Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2012, 3 (4):400-403

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

HPLC determination of ketorolac tromethamine in tablet dosage forms

Boyka G. Tsvetkova*, Ivanka P. Pencheva, Plamen T. Peikov

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University – Sofia

---

ABSTRACT

A simple, specific, precise and accurate reversed phase liquid chromatographic (RP-LC) method has been developed for the determination of ketorolac tromethamine (KTR) in tablet dosage forms. Acetylsalicylic acid was used as internal standard. The chromatographic separation was achieved on a LiChrosorb C_{18}, 250 mm x 4.6 mm, 5 µm column at a detector wavelength of 230 nm and a flow rate of 1.0 ml/min. The mobile phase was composed of 0.1M sodium acetate buffer pH 5.0, acetonitrile and tetrahydrofuran (23:75:2 v/v/v). The retention time of KTR was 3.05 min. The method was validated for the parameters like specificity, linearity, precision, accuracy, limit of quantitation and limit of detection. The method was found to be specific as no other peaks of impurities and excipients were observed. The square of correlation coefficient (R^2) was 0.9999 while relative standard deviations were found to be <2.0%. This method can be used for the routine quality control and assay of KTR in pharmaceutical preparations.

Key words: ketorolac tromethamine, liquid chromatography, validation, quality control

---

INTRODUCTION

Ketorolac tromethamine [(+/-)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid, 2-amino-2-(hydroxymethyl)-1,3-propanediol] is a highly potent member of nonsteroidal anti-inflammatory drugs. The compound shows potent prostaglandin cyclooxygenase inhibitory activity [1]. Ketorolac, when administered intramuscularly or orally, is a safe and effective analgesic agent for the short-term management of acute postoperative pain and can be used as an alternative to opioid therapy [2]. The official method for determination of KTR is titration with perchloric acid in anhydrous acetic acid media [3]. A spectrophotometric method has been developed for the estimation of ketorolac tromethamine, based on its reaction with 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) in presence of Fe (III) ion [4]. Manal et al. described spectrophotometric determination of KTR via its oxidation with cerium (IV) ion [5]. Flow-injection methods with spectrophotomeric detection for determination of KTR [6, 7] as well as fluorimetric assay [8] have been described. Also, several types of analytical procedures have been proposed for the analysis of KTR in pharmaceutical formulations. The procedures include HPLC [9-11], HPTLC [12], capillary electrochromatography [13], micellar electrokinetic chromatography [14] and differential pulse polarography [15].

The aim of the present work was to develop and validate the rapid and sensitive high performance liquid chromatography method for determination of ketorolac tromethamine in tablets.

MATERIALS AND METHODS

Chemicals, reagents and chromatographic conditions

Tablets containing ketorolac tromathamine (10 mg) were obtained commercially. Analytically pure powder KTR was procured as gift sample from Bulgarian Drug Agency. LC-grade acetonitrile and tetrahydrofuran were supplied from Merck (Germany). All other chemical reagents were of analytical grade. Chromatographic separation was

performed on modular HPLC system LC-10A Shimadzu (Japan) arranged with a LC-10A pump, solvent degasser DGU-3A, Rheodyne injector, column oven CTO-10A, SPD-M10A fixed wavelength detector and communication bus module CBM-10A. A LiChrosorb C_{18}, 250 mm x 4.6 mm, 5 µm column was used as a stationary phase. The separation was performed isocratically with a mobile phase consisting 0.1M sodium acetate buffer pH 5.0, acetonitrile and tetrahydrofuran (23:75:2 *v/v/v*) at a flow rate of 1.0 ml/min. The analysis was carried out at an ambient temperature and injection volume was 20 µl. The UV detector was set at 230 nm.

Preparation of 0.1 M acetate buffer pH 5.0
13.6 g of sodium acetate was dissolved in sufficiently quantity of distilled water and made to produce 1000 ml and adusted to pH 5.0 with glacial acetic acid.

Standard solutions and calibration curve
Standard stock solutions of KTR (100 µg/ml) and acetylsalicylic acid (80 µg/ml, I.S.) were prepared in methanol. Subsequent dilutions were made in same diluent to achieve the concentrations of calibration solutions in the range 10 – 50 µg/ml. The concentration of the I.S. in the working solution was 20 µg/ml.

Sample preparation
Twenty tablets were accurately weighed and finely powdered. The powder equivalent to 10 mg KTR was weighed accurately and dissolved in 100 ml methanol. The solution was filtered through 0.2 µm membrane filter. Five ml of the resulting solution was mixed with 5.00 ml of I.S. (80 µg/ml) and was further diluted to 20 ml to get a solution having a concentration of 25 µg/ml of KTR and 20 µg/ml of I.S.

RESULTS AND DISCUSSION

The proposed method was validated with respect to selectivity, linearity, precision, accuracy, limit of quantitation (LOQ) and limit of detection (LOD) according to ICH requirements [16-22] to show it could be used for determination of KTR in pharmaceutical formulations.

Selectivity
From the chromatogram shown in Fig. 1, it is evident, that under the chosen chromatographic conditions KTR (Tr=3.05 min) and internal standard (Tr=6.96 min) were completely separated. The specificity of the proposed method was confirmed by injecting blank sample. The specificity analysis revealed the HPLC method did not suffer interference by the formulation excipients, since there was not another peaks on the retention times of KTR and internal standard.

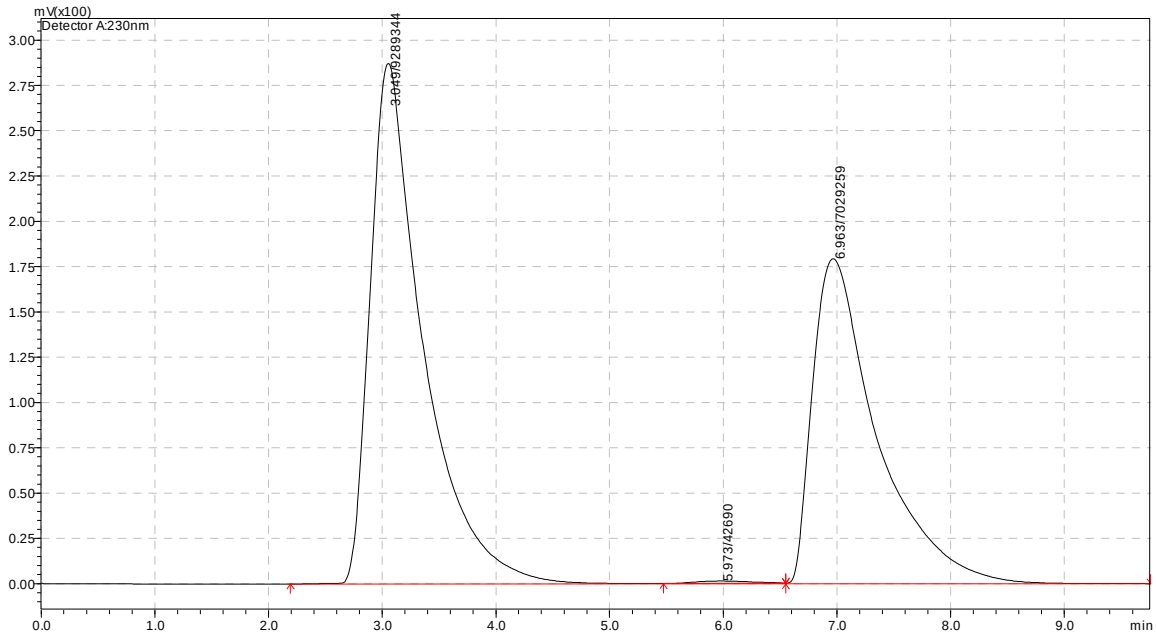

Fig.1. Chromatogram from analysis of sample

System suitability test

A system suitability test was an integral part of the method development to verify that the system is adequate for the analysis of KTR to be performed. The system suitability studies were carried out to determine theoretical plates, resolution and tailing factor. The results were given in **Table 1**.

Table 1. System suitability parameters for analysis of KTR

Parameter	KTR
Retention time (min)	3.05
Resolution	3.08
Tailing factor	0.83
Theoretical plates	5670

Linearity, limit of detection and limit of quantification

Calibration curve was constructed in the range of 10–50 µg/ml for KTR to encompass the expected concentration in measured samples. The results showed an excellent linearity between peak area ratios (KTR/internal standard) and concentration. The equation of the calibration curve was obtained by the least-squares linear regression analysis and calculated as: *y=125647.2x-1124.1* with square of correlation coefficient R^2 of 0.9999. The LOD was calculated to be three times the standard deviation of baseline noise. The LOQ was measured as the lowest of analyte that could be reproducibly quantified above the baseline noise, i.e. for which duplicate injection resulted in an RSD ≤ 2%. The LOD and LOQ for KTR were 0.1 µg/ml and 0.5 µg/ml, respectively.

Precision

The precision of analytical method was investigated by performing six consecutive replicate injections of the same standard solution. The standard deviation (S_d) and relative standard deviation (RSD) obtained were listed in **Table 2**.

Table 2 Precision of the HPLC method

Amount claimed (mg/tablet)	Amount found (mg/tablet)	Percentage purity obtained	Mean± S.D.	RSD %
10.00	9.96	99.60	99.47± 1.356	1.36
	9.81	98.10		
	10.08	100.8		
	9.79	97.90		
	10.12	101.2		
	9.92	99.20		

Accuracy

Accuracy was determined by applying the proposed method to synthetic mixtures of the drug product components to which known quantities of KTR substance had been added (corresponding to 75, 100 and 125 % of the label claim of the drug). The accuracy was expressed as the percentage of analyte recovered by the assay. Mean recoveries for KTR from the specific formulations were shown in **Table 3**. The results indicated good accuracy of the method for the determination of analysed drug as revealed by mean recovery data.

Table 3 Accuracy of the HPLC method

Level (%)	Theoretical concentration (µg/ml)	Observed concentration (µg/ml)	Mean recovery (%) ± SD	RSD (%)
75	18.61	18.56	99.60±0.431	0.43
		18.62		
		18.48		
100	24.93	24.81	99.67±0.477	0.48
		24.97		
		24.75		
125	31.28	31.20	99.84±0.322	0.32
		31.15		
		31.34		

Solution stability

Sample solution stability was evaluated by storing the solution at ambient temperature and at 2-5ºC and analysis after 12, 24, 36, and 48 h. The responses from the aged solutions were compared with those from freshly prepared standard solution. The results showed that the retention time and peak area of KTR remained almost unchanged and

no significant degradation within the indicated period occurred. **Table 4** showed the results obtained from evaluation of stability.

Table 4. Results from study of solution stability

Time (h)	Assay (%), test solution stored at 2-5ºC	Assay (%), test solution stored at ambient temperature
Initial	99.91	99.87
12	99.84	99.85
24	99.37	99.51
36	99.41	99.60
48	99.15	99.47

CONCLUSION

The newly developed RP-LC method for determination of ketorolac tromethamine in dosage forms is specific, precise, accurate and rapid. Hence the proposed method is suitable for the quality control of the raw materials, formulations and dissolution studies.

REFERENCES

[1] W.H. Rooks 2^{end}, *Pharmacotherapy*, **1990**, 10, 30S.
[2] W.H Rooks, P.J. Maloney, L.D. Shott, M.E. Schuler, H. Sevelius, A.M. Strosberg, L. Tanenbaum, A.J. Tomolonis, M.B. Wallach, D. Waterbury, *Drug Exp. Clin. Res*., **1985**, 11, 479.
[3] British Pharmacopoeia, **2007**.
[4] D.M. Shingbal, U.C. Naik, *Indian Drugs*, **1997**, 34, 608.
[5] E. Manal, A.El-Brashy, A. Fatma, W. Talaat, *J. AOAC Int.*, **2007**, 90, 941.
[6] B. Kamath, K. Shivram, S. Vangani, *Anal. Lett.*, **1994**, 27, 103.
[7] B. Kamath, K. Shivram, A. Shah, *J. Pharm. Biomed. Anal.*, **1994**, 12, 343.
[8] M.S. Prakash, S. Meena, *Indian Drugs*, **1996**, 12, 343.
[9] S. N. Razzaq, I. Mariam, I.U. Khan, M. Ashaq, *J. Liq. Chrom. Rel. Tech.*, **2012**, 35, 651.
[10] J. Squella, I. Lemus, J. Sturm, L. Nunez-Vergara, *Anal. Lett.*, **1997**, 30, 553.
[11] B.P. Reddy, M.V. Suyanarayana, S. Vemkatraman, G.L. Krupadanam, C.S. Sastry, *Indian Drugs*, **1993**, 30,176.
[12] P.V. Devarajan, S.P. Gore, S.V. Chavan, *J. Pharm. Biomed. Anal.*, **2000**, 22, 679.
[13] S. Orlandini, S. Furlanetto, S. Pinzauti, G. D'Orazio, S. Fanali, *J. Chrom. A.*, **2004**, 1044, 295.
[14]. S. Orlandini, S. Fanalo, S. Furlanetto, A. Marras, S. Pinzauti, *J. Chrom. A.*, **2004**, 1032, 253.
[15] J. Sturm, H. Canelo, L. Nunez-Vergara, J. Squella, *Talanta*, **1997**,44, 931.
[16] ICH, Q2B, Validation of Analytical Procedures: Methodology, International Conference on Harmonization, Geneva, November, **1996.**
[17] M. Sugumaran, M. Poornima, M. Yogesh Kumar, S. Ramarajasekhar, *Der Pharmacia Sinica*, **2011**, 2, 12.
[18] M. Mohideen, M. Shivakanth, P. Suresh Kumar, S. Navaneetha Krishnan, Y. Surendranath, T. Satyanarayana, *Der Pharmacia Sinica*, **2011**, 2, 114.
[19] R. Narenda Kumar, G. Nageswara Rao, P.Y. Naidu, *Der Pharmacia Sinica*, **2011**, 2, 125.
[20] A. Shrivastava, A. Chakraborty, S. Rambhade, U. Patil, *Der Pharmacia Sinica*, **2011**, 2, 263.
[21] M. Narenda Kumar, V. Krishna Reddy, H. Kumar Sharmal, T. Mastanainah, *Der Pharmacia Sinica*, **2012**, 3, 450.
[22] R. Manmodel, A. Dhamankar, J. Manwar, S. Laddha, *Der Pharmacia Sinica*, **2011**, 2, 81.