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Research Article

A NEW REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF KETOROLAC TROMETHAMINE AND TRAMADOL HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Objective: This study was embarked upon to develop a new, simple, rapid, validated reversed-phase high-performance liquid chromatography (HPLC) method for the estimation of ketorolac tromethamine (KET) and tramadol hydrochloride (TDL) in pharmaceutical dosage forms.

Methods: The HPLC method was developed on Shishiedo C18 column (250 mm × 4.6 mm i.d, 5 µ) using methanol: 50 mM phosphate buffer (pH 6.0) in the ratio of 52:48 at 282 nm.

Results: Retention time for the drugs was found to be 5.1 and 6.9 minutes for tramadol and ketorolac, respectively. The limit of detection for tramadol and ketorolac were found to be 1.0 and 0.1 µg/ml, limit of quantitation for tramadol and ketorolac were found to be 5.0 and 0.5 µg/ml, respectively. Linearity was established in the range of 20.0-30.0 µg/ml and 8.0-12.0 µg/ml for TDL and KET, respectively. The method was precise with % relative standard deviation <2 for both intra- and interday precision. The accuracy of the method was performed over three levels of concentration, and the recovery was in the range of 98-102%.

Conclusion: From the found experimental data, it can be concluded that the developed method is accurate, precise, and selective and can be employed successfully for the estimation of KET and TDL in Pharmaceutical dosage forms.

Keywords: Reversed-phase high-performance liquid chromatography, Ketorolac tromethamine, Tramadol hydrochloride.

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INTRODUCTION

Tramadol hydrochloride (TDL) (Fig. 1) and ketorolac tromethamine (KET) (Fig. 2) are analgesic drugs commonly used in combination in post-operative pain management [1]. Tramadol HCl is a synthetic, centrally acting analgesic with no anti-inflammatory activity and one of the most interesting and useful weak opioids for treatment of moderate to moderately severe pain with weak µ-receptor agonist properties and noradrenergic and serotonergic neurotransmission effects [2-8].

This study is to develop a reversed-phase high-performance liquid chromatography (RP-HPLC) method for KET and TDL. A literature survey reveals several analytical methods for estimation of KET and TDL individually and in combination with them and other drugs based on ultraviolet (UV) [9-11], HPLC [12-23], liquid chromatography–mass spectrometry [24], and ion-pair chromatography [25] were reported. However, there are few methods reported for the method development of TDL and KET; the present aim is to develop a more precise, accurate and simple RP-HPLC method for the estimation of TDL and KET. The molar absorptivity of TDL and KET was found maximum at 282 nm. The validated method was used for the quantification of marketed formulation containing TDL and KET.

METHODS

Chemicals and reagents

KET and TDL working standards were procured from Hetero Laboratories Ltd. Commercially available as GAMMADOL (tramadol – 25 mg + ketorolac – 10 mg) tablets were purchased from the local pharmacy. Orthophosphoric acid and HPLC grade methanol were purchased from Merck Specialties Pvt. Ltd., Mumbai. HPLC grade water was purchased from Thermo Fisher Scientifics Ltd., Mumbai.

Instrumentation and analytical conditions

RP-HPLC method was performed on the HPLC system (Shimadzu) consisting of binary gradient pump with UV detector (LC-20AD). Rheodyne injector with 20 µl fixed loop was used for injecting sample in this study.

Preparation of solutions

Preparation of standard solutions

About 10 mg of tramadol and ketorolac working standards were accurately weighed and transferred into two 10 ml volumetric flasks and dissolved in methanol and water (50:50) solution and made up to the volume with the same solvent to produce a 1 mg/ml of tramadol and ketorolac, respectively. The both solutions were scanned in UV spectrophotometer from 200-400 nm and the iso absorptive point is taken for method development (Fig. 3). The stock solutions were stored in refrigerator at −20±2°C until analysis.

The stock solutions were diluted to suitable concentrations with methanol and phosphate buffer (50:50) solution to obtain calibration curve (CC) standards and quality control samples.

Preparation of the sample solutions

About 20 tablets were taken and their average weight was calculated; tablets were crushed to fine powder and powder equivalent to 25 mg of tramadol was taken into 10 ml volumetric flask and methanol: water (50:50) was added to obtain a concentration of TDL (2500 µg/ml) and KET (1000 µg/ml). 1 ml of the above solution was taken in a 10 ml volumetric flask and diluted to 10 ml with diluent methanol: buffer (50:50 v/v) to obtain a concentration of 250 µg/ml TDL and 100 µg/ml KET. 1 ml of the above solution was taken in a 10 ml volumetric flask and diluted to 10 ml

with diluent methanol: buffer (50:50 v/v) to obtain a concentration of 25 µg/ml TDL and 10 µg/ml KET.

Optimized analytical method

- Stationary phase: Shiseido C_{18} (250 × 4.6 mm i.d., 5 m)
- Mobile phase: Methanol: 50 mM phosphate buffer (pH 6.0)
- Mobile phase ratio: 52:48
- Flow rate: 1.0 ml/min
- Sample volume: 20 ml
- Detection: 282 nm
- Drug diluent: Methanol: buffer (50:50).

Method validation

The developed methods were validated according to International Conference on Harmonization (ICH) guidelines for validation of analytical procedures. Validation was done as per ICH guideline Q2 (R1).

System suitability

The system suitability of the HPLC method was determined by making six replicate injections from freshly prepared standard solutions and analyzing each solute for their retention time, theoretical plates number (N) and tailing factors (T).

Specificity

It is the ability to assess unequivocally the analyte in the presence of impurities, degradants, and matrix. To determine this, 20 µl of blank, standard and sample solutions were injected separately in triplicate, and respective chromatograms were recorded under the optimized conditions.

Linearity

The CCs were obtained with concentrations of the standard solutions of 20-30 and 8-12 µg/ml for TDL and KET, respectively. Linearity was evaluated by regression analysis, which was calculated by the least square regression method.

Accuracy

To check the degree of accuracy recovery studies were performed in triplicate by the standard addition method at 80%, 100%, and 120% levels.

Precision

Precision was checked by analyzing the samples at different time intervals of the same day (intraday precision) as well as on different days (interday precision).

Table 1: System suitability parameters of KET, TDL

S.No.	Parameters	Tramadol	Ketorolac
1	Theoretical plates	4987	7435
2	Resolution factor	-	5.90
3	Asymmetric factor	0.23	0.35

KET: Ketorolac tromethamine, TDL: Tramadol hydrochloride

Table 2: Linearity of KET and TDL

TDL		KET	
Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area
0	0.00	0	0.00
20.0	89,925	8.0	23,247
22.5	123,011	9.0	34,034
25.0	163,196	10.0	42,297
27.5	202,372	11.0	48,858
30.0	243,171	12.0	56,133
Correlation coefficient	0.998	Correlation coefficient	0.999

KET: Ketorolac tromethamine, TDL: Tramadol hydrochloride

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were calculated using the values of signal to noise (S/N) ratio for two drugs. For LOD S/N should be 3:1 and 10:1 for LOQ.

Robustness

Robustness was determined by analysis of samples under slight variations in chromatographic conditions. The flow rate of the mobile phase was changed from 0.9 to 1.1 ml/min. The ratio of the organic phase was changed by 2%, i.e., 54% and 50% of the aqueous phase. The effect of retention time and peak parameter was studied.

Assay of pharmaceutical dosage form

About 20 µl of each standard and sample solution were injected and from the peak areas of KET, and TDL amount of each drug in samples was computed.

RESULTS AND DISCUSSION

System suitability

About 20 µl of working standard solution containing 1 µg/ml of KET and 1.5 µg/ml of TDL was prepared and injected into the system under optimized chromatographic conditions. Chromatograms were recorded and studied for different system suitability parameters such as tailing factor, theoretical plates, resolution and peak area, peak heights were also studies. Six different working standard solutions were injected to study this parameter, and all the suitability parameters were found to be within the limits. The system suitability parameters were shown in Table 1.

Specificity

The HPLC chromatograms were recorded for blank (Fig. 4) and standard (Fig. 5) under optimized analytical conditions and compared with that of the standard solution with no additional peaks. The two peaks were completely separated in HPLC chromatogram, and the resolution was found to be more than 2. Even in the presence of excipients of the sample no interfering peaks were found in HPLC chromatogram.

Linearity

The CCs of KET and TDL were constructed in the concentration range of 8-12 µg/ml, 20-30 µg/ml of KET and TDL, respectively. The plots obtained from linear regression and residuals analysis are given in (Table 2 Fig. 6).

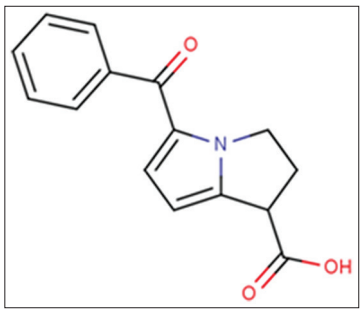

Fig. 1: Chemical structure of ketorolac tromethamine

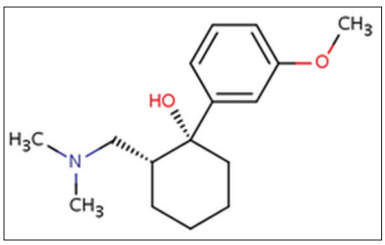

Fig. 2: Chemical structure of tramadol hydrochloride

Accuracy

The accuracy for proposed method was determined, recovery studies were performed in mentioned levels and recorded (Tables 3 and 4), obtained results were found to be within the limits of 98-102%, indicating an agreement between the true value and found value.

Precision

Precision was calculated as intra- and interday variations for the drugs. Percent relative standard deviations for estimation of KET and TDL under intra- and interday variations were found to be <2 (Tables 5 and 6).

Table 3: Accuracy of TDL

Level	Concentration of sample (µg/ml)	Concentration of standard (µg/ml)	Amount of drug recovered (µg/ml)	Recovery (%)	Mean recovery (%)
Level I	20.0	20.0	39.24	98.10	98.50
			39.42	98.55	
			39.54	98.85	
Level II	25.0	20.0	44.14	98.08	98.32
			44.25	98.33	
			44.35	98.55	
Level III	30.0	20.0	50.35	100.70	100.47
			50.21	100.42	
			50.15	100.30	

TDL: Tramadol hydrochloride

Table 4: Accuracy of KET (±SD)

Level	Concentration of sample (µg/ml)	Concentration of standard (µg/ml)	Amount of drug recovered (µg/ml)	Recovery (%)	Mean recovery (%)
Level I	8.0	10.0	18.08	100.44	100.38±1.02
			17.98	99.88	
			18.15	100.83	
Level II	10.0	10.0	19.89	99.45	100.03±0.98
			20.14	100.70	
			19.99	99.95	
Level III	12.0	10.0	21.90	99.54	99.89±1.01
			22.05	100.22	
			21.98	99.90	

SD: Standard deviation, KET: Ketorolac tromethamine, TDL: Tramadol hydrochloride

Table 5: Intraday precision of KET and TDL

S.No	TDL			KET		
	LQC	MQC	HQC	LQC	MQC	HQC
	20.0 µg/ml	25.0 µg/ml	30.0 µg/ml	8.0 µg/ml	10.0 µg/ml	12.0 µg/ml
1	92,685	170,856	224,587	124,568	245,698	365,987
2	93,856	171,232	225,634	125,879	245,273	365,249
3	91,876	173,985	226,589	123,569	245,859	364,690
Mean±SD	92,802.6±998.70	172,024.3±1708.36	225,603.3±1001.35	124,672.0±1158.50	245,610.0±302.74	365,308.7±650.55
CV (%)	1.07	0.99	0.44	0.92	0.12	0.17
N	3	3	3	3	3	3

LQC: Lower quality control, MQC: Middle quality control, HQC: Higher quality control, SD: Standard deviation, KET: Ketorolac tromethamine, TDL: Tramadol hydrochloride, CV: Coefficient of variation

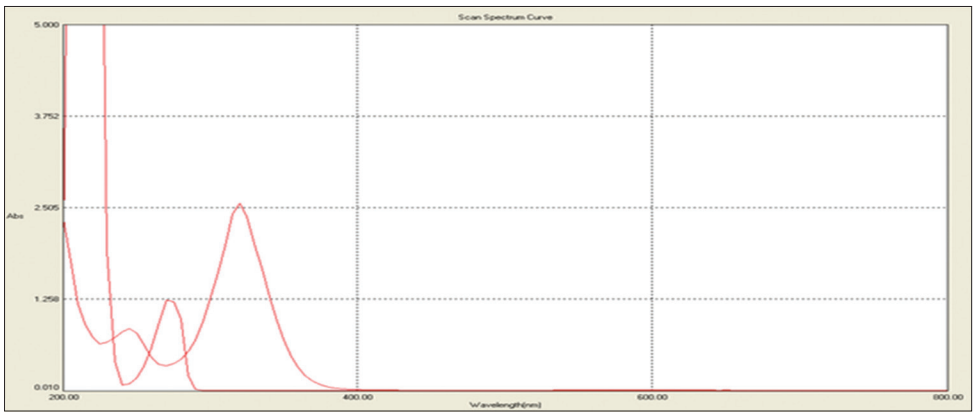

Fig. 3: Overlay spectrum of ketorolac tromethamine and tramadol hydrochloride

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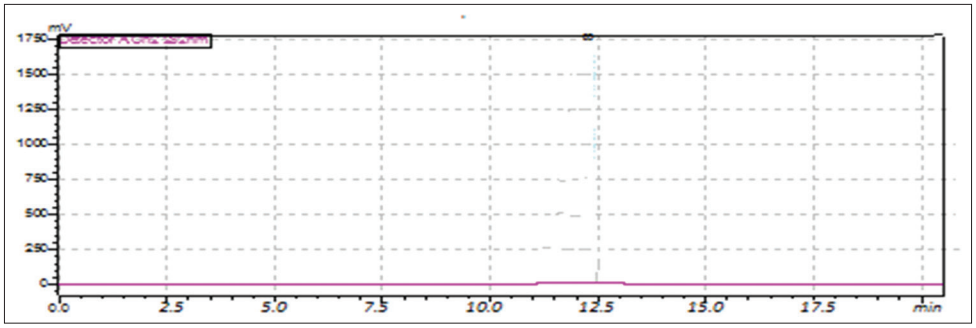

Fig. 4: Chromatogram of blank

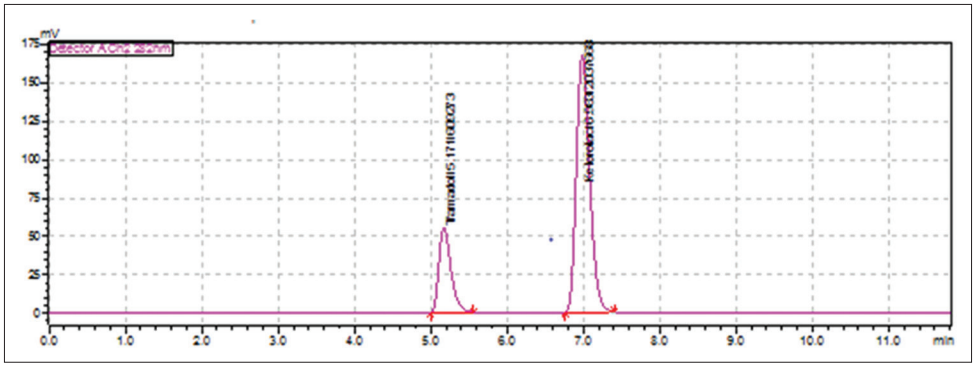

Fig. 5: Chromatogram of well resolved peaks of ketorolac tromethamine and tramadol hydrochloride

Table 6: Intraday precision of KET and TDL

S.No	TDL			KET		
	LQC	MQC	HQC	LQC	MQC	HQC
	20.0 µg/ml	25.0 µg/ml	30.0 µg/ml	8.0 µg/ml	10.0 µg/ml	12.0 µg/ml
1	92,596	171,256	224,365	124,956	245,659	365,789
2	93,013	170,986	225,012	125,032	245,498	365,653
3	92,858	172,013	226,124	123,998	243,365	364,986
Mean±SD	92,822.3±210.79	171,418.3±532.39	225,167.0±889.68	124,662.0±576.29	244,840.7±1280.49	365,476.0±429.76
CV (%)	0.22	0.31	0.39	0.46	0.52	0.11
N	3	3	3	3	3	3

CV: Coefficient of variation, LQC: Lower quality control, MQC: Middle quality control, HQC: Higher quality control, SD: Standard deviation, KET: Ketorolac tromethamine, TDL: Tramadol hydrochloride

LOD and limit of quantitation (LOQ)

The LOD and LOQ were calculated according to the S/N ratio of the respective drugs. The concentration of the drugs is reduced with definite interval of concentration and injected into HPLC. The concentration with S/N 3:1 is taken as LOD and concentration with S/N 10:1 is taken as LOQ (Table 7).

Robustness

For robustness studies, conditions like flow rate and concentration of organic phase were changed, and method was performed. In all deliberately varied conditions, percent relative standard deviations for peak areas, retention times, theoretical plates, and tailing factor were found to be <2% (Table 8).

Assay

The percent of the assay was calculated using absorbances using peak areas of standard and sample. The experimental values obtained for the determination of KET and TDL in Pharmaceutical formulation were within the claimed limits (Table 9).

Table 7: LOD and LOQ of KET and TDL

Drug	LOD (µg/ml)	LOQ (µg/ml)
KET	0.5	0.1
TDL	5.0	0.5

KET: Ketorolac tromethamine, TDL: Tramadol hydrochloride, LOD: Limit of detection, LOQ: Limit of quantification

Table 8: Robustness parameters of KET and TDL

S.No.	Parameter	KET Rt (min)	TDL Rt (min)
1	Initial flow	6.95	5.1
2	Flow 0.9 ml/min	6.99	5.3
3	Flow 1.1 ml/min	6.80	5.0
4	Initial organic phase concentration	6.95	5.1
5	Organic phase, 2% less	7.00	5.4
6	Organic phase, 2% more	6.90	5.2

KET: Ketorolac tromethamine, TDL: Tramadol hydrochloride

CONCLUSION

The proposed RP-HPLC were developed and validated as per ICH guidelines. The standard deviation and % relative standard deviation calculated for the proposed methods are low, indicating a high degree of precision of the method. The results of the recovery studies performed

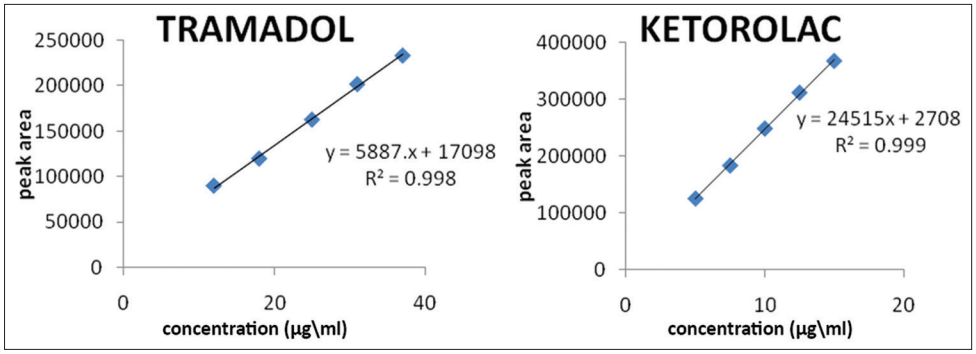

Fig. 6: Graph of linearity of ketorolac tromethamine and tramadol hydrochloride

Table 9: Assay data of marketed formulation

Drug	Amount labeled (mg)	Amount found	% Assay
KET	10	9.65 mg	99.36
TDL	25	24.74 mg	99.71

KET: Ketorolac tromethamine, TDL: Tramadol hydrochloride

show the high degree of accuracy of the proposed methods. The RP-HPLC method could selectively quantify KET and TDL present in the formulations. From the found experimental data, it can be concluded that the developed method is accurate, precise and selective and can be employed successfully for the estimation of KET and TDL in pharmaceutical dosage form.

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