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Original Article

RP-HPLC-METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF DEXKETOPROFEN TROMETAMOL AND TRAMADOL HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: For the measurement and quantification of Dexketoprofen Trometamol and Tramadol Hydrochloride, a rapid, sensitive, and reliable RP-HPLC technique using the Waters HPLC System with PDA detection was designed and validated.

Methods: Chromatography was carried out on an Inertsil-ODS C18 (250 x 4.6 mm, 5) column with a flow rate of 1.0 ml/min and effluent of 240 nm using filtered and mixed Degassed Methanol: Buffer (75:25) v/v as a mobile phase.

Results: Dexketoprofen Trometamol and Tramadol hydrochloride has a retention time of 3.617 min and 5.013 min, respectively. % recovery for Dexketoprofen trometamol and tramadol hydrochloride was found to be 100.21% and 100.20%, respectively. LOD and LOQ values obtained for Dexketoprofen trometamol and tramadol hydrochloride are 0.074µg/ml, 0.225µg/ml and 0.175µg/ml, 0.531µg/ml respectively. Regression equation of Dexketoprofen trometamol is y = 7446.1273x-284.0702 and y = 39535.8782x-1580.4211 of Tramadol hydrochloride.

Conclusion: Dexketoprofen trometamol and tramadol hydrochloride has developed and validated in pharmaceutical dosage form using RP-HPLC method.

Keywords: Reverse phase-high performance liquid chromatography, Validation, Dexketoprofen trometamol, Tramadol hydrochloride

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INTRODUCTION

Dexketoprofen trometamol is a non-steroidal anti-inflammatory drug with antianalgesic, antipyretic and anti-inflammatory properties and having an IUPAC name of (2S)-2-(3-benzoylphenyl) propanoic acid; 2-amino-2-(hydroxymethyl)propane-1,3-diol [1]. The structure of Dexketoprofen trometamol is shown in fig. 1 [2].

Tramadol hydrochloride is a centrally acting synthetic opioid analgesic and serotonin reuptake inhibitor having an IUPAC name of 2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexan-1-ol hydrochloride [3]. The structure of tramadol hydrochloride is shown in fig. 2 [4].

Both drugs work to generate good analgesia at a relatively low dosage for the treatment of moderate to severe acute pain [5].

HPLC is a widely used analytical technique for the separation and quantification of several mixtures [6]. Several literatures show different methods for the estimation of Dexketoprofen trometamol and Tramadol hydrochloride [7-11]. The current work aims to develop and validate an operative HPLC method for simultaneous determination with good retention and resolution.

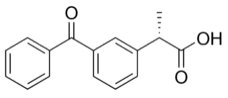

Fig. 1: Structure of dexketoprofen trometamol [2]

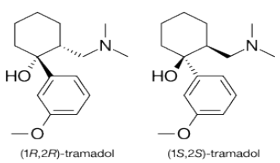

Fig. 2: Structure of tramadol hydrochloride [4]

MATERIALS AND METHODS

The API Dexketoprofen trometamol and Tramadol hydrochloride was obtained from Metro chem api pvt Ltd, Hyderabad. The marketed formulation used is Skudexa (Dexketoprofen trometamol 25 mg, Tramadol hydrochloride 75 mg), Metrochem api pvt Ltd, Hyderabad, India. Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen orthophosphate, and Orthophosphoric acid are from Rankem. Denver Electronic balance, BVK Enterprise p^H meter and Ultrasonicator, Thermo Scientific Hot air oven and Refrigerator, Millipore BM2EA9672R, WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and autosampler integrated with Empower 2 Software. UV-VIS spectrophotometer PG Instruments T60 was used for measuring absorbances of Dexketoprofen trometamol and Tramadol hydrochloride solutions.

Methodology

Diluent

Based on the solubility of the drugs, Acetonitrile and methanol taken in theratio of 50:50v/v.

Preparation of solutions

Preparation of standard stock solutions

Accurately weighed and transferred 10 mg of Dexketoprofen trometamol A standard working drug transferred into a dry clean 10 ml volumetric flask and add 5 ml of diluent and sonicated for 20 min then make up the volume withdiluent (i.e. 1000µg/ml or 1000 ppm).

Preparation of standard working solution (100%)

1 ml of the resulting solution was pipetted out and transferred into a 10 ml volumetric flask and made up to 10 ml with diluent (i.e.100µg/ml or100 ppm).

Preparation of sample stock solution and sample working solution (100% solution)

20 tablets were weighed and powdered which are equivalent to 100 mg and then transferred into a 100 ml of the volumetric flask, 50 ml

of diluent was added and sonicated for 25 min. Further, the volume was made up with diluent and filtered through 0.45 µm filter paper. To 10 ml volumetric flask, transfer 1 ml of filtered sample stock solution and maeupto10 ml with diluent.

Optimization of chromatographic conditions

By changing the method development parameters like mobile phase ratios, buffers, flow rate, columns and run time used for the analysis of dexketoprofen trometamol and tramadol hydrochloride. Sustainable retention time, good resolution, tailing factor and theoretical plates were observed with optimized chromatographic conditions, as mentioned in table 1. The optimized chromatogram is shown in fig. 3. According to ICH guidelines, method validation and optimization were performed [12].

Method validation

As per the ICH guidelines, method validation was performed [13]. The method was validated for the parameters like system suitability, specificity, linearity, precision (system precision and repeatability), accuracy, the limit of detection and limit of quantification, robustness, and assay as per ICH guidelines.

Table 1: Optimized chromatographic conditions

S. No.	Parameters	Method
1	Stationary phase (column)	Inertsil-ODS C18(250x4.6 mm, 5 µ)
2	Mobile phase	Methanol: Buffer (75:25) v/v
3	Flow rate (ml/min)	1.0 ml/min
4	Run time (minutes)	10 min
5	Column temperature (°C)	Ambient
6	Injection volume	20 µl
7	Detection wavelength	240 nm
8	Drug RT (min)	3.617 min for Dexketoprofen trometamol and 5.013 min for Tramadol Hcl

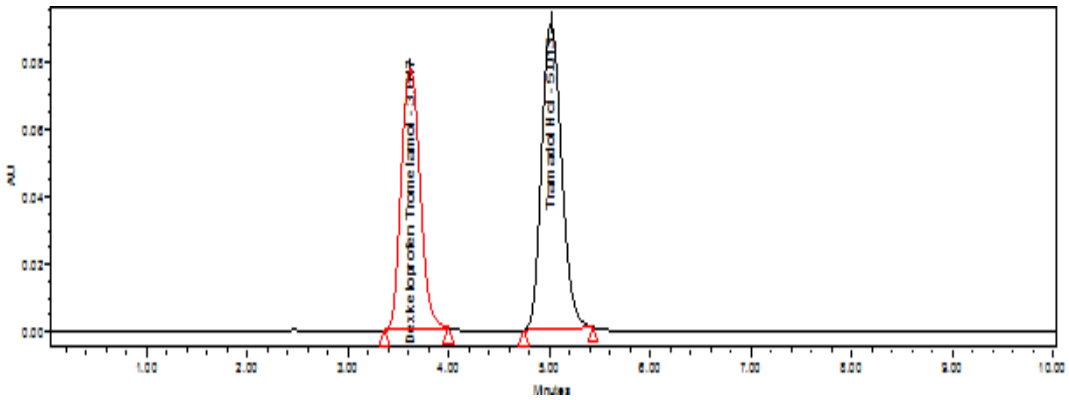

Fig. 3: Optimized chromatogram

System suitability

This test should be carried out to verify that the analytical system is working properly and can give accurate and precise results. Standard solutions of Dexketoprofen trometamol (100 ppm) and Tramadol hydrochloride (150 ppm) were injected five times and the parameters like peak tailing, resolution and USP plate count were determined and the results of system suitability were shown in table 2. According to ICH guidelines, the plate count should be more than 2000, the tailing factor should be less than 2, and the resolution must be more than 2. All the system suitability parameters were passed and were within the limits.

Table 2: System suitability parameters for dexketoprofen trometamol and tramadol ydrochloride

S. No.	Dexketoprofen trometamol			Tramadol hydrochloride			
Injection	Rt (min)	USP plate count	Tailing	Rt (min)	USP plate count	Tailing	Resolution
1	3.617	21023.8	1.094	5.013	8325.8	1.05	3.6
2	3.621	21010.5	1.101	5.019	8384.5	1.07	3.6
3	3.619	21036.8	1.076	5.018	8314.8	1.05	3.6
4	3.616	21027.2	1.059	5.018	8372.7	1.05	3.6
5	3.617	21084.6	1.107	5.018	8392.0	1.08	3.6

Specificity

The specificity of the method is performed by separately injecting the blank, standard, and sample solutions. The interference observed (if any) at the retention times of each analyte in all the chromatograms is evaluated. Chromatograms were as shown in fig. 4 and fig. 5. Retention times of Dexketoprofen trometamol and Tramadol hydrochloride were 3.617 min and 5.018 min, respectively. The method is specified as no interfering peaks were observed in blankat retention times of the drugs.

Linearity

Standard solutions of 25%, 50%, 75%, 100%, 125%, and 150% concentrations were prepared by taking 0.25, 0.5, 0.75, 1.0, 1.25, 1.5 ml each from two standard stock solutions and make up to 10 ml [14]. Six linear concentrations of Dexketoprofen trometamol (20-80 µg/ml) and tramadol hydrochloride (20-80 µg/ml) were injected in a duplicate manner. Peak areas were recorded for each injected concentration and the calibration curves-concentration vs. peak area were constructed fig. 6. and fig. 7 and the chromatograms were shown in fig. 8-14. The results were given in table 3 and table 4.

Linearity equations obtained for Dexketoprofen trometamol was y = 7446.1x-284.0 and of Aclidinium bromide was y =39536x-1580.4. Correlation coefficient obtained was 0.9996 and 0.999 for the two drugs.

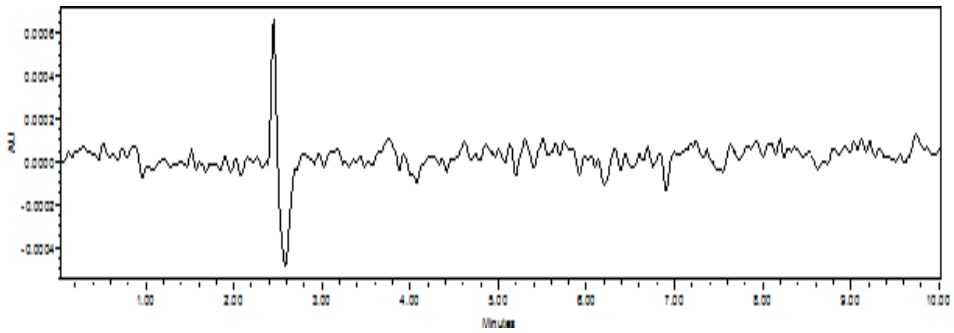

Fig. 4: Chromatogram of blank

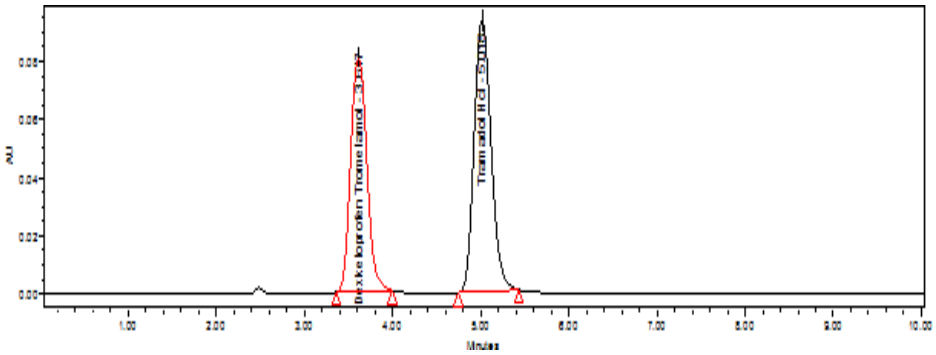

Fig. 5: Chromatogram of standard

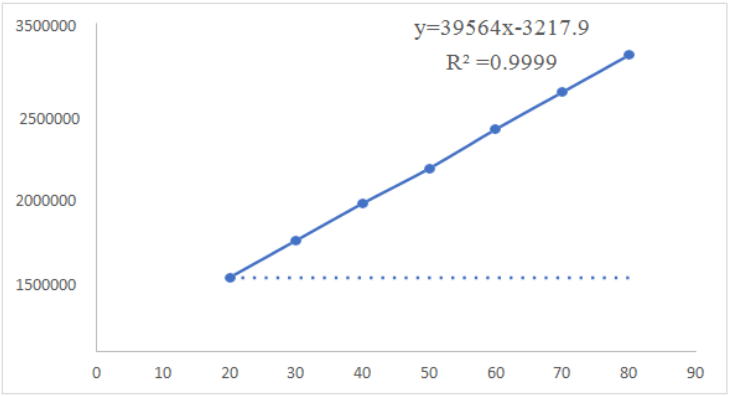

Fig. 6: Calibration curve of dexketoprofen trometamol

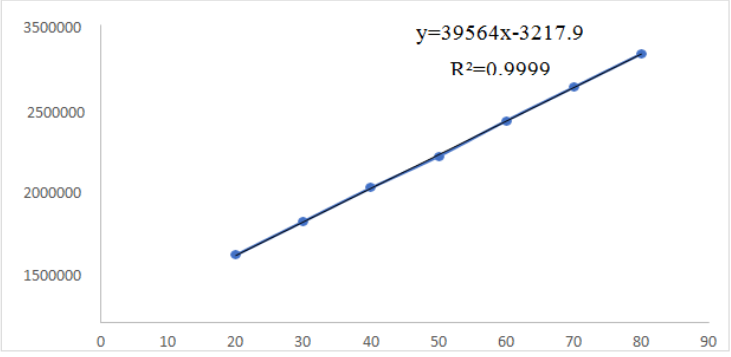

Fig. 7: Calibration curve of tramadol hydrochloride

Table 3: Results for linearity of dexketoprofen trometamol

Dexketoprofen trometamol	
Conc. (µg/ml)	**Peak area**
20	149024
30	223539
40	298048
50	368512
60	447072
70	521584
80	596096

Table 4: Results for linearity of tramadol hydrochloride

Tramadol hydrochloride	
Conc. (µg/ml)	**Peak area**
20	791282
30	1186923
40	1582564
50	1955684
60	2373864
70	2769487
80	3165128

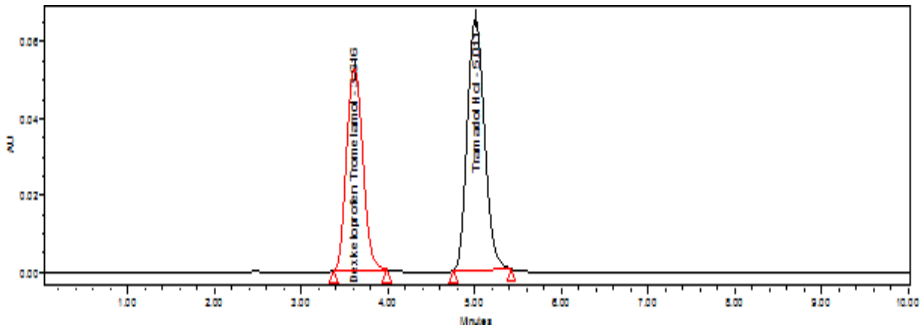

Fig. 8: Chromatogram for linearity 20%

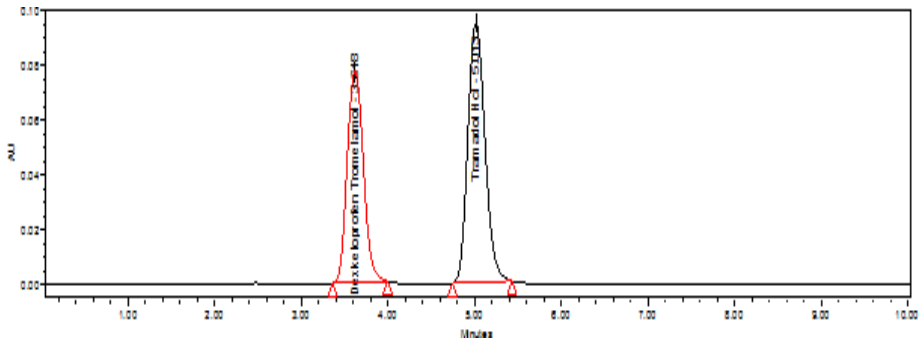

Fig. 9: Chromatogram for linearity 30%

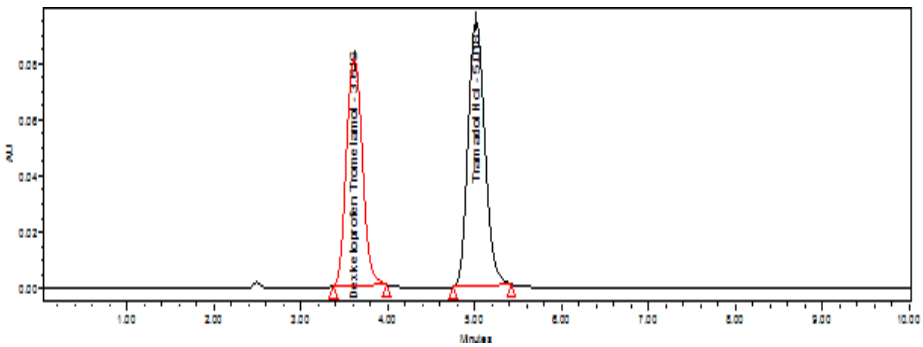

Fig. 10: Chromatogram for linearity 40%

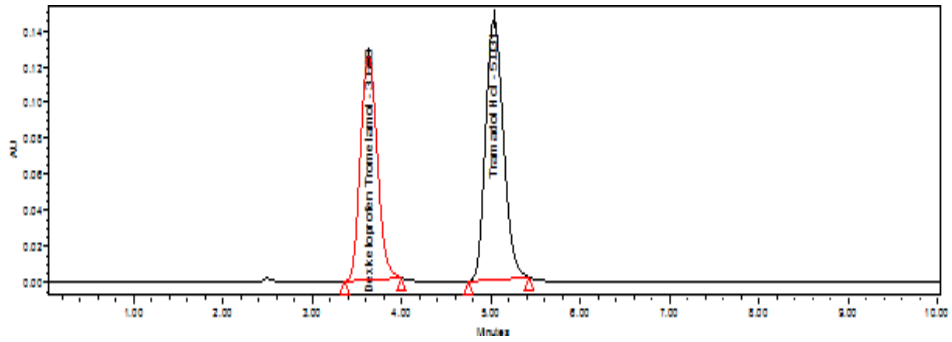

Fig. 11: Chromatogram for linearity 50%

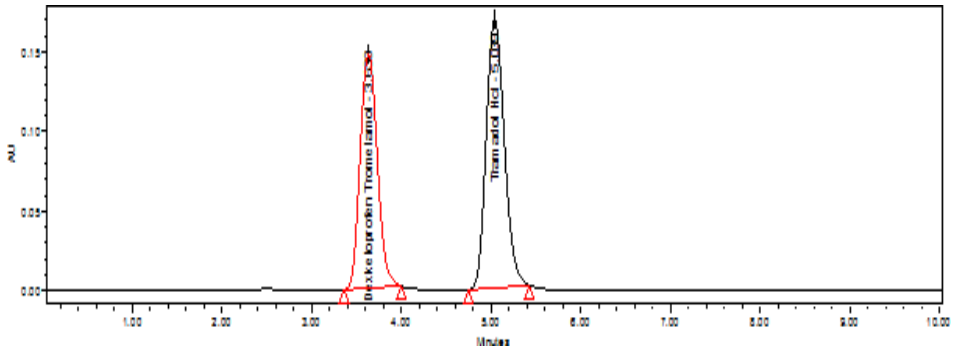

Fig. 12: Chromatogram for linearity 60%

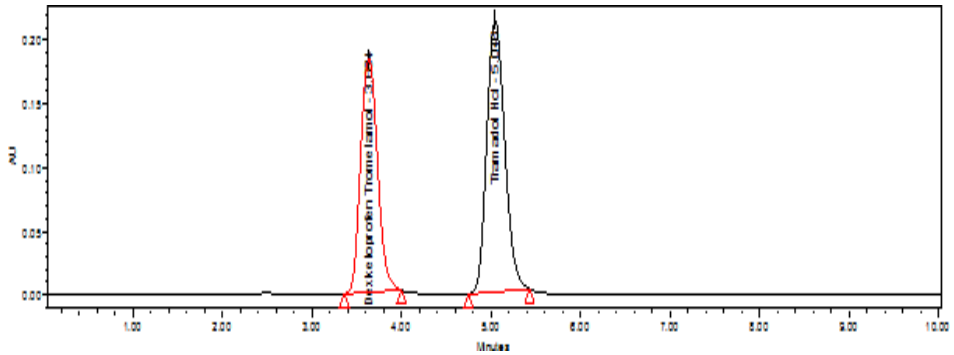

Fig. 13: Chromatogram for linearity 70%

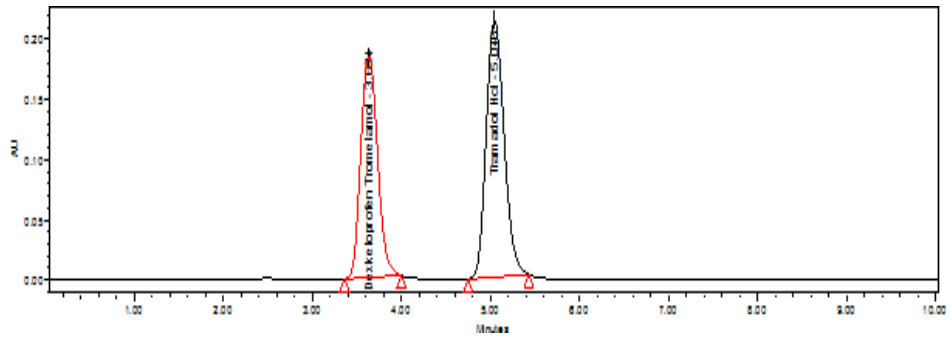

Fig. 14: Chromatogram for linearity 80%

Precision

System precision

System precision was determined by injecting 15 µl standard solution six times and the chromatograms were recorded. Average area, standard deviation, and %RSD were calculated for two drugs and the results are shown in table 5. %RSD was obtained as 0.88% and 1.80%, respectively for Dexketoprofen trometamol and Tramadol hydrochloride. As the limit of precision was less than 2, the method is precise. Chromatograms were shown in fig. 15-19.

Table 5: Results for system precision of dexketoprofen trometamol and tramadol hydrochloride

S. No.	System precision	
	Area of dexketoprofen trometamol	Area of tramadol hydrochloride
1.	298165	1586497
2.	298248	1584565
3.	298469	1583564
4.	298360	1587545
5.	298604	1587021
Mean	298582	1583659
SD	298404	1585425
%RSD	178.493	1828.610

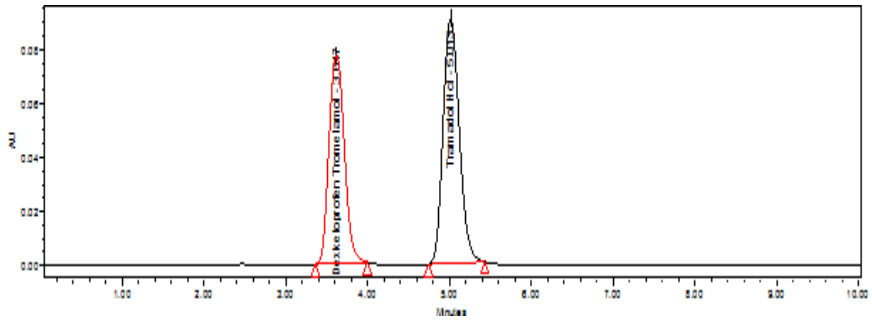

Fig. 15: Chromatogram for system precision-1

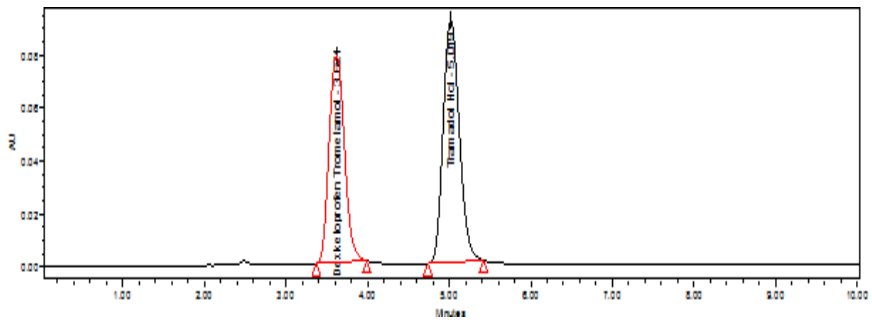

Fig. 16: Chromatogram for system precision-2

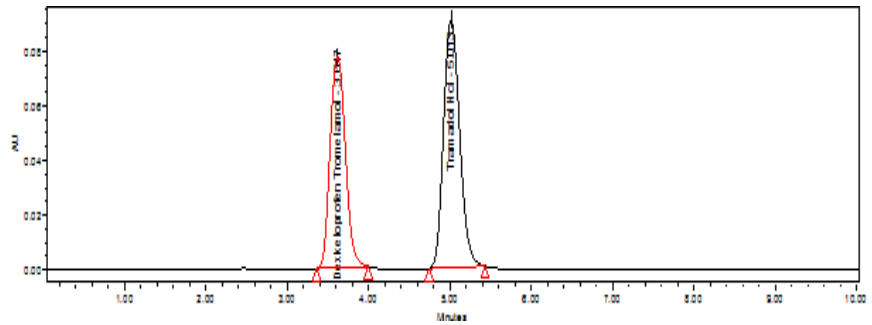

Fig. 17: Chromatogram for system precision-3

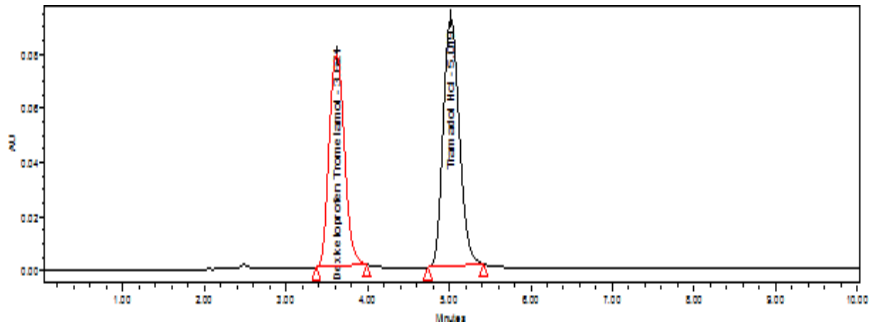

Fig. 18: Chromatogram for system precision-4

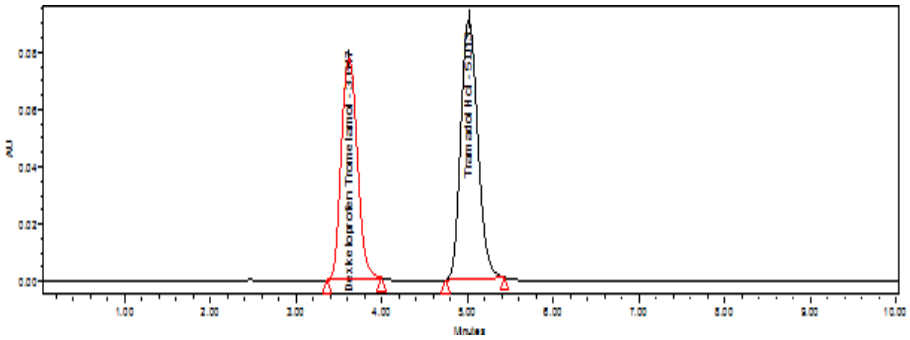

Fig. 19: Chromatogram for system precision-5

Repeatability

Repeatability (Method precision) was determined by multiple sampling from a sample stock solution and six working sample solutions of the same concentrations were prepared, 15 µl injection from each working sample solution was given, and obtained areas were mentioned in table 6. Average area, standard deviation, and % RSD were calculated for two drugs and obtained as 0.089% and 0.135%, respectively for dexketoprofen trometamol and Tramadol hydrochloride. As the limit of precision was less than 2 the method is repeatable.

Table 6: Results for repeatability of dexketoprofen trometamol and tramadol hydrochloride

Repeatability		
S. No.	**Area of dexketoprofen trometamol**	**Area of tramadol hydrochloride**
1.	298598	1586845
2.	298348	1583485
3.	298168	1584697
4.	298264	1585509
5.	298433	1587946
6.	298680	1583965
MEAN	298415	1585407
SD	196.130	1720.599
%RSD	0.065	0.108

Accuracy

Accuracy of the method was determined by recovery studies. To the pre analysed sample, the reference standards of the drugs were added at the level of 50%, 100%, 150%. Triplicate injections were given for each level of accuracy and mean % recovery was obtained in the range of 99.693%-99.973% and 100.02%-100.06% for Dexketoprofen trometamol and Tramadol hydrochloride, respectively. The results were shown in table 7 and table 8.

Table 7: Accuracy results for dexketoprofen trometamol

% Level	**Amount spiked (µg/ml)**	**Amount recovered (µg/ml)**	**% Recovery**	**Statistical analysis of % recovery**	
50%	20	20.14	100.70	MEAN	100.63
	20	20.11	100.56		
	20	20.12	100.62	%RSD	0.068
100%	40	40.07	100.18	MEAN	100.25
	40	40.10	100.26		
	40	40.12	100.31	%RSD	0.065
150%	80	60.08	100.13	MEAN	100.21
	80	60.13	100.22		
	80	60.16	100.27	%RSD	0.068

Table 8: Accuracy results for tramadol hydrochloride

% Level	**Amount spiked (µg. ml)**	**Amount recovered (µg. ml)**	**% Recovery**	**Statistical analysis of % recovery**	
50%	20	20.05	100.27	MEAN	100.28
	20	20.05	100.28		
	20	20.06	100.30	%RSD	0.016
100%	40	40.12	100.30	MEAN	100.38
	40	40.15	100.38		
	40	40.18	100.40	%RSD	0.083
150%	60	60.08	100.13	MEAN	100.20
	60	60.12	100.20		
	60	60.16	100.27	%RSD	0.067

Limit of detection and limit of quantification

LOD and LOQ were calculated from the standard deviation of response from precision and slope from linearity [15]. The limit of detection values of Dexketoprofen trometamol and Tramadol hydrochloride were 0.074 and 0.175 µg/ml and limit of quantification values of Dexketoprofen trometamol and tramadol hydrochloride were 0.225 and 0.531µg/ml respectively. The LOD and LOQ result were shown in table 9. which indicates that the proposed method can be used for the detection and quantification of Dexketoprofen trometamol and Tramadol hydrochloride in a very wide concentration range.

Table 9: Results for lod and loq of dexketoprofen trometamol and tramadol hydrochloride

Drug	LOD (µg/ml)	LOQ (µg/ml)
Dexketoprofen trometamol	0.074	0.225
Tramadol hydrochloride	0.175	0.531

Table 10: Robustness results for dexketoprofen trometamol and tramadol hydrochloride

S. No.	Condition	% RSD of dexketoprofen trometamol	% RSD of tramadol hydrochloride
1	Flow rate (-) 0.8 ml/min	0.09	0.12
2	Flow rate (+) 1.2 ml/min	0.06	0.08
3	Mobile phase (-) 72A: 25B	0.13	0.09
4	Mobile phase (+) 85A: 15B	0.06	1.60
5	Temperature (-) 25 °C	0.90	0.06
6	Temperature (+) 35 °C	0.93	0.89

Robustness

Robustness conditions like Flow rate minus (0.8 ml/min), Flow rate plus (1.2 ml/min), mobile phase minus (72A: 25B), mobile phase plus (85A: 15B), temperature minus (25 °C), and temperature plus (35 °C) were maintained and samples were injected in a duplicate manner. Results were given in table 10. System suitability parameters were not much affected and %RSD was within the limit. Hence the method was considered to be robust.

CONCLUSION

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to beat 244 nm for Dexketoprofen Trometamol and 255 nm for Tramadol Hydrochloride and the peaks purity was excellent. Injection volume was selected tobe 20 µl, which gave a good peak area. The column used for study was Inertsil C18, ODSchosen good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow ratewasfixedat1.0 ml/min because of goodpeakarea, satisfactory retention time and good resolution. Different ratios of mobile phase were studied, mobile phase with ratio of Methanol: Buffer (75:25)v/v was fixed due to good symmetrical peaks and for good resolution. So, this mobile phase was used for the proposed study. Both system and method precision were found to be accurate and well within range. Linearitystudy was correlation coefficient and curve fitting were found to be. The analytical method was found linearity over the range of 20-70 ppm of the target concentration for both the drugs. The analytical passed both robustness and ruggedness tests. On both cases, the relative standard deviation was well satisfactory.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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