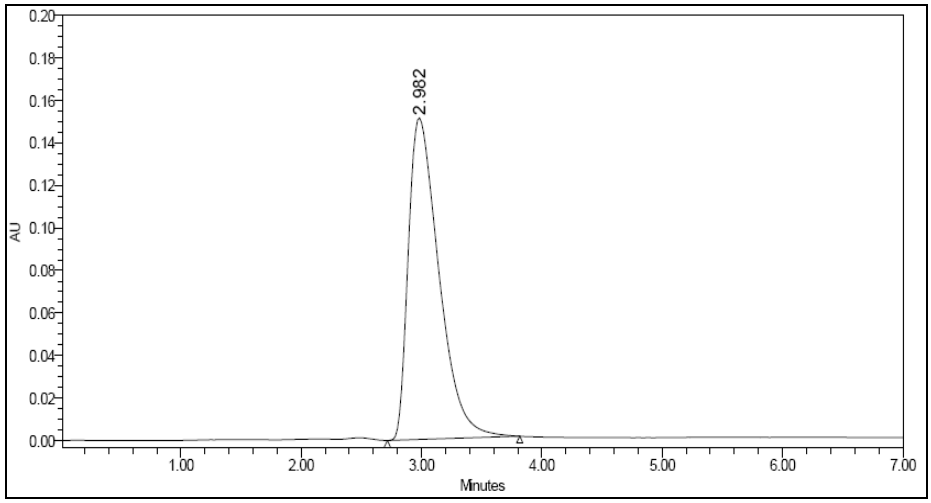

FIG 1a: HPLC CHROMATOGRAMS OF KETOPROFEN *IN VITRO*

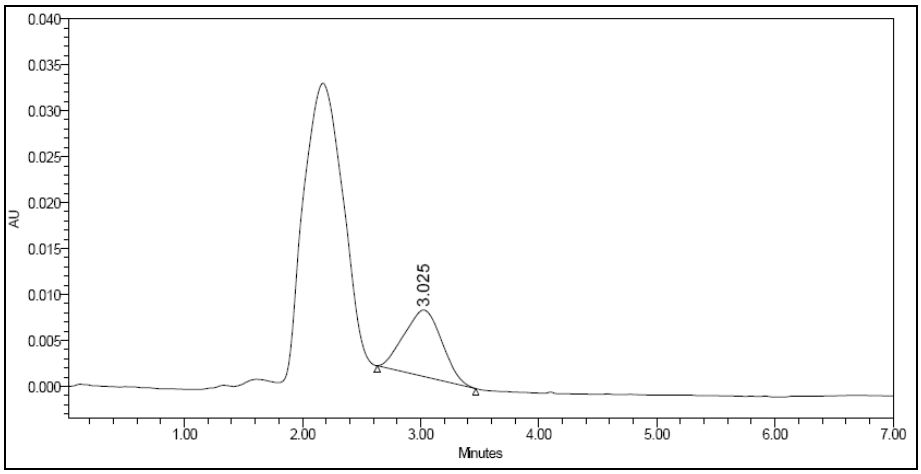

FIG 1b: HPLC CHROMATOGRAM OF KETOPROFEN *IN VIVO*

Calibration curve for estimation of *in vitro* samples:

Various concentrations of standard samples were prepared and injected into HPLC column. Peak area values of each prepared standard sample was calculated and taken on Y axis and concentration was taken on X axis and calibration curve was plotted. Regression analysis was done and R^2 value and slope was calculated which was used for the estimation of unknown concentration. Calibration curve obtained is shown in **Fig. 2**.

R^2 value was found to be 0.9999. Regression equation was found to be y= 134.31x-388.73.

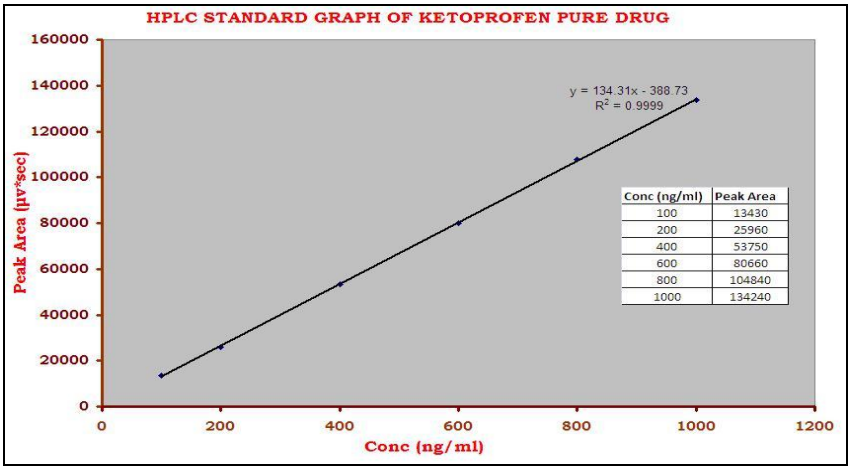

FIG. 2: HPLC STANDARD GRAPH OF KETOPROFEN WITH IN VITRO STANDARD SAMPLES

Determination of unknown concentrations of *in vitro* samples:

In vitro diffusion study was conducted for marketed gel and samples were analysed for diffused ketoprofen content by developed HPLC method. Calculated amount of drug diffused values are given in **Table 1.**

TABLE 1: AMOUNT OF KETOPROFEN DIFFUSED

Formulations	Amount of Drug Diffused within (MEAN ± SD)				
	30 min	60 min	120 min	240 min	360 min
Marketed Gel	2.67 ± 0.34	3.28 ± 0.44	3.99 ± 0.32	7.59 ± 0.21	9.96 ± 0.12

Calibration curve for estimation of *in vivo* samples:

Various concentrations of standard samples were mixed with plasma and processed as per the procedure given above. The prepared samples were injected into HPLC column. Ratio of peak areas of internal standard (naproxen) and test sample ketoprofen values of each prepared standard sample was calculated and taken on Yaxis and concentration was taken on X axis and calibration curve was plotted. Regression analysis was done and R^2 value and slope was calculated which was used for the estimation of unknown concentration. Calibration curve obtained is shown in **Fig.3**.

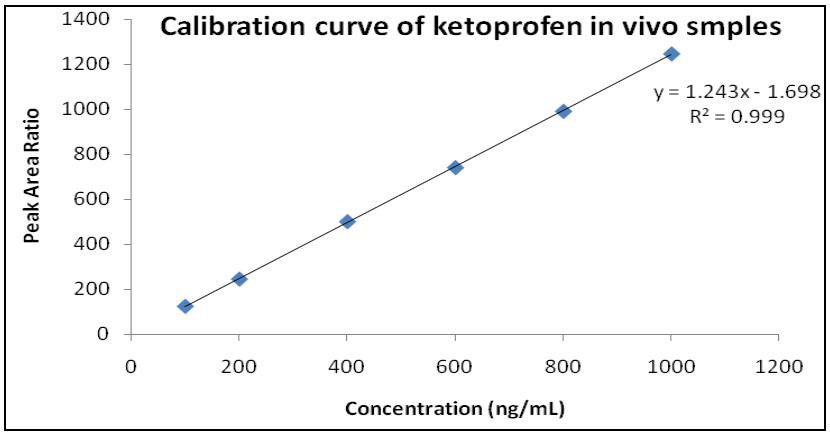

FIG 3: CALIBRATION CURVE OF KETOPROFEN *IN VIVO* SMPLES

Determination of unknown concentrations of *in vivo* samples:

In vivo study was conducted in male albino rabbits as per the procedure given above. Plasma samples were analysed by developed HPLC method to check the method feasibility and applicability. Obtained plasma concentration values are given in the **Table 2**.

TABLE 2: PLASMA CONCENTRATION VALUES OF *IN VIVO* SAMPLES

Formulation	Plasma Concentration (ng/ml) at				
	40 min	80 min	120 min	180 min	240 min
Market Gel	421.41	1194.39	1169.58	946.76	488.27

CONCLUSION: A novel RP-HPLC method was developed successfully for estimation of *in vitro* samples and *in vivo* samples of ketoprofen successfully**.**

REFERENCES:

1. Carlo P, Colin B: Nonsteroidal Anti-Inflammatory Drugs and the Heart. Contemporary Reviews in Cardiovascular Medicine 2014; 129: 907-916.
2. British Pharmacopoeia. London, Great Britain, 2015.
3. El-Kommos ME, Mohamed NA, Hakiem AF: Extractive spectrophotometric determination of some nonsteroidal anti-inflammatory drugs using methylene blue. Journal of AOAC International 2013; 96:737-744
4. Blanko M, Coello J, Iturriaga H, Maspoch S and Alaoui-Ismaili S: UV-spectrophotometric determination of ketoprofen and paraben in a gel preparation by partial least-squares calibration. Fresenius Journal of Analytical Chemistry 1997; 357: 967-972.
5. Niraimathi V, Suresh AJ, Alageswaram A: UV Spectroscopic determination of fenofibric acid by using hydrotrophy. International Journal of Pharma Sciences and Research 2015; 6:451-459

6. Kormosh Z, Hunka I and Basel Y: Spectrophotometric determination of ketoprofen and its application in pharmaceutical analysis. Acta Poly Pharmacy and Drug research 2012; 66:3-9.
7. Dvorac J, Hajkova R, Matysova L, Novakova L, Koupparis M and Solich P: Simultaneous determination of ketoprofen and its degradation products in the presence of preservatives in pharmaceuticals. Journal of Pharmaceutical and Biomedical Analysis 2014; 36:625-629.
8. El-Sadec M, El-Adi S and Abou-Kull M: Spectrophotometric determination of ketoprofen in pharmaceutical preparations by means of charge transfer complex formation. Talanta 1993; 40: 585-588.
9. Labbozzetta S, Valvo L, Bertocchi P, Alimouti S, Gandiano M and Manna L: Focused microwave-assisted extraction and LC determination of ketoprofen in the presence of preservatives in a pharmaceutical cream formulation. Chromatographia 2009; 69:365-368.
10. Novakova L, Matysova L, Solichova D, Koupparis M and Solich P: Comparison of performance of C18 monolithic rod columns and conventional C18 particle packed columns in liquid chromatographic determination of Estrogel and Ketoprofen gel. Journal of Chromatography B 2004; 813:191-197.
11. Wong C, Yeh M and Wang D: High-performance liquid chromatographic determination of ketoprofen in pharmaceutical dosage forms and plasma. Journal of Liquid Chromatography 1992; 15:1215-1225.
12. Bempong D and Bhattacharyya L: Development and validation of a stability-indicating high-performance liquid chromatographic assay for ketoprofen topical penetrating gel. Journal of Chromatography A 2005; 1073:341-346.
13. Mannucci C, Bertini J, Cocchini A and Perico F: High performance liquid chromatography simultaneous quantitation of ketoprofen and parabens in a commercial gel formulation. Journal of Liquid Chromatography 1992; 15:327-335.
14. Zhuang Y, Cao D and Ge D: Flow injection analysis of ketoprofen based on the order transform second chemiluminiscence reaction. Spectrochim Acta part B 2012; 85: 139-144.
15. Zhuang Y and Song H: Sensitive determination of ketoprofen using flow injection with chemiluminiscence detection. Journal of Pharmaceuical and Biomedical Analysis 2014; 44: 824-828.
16. Aboul-Enein H, Dal A and Tuncel M: A validated method development for ketoprofen by a flow-injection analysis with UV-detection and its application to pharmaceutical formulations. IL Pharmacon 2013; 58: 419-422.
17. Emara K, Ali A and Maali N: The polarographic behaviour of ketoprofen and its degradation products in the presence of preservatives in pharmaceuticals. Journal of Pharmaceutical and Biomedical Analysis 2014; 36: 625-629.

How to cite this article:
Lele AC, Khambete MP, Raju A, Ray M, Rajan MGRA and Degani MS: Design and Synthesis of Novel *Mycobacterium Tuberculosis* DHFR Inhibitors. Int J Pharm Sci Res 2016; 7(5): 2152-56.doi: 10.13040/IJPSR.0975-8232.7(5).2152-56.