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Research Article

HPTLC Method Development for the Simultaneous Estimation of Ketorolac Tromethamine and Tramadol Hydrochloride from a Formulation

Vrushali S Tambe[1]*, Bijal P Gujarathi[1], Archana Karnik[2], SN Dhole[1]

[1]*Department of Quality Assurance, P. E. S's, Modern College of Pharmacy (For Ladies), Maharashtra, India*

[2]*Indira College of Pharmacy, Pune, India*

***Corresponding Author:** Vrushali S Tambe, Department of Quality Assurance Technique, PES's Modern College of Pharmacy (For Ladies), Maharashtra, India.

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Abstract

Objective: The study was aimed to develop simple, specific, accurate HPTLC method for simultaneous determination of the Ketorolac Tromethamine and Tramadol HCl in pharmaceutical dosage form.

Material and Method: A rapid, selective and simple high performance thin layer chromatographic method was developed and validated for their simultaneous estimation in a mixture. Well resolved peaks were observed for both the drugs on aluminium sheet with silica gel 60 F_{254} as the stationary phase. The solvent system consisted of ethyl acetate: methanol: 25% ammonia solution [8.5: 1.5: 0.5 v/v/v]. The λmax were observed at 282nm and 271nm for Ketorolac Tromethamine and Tramadol HCl respectively. Spectrodensitometric scanning-integration was performed at a wavelength of 282 nm.

Results: This system was found to give compact spots for both Ketorolac [Rf value of 0.08 ± 0.01] and Tramadol [Rf value of 0.52 ± 0.02]. The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 200-700 ng/band for ketorolac [r^2 =0.999] and 500-1750 ng/band for Tramadol [r^2 = 0.995]. The LOD and LOQ were found to be 0.3912 ng/band and 1.7930 ng/band for Ketorolac and 4.6370 ng/band and 7.7551 ng/band for Tramadol, respectively. The peak purity of both drugs was found to be always more than 0.995 proving the specificity of the method.

Conclusion: The method was validated for linearity, LOD, LOQ, specificity, accuracy and precision as per ICH guidelines. The proposed method has demonstrated to have a potential use in simultaneous analysis of Ketorolac tromethamine and Tramadol hydrochloride from a tablet formulation.

Keywords: Ketorolac Tromethamine; Tramadol Hydrochloride; HPTLC Method; Simultaneous Estimation

Abbreviations

KETO: Ketorolac Tromethamine; TRAM: Tramadol Hydrochloride

Introduction

Two drugs are used in this study are Ketorolac Tromethamine [KETO, Figure 1a, NSAID] and Tramadol Hydrochloride [TRAM, Figure 1b, Opioid analgesic]. The combination of KETO/TRAM is a rational therapy for pain by different mechanisms of action. Ketorolac is a carboxylic acid derivative mainly used for its analgesic activity. Tramadol is a centrally acting analgesic used to produce pain relief. The combination of ketorolac and tramadol analgesic efficacy is higher than each of its component individually and has a faster onset of action. Literature revealed analytical methods viz. HPLC [1-8], UPLC [9], HPTLC [10-13] and Spectrophotometric techniques [14-19] for analysis of individual drugs as well as in combinations with other drugs. But no single HPTLC method has been reported for the simultaneous estimation of KETO and TRAM in a formulation.

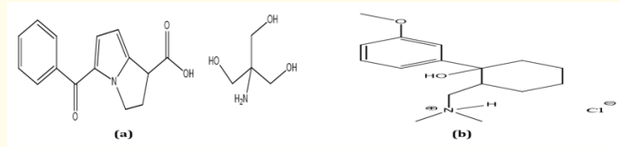

Figure 1: (a) Structure of Ketorolac Tromethamine. (b) Structure of Tramadol hydrochloride.

HPTLC is a reliable, fast and accurate for quantitative drug analysis. Moreover, many samples can be run simultaneously using a small quantity of mobile phase, thus minimizing analysis time and cost per analysis. So here an attempt has been made to develop simple, accurate, sensitive, rapid, economic and specific HPTLC method for simultaneous estimation of KETO and TRAM from a formulation.

Material and Method

Materials

KETO and TRAM were gifted by Dr. Reddy's Laboratories Ltd, India and Alkem Laboratories Ltd, India, respectively. Methanol was purchased from Merck Specialities Pvt. Ltd., India. Ethyl acetate and 25% Ammonia solution were purchased from Analab fine chemicals, Mumbai, India and were of analytical grade.

Instrumentation

Auminum-backed TLC plates (20 cm × 10 cm), coated with 250 µm layer of silica gel 60 F_{254} [E. Merck, Darmstadt, Germany supplied by Anchrom Technologists, Mumbai] were used as a stationary phase. Samples were applied to the plates as 8 mm bands, by means of 100 microlitre Linomat V applicator [Camag, Muttenz Switzerland] equipped with a Hamilton syringe [Bonaduz., Switzerland].

Method development

The pure drugs in methanol were applied as bands on the TLC plates and run in different solvent systems. After initial trials with neat solvents, dichloromethane: methanol in varying ratios was tried. Ethyl acetate: methanol [9:1 v/v] ratio was used. A mobile phase consisting of ethyl acetate: methanol: 25% ammonia solution in varying ratios, 9:1:0.5 v/v/v and 9:0.5:0.5 v/v/v were tried. To increase the compactness of band methanol concentration was increased. Finally the mobile phase ethyl acetate: methanol: 25% ammonia solution [8.5:1.5:0.5 v/v/v] was chosen for further analysis.

Development of mobile phase

Ethyl acetate: methanol: 25% ammonia solution [8.5:1.5:0.5 v/v/v] was used as a mobile phase, in a Camag 20 cm × 10 cm twin trough glass chamber [Camag, Muttenz, Switzerland]. The optimized chamber saturation time for mobile phase was 10 min at room temperature. The average development time was 20 min.

Detection of band

The slit dimensions were 4.00 mm × 0.45 mm and the scanning speed was 20mm s-1. Application rate of 1 µl/s was used and the space between two bands was 10 mm. Densitometric scanning was performed on Camag TLC Scanner 3 in the reflectance absorbance mode at 282 nm for all measurements and operated by WinCATS software version 3.15 supplied by Anchrom technologists, [Mumbai].

Method validation

Validation of HPTLC method was done as per ICH guidelines for following parameters.

Linearity

Stock solution containing KETO [0.1 mg/ml] and TRAM [0.25 mg/ml] was prepared in methanol. Aliquots (2- 7 µl) from stock solutions of KETO and TRAM were applied to get final concentration in the range of 200-700 ng/band for KETO and 500-1750 ng/band for TRAM in three replicates. Peak areas of developed spots were measured and average peak area was used to plot calibration curve. The results are given in Table 1 and 2.

Sr. No.	KETO		TRAM	
	Conc. [ng/band]	Peak Area*	Conc. [ng/band]	Peak Area*
1	200	1782.97	500	601.78
2	300	2357.50	750	1116.63
3	400	2990.21	1000	1564.81
4	500	3579.32	1250	1982.58
5	600	4111.32	1500	2345.39
6	700	4712.28	1750	2951.40

Table 1: Standard calibration curve data for KETO and TRAM.

*Mean of three estimations.

Parameters	KETO	TRAM
Linearity range [ng/band]	200 - 700	500 -1750
Regression coefficient	0.999	0.995
Intercept	620.2	277.7
Slope	5.856	1.811

Table 2: Statistical data of regression equation for KETO and TRAM.

Limit of detection and Limit of quantitation [LOD and LOQ]

LOD was calculated from the formula LOD = 3.3 σ/S while LOQ was calculated from the formula LOQ = 10 σ/S, where σ = Standard deviation of the response of calibration curve, S = Slope of the calibration curve.

Specificity

The specificity of the method was ascertained by comparing densitogram of developed mixture with that of diluents, mobile phase, standard drug sample of KETO and TRAM. The application volume for each spot was 5 µl. The band for KETO and TRAM in synthetic mixture was confirmed by comparing the Rf and spectra of the band with that of standard. The mobile phase resolved both the drugs very efficiently, as shown in Figure 2.

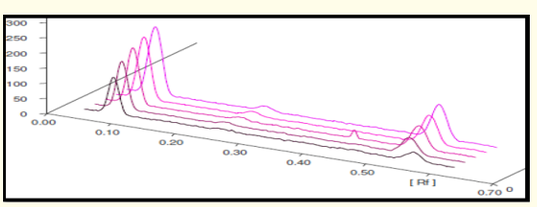

Figure 2: Chromatogram for KETO and TRAM.

Accuracy

The accuracy was determined by applying the proposed method to the formulation blend to which known quantity of KETO and TRAM has been added [corresponding to 80%, 100% and 120% w/w of the label claim of the drug]. At each level, three determinations were carried out by applying each sample three times on TLC plate. The mixtures were analysed by running chromatogram in optimized mobile phase. The mean recoveries for KETO and TRAM were calculated. For 80%, 8 mg of KETO and 20 mg of TRAM was added. For 100%, 10 mg of KETO and 25 mg of TRAM was added and for 120%, 12mg of KETO and 30 mg of TRAM was added and analysed as per the assay procedure.

Precision

Sample solution of concentration 500ng/band for KETO and 1250 ng/band for TRAM were used for studying degree of precision of the developed method. The reproducibility of sample application and measurement of peak area of drug was done six times from the same solution. The intra-day precision was determined by repeating the sample application six times on the same day at different time intervals. The inter-day precision was determined by repeating the sample application six times on different day. For method precision, above solution was prepared six times by independent weighting, applied separately on plate and analysed for peak area.

Analysis of developed formulation containing KETO and TRAM

A triturated mixture equivalent to 10 mg of KETO and 25 mg of TRAM was weighed and dissolved in 50 ml of methanol and then volume was made up to 100 ml using methanol so as to get a solution of 0.1 mg/ml of KETO and 0.25 mg/ml of TRAM. The 5µl of this solution was applied on the plate. The densitogram of six replicates was recorded. The peak area was measured and % content was determined by regression equation.

Result and Discussion

Development of optimum mobile phase

Well resolved bands were observed in a mobile phase consisting of ethyl acetate: methanol: 25% ammonia solution in varying ratios, 9:1:0.5 v/v/v and 9:0.5:0.5 v/v/v. To increase the compactness of band, methanol concentration was increased. Finally the mobile phase ethyl acetate: methanol: 25% ammonia solution [8.5:1.5:0.5 v/v/v] gave well resolved compact bands with an Rf value of 0.08 ± 0.01 for KETO and 0.52 ± 0.02 for TRAM with 20 minutes chamber saturation time.

Method validation

Linearity

From stock solution, KETO and TRAM were spotted on the TLC plate to obtain final concentration 200-700 ng/band and 500-1750 ng/band respectively. The linearity correlation was obtained between the peak area and the corresponding concentration as shown in Table 1 and 2. The regression equations were calculated as Y=5.856x+629.2, r^2= 0.999 for KETO (Figure 3) and Y=1.811x-277.7, r^2= 0.995 for TRAM (Figure 4). The low Y intercept value and correlation coefficient close to 1 proves linearity of method.

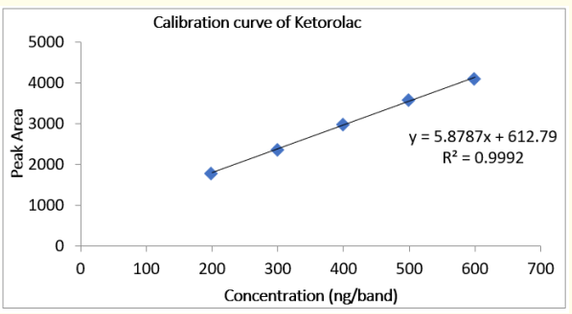

Figure 3: Calibration Curve of KETO.

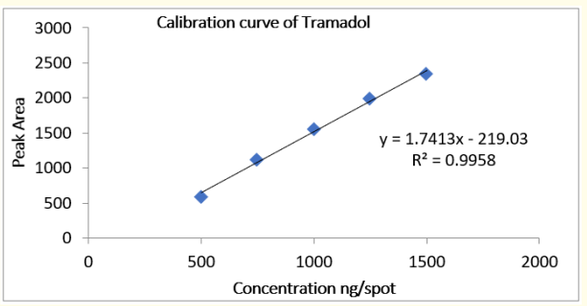

Figure 4: Calibration Curve of TRAM.

Limit of detection and limit of quantitation

The LOD and LOQ for KETO were found to be 0.3912 ng/band and 1.7930 ng/band, respectively. The LOD and LOQ for TRAM were found to be 4.6370 ng/band and 7.7551 ng/band, respectively. Sensitivity of method was proved by low LOD.

Specificity

The chromatogram of formulation showed only two peaks for KETO and TRAM (Figure 5), indicating that there is no any interference of any of the excipients of the formulation.

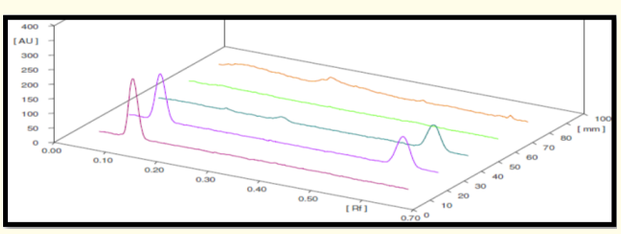

Figure 5: Chromatogram of formulation containing only two peaks KETO and TRAM.

Accuracy

The % recovery was found to be within the limits of the acceptance criteria i.e. 95% w/w to 105% w/w and the results are given in Table 3.

Drug [ng/spot]	Amount added [%]	% Recovery [w/w]	± SD	%RSD
KETO	80	95.08	0.3890	0.4091
	100	97.15	0.8714	0.897
	120	97.29	0.6240	0.6413
TRAM	80	98.92	0.5190	0.5247
	100	95.92	0.6929	0.7224
	120	96.52	0.3120	0.3233

Table 3: Recovery analysis of KETO and TRAM.

Precision

Precision of the method was assessed in terms of repeatability, intra-day and inter-day precision as per the recommendations stated in the ICH guidelines. The results were found to be well within the acceptance limits with % RSD less than 2.

Analysis of tablet formulation

The mean % drug content was found to be 99.40% w/w and 102.09% w/w for KETO and TRAM respectively. The mean % drug content with %RSD of mixture is given in Table 5.

Drug	Conc [ng/band]	Repeatability	Intra-day	Inter-day
		Peak area ± RSD	Peak area± RSD	Peak area ± RSD
KETO	500	3547.98 ± 0.5425	3572.20 ± 1.1189	3514.34 ± 1.4549
TRAM	1250	1853.52 ± 1.7621	1843.36 ± 0.9622	1843.25 ± 1.8224

Table 4: Precision of KETO and TRAM.

*mean of six readings.

Drug	Label Claim [ng/band]	%Mean ± SD*	% RSD
KETO	500	99.40 ± 1.716	1.817
TRAM	1250	102.09 ± 1.586	1.454

Table 5: Assay results for KETO and TRAM.

*Mean of six replicates.

Conclusion

HPTLC proved to be a useful method for simultaneous estimation of KETO and TRAM from a tablet formulation. It was found to be a simple, rapid, accurate, precise, specific and economical method. The developed method can be used for routine analysis of two drugs in combined dosage forms.

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