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Research Article

REVALIDATION AND ANALYTICAL EVALUATION OF KETOROLAC TROMETHAMINE BY HPTLC USING REFLECTANCE SCANNING DENSITOMETRY

P.L.K.M. Rao, V. Venugopal, Ch.S.G. Teja*, D.V.N.S. Radhika, K. Kavitha, S. Lavanya, Manasa T. and Pavani A.

Department of pharmaceutical analysis, SLC'S college of pharmacy, Hyderabad, Andhra Pradesh, India.

*Corresponding Author: sriguruteja@yahoo.co.in

ABSTRACT

A new quantitative densitometric High Performance Thin Layer Chromatographic method was developed and validated for the analysis of ketorolac tromethamine both in bulk and formulations where the formulation was separated and identified on a silica gel 60 F254 HPTLC plates with toluene: ethyl acetate : acetic acid (6:2:1 v/v) as mobile phase. Densitometric quantification was performed at λ= 320 nm by reflectance scanning, which facilitated well-resolved band for the main drug (R_f 0.61+0.02). Response to ketorolac tromethamine was a linear function of concentration in the range of 50-300ng/spot. The minimum amount of ketorolac tromethamine that could be authentically detected and LOD and LOQ were quantified as 16.22 and 54.13 ng/spot, respectively. The proposed method was validated with respect to linearity, precision, accuracy, specificity and robustness.

Keywords: Ketorolac tromethamine, TEG, HPTLC, Reflectance scanning densitometry.

INTRODUCTION

Ketorolac tromethamine

Systematic (IUPAC) name: (±)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid,2-amino-2-(hydroxymethyl)-1,3-propanediol

Ketorolac tromethamine (Brand name **Toradol and Acular**) is a nonsteroidal anti-inflammatory drug (NSAID) in the family of heterocyclic acetic acid derivative, often used as an analgesic, antipyretic and anti-inflammatory. Ketorolac acts by inhibiting the bodily synthesis of prostagladins. Literature review revealed different analytical methods such as UV and HPLC for the quatitative determination of ketorolac. The present work deals with analytical evaluation of ketorolac in tablets by HPTLC using reflectance scanning densitometry. The main objective of the work was to develop simple, fast, sensitive and accurate method.

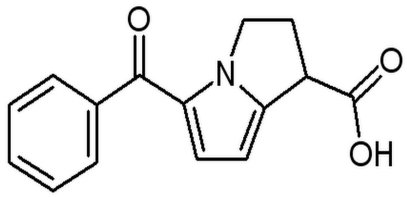

Fig. 1: Structure of Ketorolac

EXPERIMENTAL

INSTRUMENTS

A CAMAG TLC system comprising of a Linomat-5 applicator using rheodyne injector and CAMAG TLC III scanner, silica gel G60F254, 10x10 cm TLC plate was used as a stationary phase. Toulene, Glacial acetic acid, Ethyl acetate were used of AR Grade. The plates were developed in a CAMAG twin

trough glass chamber (20 x 10 cm) by ascending method.
Distance of solvent front 80mm, band length 5mm and detection wavelength 320nm were used for the present study.

CHEMICALS

Ethyl acetate, Toulene, Glacial acetic acid, Methanol. All chemicals and reagents used were of Chromatographic grade and were purchased from Merck chemicals, India.

PREPARATION OF STANDARD STOCK SOLUTION

A stock solution of KT (1mg/ml) was prepared by dissolving the authentic sample in methanol. This solution was used to construct a calibration plot by applying 0.5,1, 1.5,2, 2.5, and 3 µl (equivalent to 50-300 ng per band) to a TLC plate. The data of peak area versus drug concentration was treated by linear least square regression to obtain the calibration graphs.

MOBILE PHASE

The mobile phase consisted of toluene: ethyl acetate: acetic acid: (6:2:1 v/v) and 9 ml of mobile phase was used for chromatography

CHROMATOGRAPHIC CONDITIONS

Stationary phase : silica gel 60F-254

Mobile phase : Toulene, ethyl acetate, acetic acid (6:2:1 v/v/v)

Lamp : deuterium

Wavelength : 200 to 400 nm

Migration distance : 8.5cm

Band width : 6mm

Distance between the tracks : 10mm

SAMPLE ANALYSIS

To determine the concentration of KT in tablets (Brand name: Ketorol-DT label claim: 10 mg per tablet), ten tablets were weighed, their mean weight determined and finely powdered. The powder equivalent to 1mg of ketorolac tromethamine was transferred into a 10ml volumetric flask containing 5ml methanol, sonicated for 30 min (Fast Clean Ultrasonic Cleaner, Enertech Electronics Pvt. Ltd, Mumbai) and the volume was made up to 10ml i.e.100ng/µl solution. The resulting sample stock solution was centrifuged at 3000 rpm for 5 min. at 25°C and the supernatant that was filtered with Whatman Filter Paper No. 41 was analyzed for drug content. Then 2µl of this solution (200ng per spot) was applied on a TLC plate which was developed and scanned as described in section 4.3. The analysis was repeated in triplicate. The possibility of excipient interference with the analysis was examined

RECOVERY STUDIES

If was performed to assess the accuracy of the analytical method. The recovery experiments were carried out in triplicate by adding a known amount of drug to the pre-analyzed sample and the percentage recovery was calculated.

RESULTS AND DISSCUSSION

The retention factor (Rf) of ketorolac tromethamine was found to be 0.61±0.02 at 320nm and the linearity range to be 50-300 ng/spot. Correlation coefficient ± SD (0.9985±0.000367) indicates good linearity between concentration and peak area. The regression of ketorolac tromethamine concentration over peak area was found to be Y= (3.961 + 6.807 X) here, Y= concentration of the drug, x= peak area ratio. The percentage recovery indicating that the proposed method is highly accurate. The densitogram and the value pertaining to evaluation are given in the table 1-5 and figure 1-3

Table 1: Linearity and Range of Ketorolac Tromethamine (HPTLC)

S. No.	Concentration ng/spot	Peak area
1	50	299.30
2	100	726.50
3	150	1056.69
4	200	1359.04
5	250	1681.24
6	300	2048.48

Table 2: Intra- and Inter- Day Precision by HPTLC method[a]

Amount (ng/spot)	Repeatability		Intermediate precision	
	Mean area (AU) ± SD	% RSD	Mean area (AU) ± SD	% RSD
50	983±9.07	0.92	964±10.89	1.12
150	2739±14.79	0.53	2695±15.25	0.56
250	4068±21.33	0.52	4025±18.56	0.46

[a]n = 6

Table 3: Recovery Studies of Ketorolac Tromethamine (n=6)

Excess of drug[a] added to the analyst (%)	Theoretical content (ng)	Amount of drug found (ng)	Recovery (%)	% RSD
0	100	102.09	102.0	0.66
50	150	148.05	98.70	0.54
100	200	198.20	99.10	0.63
150	250	253.66	101.4	0.22

[a] Matrix containing 100 ng of drug in sample

Table 4: Statistical parameters

Parameters	TLC densitometry
Linear range	50-300 ng/spot
Correlation coefficient (r) ±SD	0.9985±0.000367
Slope ±SD	6.79±0.238
Intercept±SD	8.70±1.31

Table 5: LOD and LOQ (HPTLC)

S. No.	Parameter	Ketorolac tromethamine
1	LOD	16.22 ng/spot
2	LOQ	54.13ng/spot

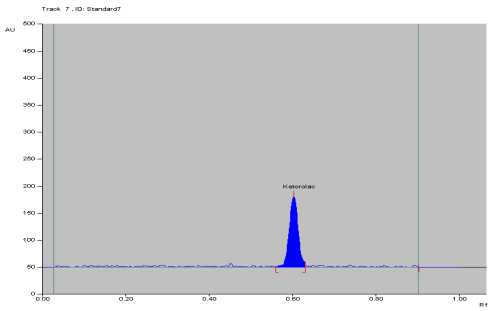

Fig. 1: A typical densitogram of ketorolac tromethamine R_f 0.61±0.02 at 320nm using toluene: ethyl acetate: acetic acid(6:2:1 v/v/v)

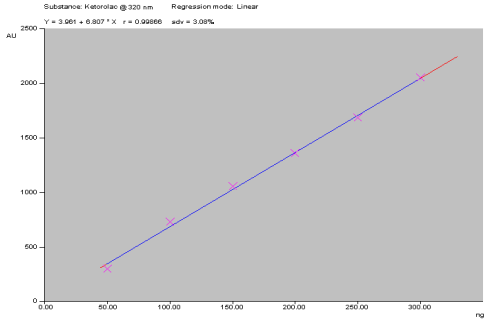

Fig. 2: Calibration curve for ketorolac tromethamine in the concentration range of 50-300ng/spot

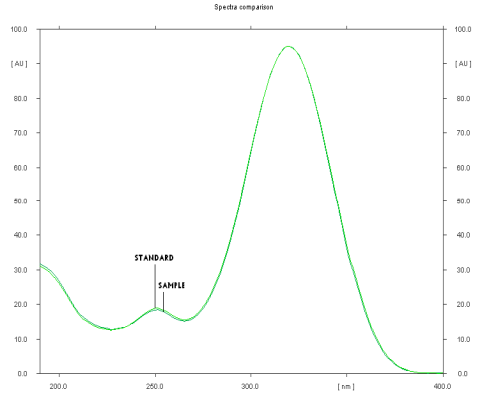

Fig. 3: Insitu UV spectra of ketorolac tromethamine standard and ketorolac tromethamine from sample

CONCLUSION

The developed HPTLC technique is simple, precise, specific and accurate and the statistical analysis proved that method is reproducible and selective for the analysis of ketorolac tromethamine in bulk and pharmaceutical formulations.

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