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HPTLC determination of ketorolac tromethamine

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Abstract

A High Performance Thin Layer Chromatography (HPTLC) method for quantification of ketorolac tromethamine, a non-narcotic and non-steroidal agent was developed. The mobile phase composition was chloroform–ethyl acetate–glacial acetic acid (3:8:0.1, v/v/v). Spectrodensitometric analysis of ketorolac tromethamine was carried out at 323 nm. The calibration curve was linear in the range of 200–700 ng. The mean values of slope, intercept and correlation coefficient were, 2941, 749583, 0.99. The method was validated for method precision, system precision, marketed sample analysis and recovery studies. The % CV for method precision studies was 1.98 ($n = 6$) and system precision study was 1.83 ($n = 6$). The average recovery was found to be 99.2%. Acid and base degraded products were adequately separated from the drug. The method was successfully used for the determination of drug from saliva. The results indicate that the method is simple, specific, selective and reliable for quantitative analysis of ketorolac tromethamine as bulk drug and from formulations. It can also be applied for the stability study of the drug and analysis of drug in biological fluids.

Keywords: Ketorolac tromethamine; HPTLC; Saliva

1. Introduction

Ketorolac tromethamine [1](Fig. 1), a NSAID, 800 times more potent than aspirin, exhibits pronounced analgesic and moderate anti-inflammatory activity. It is indicated for the short term management of moderate to severe painful states such as post operative pain, acute musculoskeletal pain, dental pain including pain after oral surgery etc.

Several LC methods [2–9] have been reported for the analysis of ketorolac tromethamine from pharmaceutical formulations and from biological samples. But all these methods are laborious and expensive.

Recently High Performance Thin Layer Chromatography (HPTLC) is widely employed for the quantification of active drugs [10–13]. HPTLC is cost effective and environmentally friendly because on a per sample basis it uses typically 5% of the solvent consumption of LC. It facilitates automated application and scanning in situ. It is the fastest chromatographic technique as several samples can be run simultaneously and is flexible

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enough to analyze different kinds of samples. Spectrophotometric methods like UV spectroscopy would not be stability indicating or suitable for analysis from biological fluids.

This paper describes a simple, rapid, precise, specific alternative to existing HPLC methods for the measurement of ketorolac tromethamine as bulk drug, from pharmaceutical dosage forms and from biological fluids.

2. Experimental

2.1. Materials

Ketorolac tromethamine USP (99.40% purity) was supplied by Ranbaxy Research Labs (India), Analytical grade organic solvents and reagents were purchased from Ranbaxy fine chemicals (India).

2.2. Standard solution

Samples for quantitative TLC were prepared in methanol at a concentration of 1000 μg/ml. Standard solution of 100 μg/ml was used for analysis.

2.3. Apparatus

Solutions of sample were applied to Silica gel 60F254 plates (10 cm × 10 cm, 0.2 mm thickness, E. Merck, Germany) using a Camag Linomat IV model equipped with a 100 μl Camag syringe. The samples were streaked in the form of narrow bands of length 3 mm, 10 mm from the bottom edge, 10 mm from margin, 5 mm apart at a constant rate of 15 s/μl using a nitrogen aspirator. Development of the plates was carried out in a Camag development chamber (12.5 cm × 12.5 cm × 5 cm) using 30 min for solvent saturation of the tank, at ambient temperature. The migration distance was 8 cm.

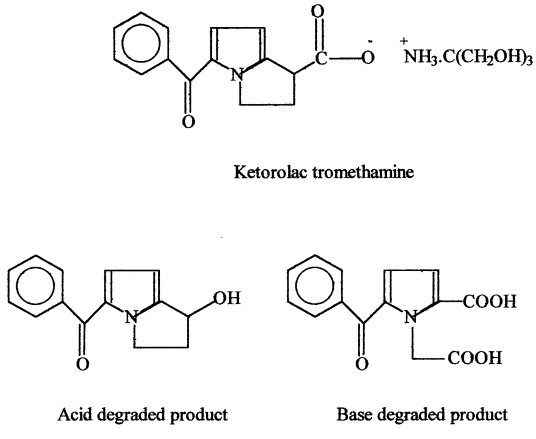

Fig. 1. Structure of ketorolac tromethamine, acid and base degraded product.

Camag HPTLC scanner II was used for densitometric evaluation of the developed plates with the following settings: photomode; absorbance/reflectance, λ, 323 nm; lamp source, deuterium; sensitivity, auto; scanning speed, 1 mm/s; and span, 10. Integration of chromatograms were performed using the Camag TLC scanner/integrator system with attenuation 32 and chart speed 25 mm/s. A solvent system consisting of chloroform–ethyl acetate–glacial acetic acid (3:8:0.1, v/v/v) was used.

2.4. Calibration curve

Calibration curve was prepared using a standard solution of 100 μg/ml in methanol ($n = 3$). One to ten microlitre volumes of standard solution were applied on the plate corresponding to concentrations of 100–1000 ng.

2.5. Stability of ketorolac tromethamine in stock solution

The stock solution of the drug was analysed at 1, 2 and 4 h after preparation by spotting 200 ng drug on the plate.

2.6. Precision study

Precision study was carried out as follows:

2.6.1. System precision

Six samples of the same concentration (200 ng) were spotted on silica gel 60 F254 plates and analysed by the proposed method.

2.6.2. Method precision

Six different samples of the same concentration (200 ng) were spotted on silica gel 60 F254 plates and analysed by the proposed method.

2.7. Ruggedness studies

The standard curve of ketorolac tromethamine was prepared and evaluated for within-day and week-to-week reproducibility.

2.8. Limit of detection and quantitation

Limit of detection and quantitation was determined by scanning the blank spot and noise was determined. Series of concentrations of the drug solution were spotted on the plate and analysed to determine LOD and LOQ by considering (S/N) ratio.

2.9. Marketed sample analysis

The drug was extracted from the tablets (label claim 10 mg/tablet) as follows:

Ten tablets were crushed to a fine powder and the equivalent to 10 mg of ketorolac tromethamine was dissolved in methanol with stirring for 30 min using a magnetic stirrer. The volume was adjusted to 50 ml with methanol to obtain a final concentration of 200 μg/ml. The resulting suspension was filtered through 0.22 μ nylon 13 mm filter and 2 μl solution analysed by HPTLC ($n = 6$). As the exact composition of the marketed formulation was not known, general tablet excipients, i.e. lactose, mannitol, magnesium sterate, talc and aerosil were stirred with methanol for 30 min and was analysed by the same manner as formulation to study the placebo effect on the analysis.

2.10. Recovery study

Recovery study was carried out by adding 25% and 50% of standard drug in a preanalysed sample of 400 ng. The resulting mixtures were analysed by HPTLC.

2.11. Degradation of ketorolac tromethamine

The degradation of ketorolac tromethamine solution (100 μg/ml) was carried out using 0.5 M hydrochloric acid and 0.5 M sodium hydroxide. The solution was boiled for 10 min with an equal volume of acid/base. Ten microlitres of the solution was spotted on the plates and analysed by HPTLC. Photodegradation of the drug was carried out by exposing the methanolic drug solution to direct sunlight at room temperature for 12 h.

2.12. Analysis of ketorolac tromethamine from saliva

2.12.1. Buccal absorption test

The buccal absorption test was performed with informed consent in six normal healthy volunteers (aged between 22 and 27 years). A solution of the drug (1 μg/ml) was prepared in a pH 7.2 phosphate buffer (0.05 M). After rinsing the mouth with buffer, 25 ml of the solution was agitated in the volunteers mouth for 5 min and expelled in a beaker. Immediately after, the mouth was rinsed with 5 ml of plain pH 7.2 buffer for 30 s and expelled in to the same beaker. The combined solutions were filtered through a G2 sintered glass filter and analyzed by HPTLC for ketorolac tromethamine. The standard solution of drug in pH 7.2 phosphate buffer was also spotted on the same plate (200 ng and 400 ng) and simultaneously analysed ($n = 3$).

3. Results and discussion

3.1. Standardization of the method

Various solvent systems were evaluated to arrive at an optimum resolution of pure drug and degraded products. The solvent system consisting chloroform–ethyl acetate–glacial acetic acid (3:8:0.1, v/v/v) gave dense, compact and well separated spots of the drugs from the degraded samples. The R_f values were found to be 0.4 which was visible under short wavelength (254 nm) ultraviolet light. Glacial acetic acid in the mobile phase liberates ketorolac from its tromethamine salt.

Table 1
Precision of the assay

Sample number	Area	
	System precision	Method precision
1	1217216	1215698
2	1272516	1258965
3	1262098	1289456
4	1298562	1245965
5	1234589	1285452
6	1219456	1269452
RSD	2.36	1.98

Table 2
Recovery studies

Sample number	Recovery level	Recovery (%)
1	500	98.32
	600	99.68
2	500	97.62
	600	101.02
Average recovery		99.2

Table 3
Within-day and week-to-week variation study of ketorolac tromethamine ($n = 3$)

	Mean slope	RSD
Within-day	2951.59	2.59
Week-to-week		
I	2945.85	3.45
II	2941.34	2.67
III	2942.59	1.89

Table 4
Marketed sample analysis

Formulation number	% Drug content ($\pm$SD)
1	100.91 ($\pm$1.489)
2	103.21 ($\pm$0.366)
3	104.75 ($\pm$0.509)
4	96.88 ($\pm$0.115)
5	97.65 ($\pm$0.683)
6	102.86 ($\pm$1.322)
Average drug content	101.37 ($\pm$0.747)

3.2. Calibration curve and validation of the method

All the standard curves were linear over the range of 200–700 ng. The mean values of coefficient of correlation, slope and intercept were 0.99, 2941 and 749583. The limit of detection for ketorolac tromethamine was 20 ng (S/N 3) and limit of quantitation was 40 ng (S/N 10). The results in Table 1 revealed excellent accuracy. The low coefficient of variation was indicative of the acceptable precision of the assay. The recovery of ketorolac tromethamine was 99.2% and is depicted in Table 2. During the stability study of drug in stock solution (methanol) there was no change in the drug concentration for 4 h.

3.3. Ruggedness studies

The results of the within-day and week-to-week variation evaluated by comparing the slopes of the standard curves of ketorolac tromethamine prepared on the same and different days are depicted in Table 3. Low percentage variation between the values indicated excellent within day and between day reproducibility of the method (ANOVA; $P > 0.05$).

3.4. Marketed sample analysis

The drug content of marketed tablets is shown in Table 4. There was no interference from the general tablet excipients. The average drug content was found to be 101.4%.

3.5. Degradation of ketorolac tromethamine

The acid degraded product of ketorolac tromethamine showed a R_f value of 0.62 while the base degraded product had a R_f value of 0.13 which is significantly different from the R_f value of ketorolac tromethamine (0.4) which is depicted in Fig. 2. The major degradation pathway for ketorolac tromethamine in aqueous solution may be autooxidative decarboxylation [14]. Depending on the previous report [9] the expected acid and base degraded products are depicted in Fig. 1. No photodegradation was observed. The developed

analytical method could therefore be considered a stability indicating method for analysis of ketorolac tromethamine.

3.6. Buccal absorption test

The standard solution of drug in pH 7.2 gave 99.39% average recovery, hence the same slope of the standard curve was utilized for the calculation of buccal absorption test results.

The results obtained from the buccal absorption test are summarized in Table 5. There was no interaction observed between saliva and drug as only one spot was observed ($R_f = 0.4$) indicating integrity of the drug. The results indicated that when a solution of ketorolac tromethamine in pH 7.2 buffer was swirled in the mouth for 5 min, on an average 73% of the drug was absorbed through the oral mucosa. Hence this method can be successfully utilized to determine the absorption of ketorolac tromethamine through oral mucosa which is very important for the development of transmucosal formulations.

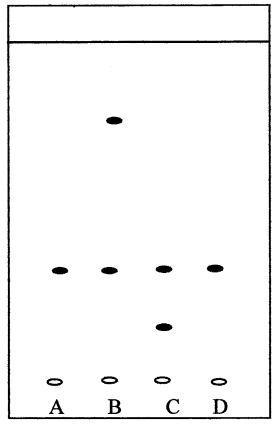

Fig. 2. Degradation of ketorolac tromethamine A, pure drug; B, acid degraded; C, base degraded; D, photodegraded.

Table 5
Buccal absorption test

Volunteer number	% Drug absorbed
1	81.0
2	78.0
3	75.0
4	68.0
5	70.0
6	66.0
Average drug absorbed	73.0

4. Conclusions

HPTLC coupled with UV densitometry using the selected solvent system provided rapid and reproducible quantification of ketorolac tromethamine from bulk drug and from pharmaceuticals. It could also be extended to study the degradation kinetics of the drug, stability of ketorolac tromethamine in pharmaceuticals and analysis of drug from biological fluids.

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