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RESEARCH ARTICLE

DEVELOPMENT AND VALIDATION OF REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR SIMULTANEOUS ESTIMATION OF TAPENTADOL HYDROCHLORIDE AND LORNOXICAM IN SYNTHETIC MIXTURE.

Prajapati Arun M[1], Soni Umang P[1]*

[1]Department of Quality Assurance, S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Ganpat Vidyanagar, Mehsana, Gujarat, India.

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Abstract:
A simple, precise, accurate and rapid reverse phase high performance liquid chromatographic method has been developed for the estimation of Tapentadol hydrochloride (TAP) and Lornoxicam(LOR) in combined synthetic mixture. The chromatographic separation was attained on reverse phase C_{18} column (Phenomenex C_{18}, 250 mm × 4.6 mm, 5µm) as stationary phase with mobile phase comprising phosphate buffer (pH-3.5) : acetonitrile (65:35, v/v) with a flow rate of 1 ml/min. Quantification was achieved with Photo Diode Array detector at 275 nm. The retention times of TAP and LOR were 2.24 min and 4.91 min respectively. The linearity of TAP and LOR were in the range of 5-35 µg/ml and 2-12 µg/ml respectively. The method was successfully applied to synthetic mixture because no chromatographic interferences from the mixture excipients were found. The method retained its accuracy and precision when the standard addition technique was applied.

Keywords: Tapentadol hydrochloride, Lornoxicam, RP-HPLC, Validation.

Introduction

Tapentadol hydrochloride (TAP)[1] is chemically 3-[(1*R*,2*R*)-3-(dimethylamino)-1-ethyl-2-methylpropyl] phenol hydrochloride (figure 1), a centrally acting analgesic. It is not official in any pharmacopoeia. Literature survey reveals HPLC, spectrophotometric method for estimation tapentadol hydrochloride in single dosage form.[2,3,4] Literature survey also reveals spectrophotometric [5] ,HPTLC [6] and HPLC [7] methods for determination of tapentadol hydrochloride with other drugs in combination. Lornoxicam (LOR)[8] is (3E)-6-chloro-3-[hydroxy(pyridin-2-ylamino)methylene]-2-methyl-2,3-dihydro-4H-thieno[2,3-e][1,2]thiazin-4-one 1,1-dioxide (figure 2), a non-steroidal anti inflammatory agent(NSAID). It is not official in any pharmacopeia. Literature survey reveals spectrophotometric [9] , HPTLC [10] and HPLC [11] methods for determination of lornoxicam in single dosage form. Literature survey also reveals spectrophotometric [12] , HPLC [13] and HPTLC [14] methods for determination of lornoxicam with other drugs in combination. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of tapentadol hydrochloride and lornoxicam in their combined synthetic mixture. There has been no report in literature on the simultaneous determination of tapentadol hydrochloride and lornoxicam in synthetic mixture. The present work describes the development of validated RP-HPLC method, which can quantify these components simultaneously from a combined synthetic mixture. The proposed RP HPLC method was validated in accordance with ICH guidelines [15], by assessing its selectivity, linearity, accuracy, precision, and limits of detection and quantification.

MATERIALS AND METHODS

APPARATUS

The separation was carried out using a RP-HPLC instrument equipped with a UV-Visible detector, a photodiode array detector, and LC-solution software (Shimadzu, LC-2010CHT, Japan,), auto sampler, Phenomenex (Torrance, CA) C18 column (150 mm × 4.6 mm i.d, 5 µm particle size) was used as stationary phase. Analytical balance (Sartorius CP224S, Germany), Triple distillation unit consisting of borosilicate glass, Digital pH meter (LI 712 pH analyzer, Elico Ltd., Ahmedabad), Ultra sonic cleaner (Frontline FS 4, Mumbai, India) were used in the study.

REAGENTS AND MATERIALS

Tapentadol hydrochloride (TAP) standard was kindly gifted by Zydus-Cadila Pharmaceuticals Ltd., Ahmedabad, Gujarat, India. Lornoxicam (LOR) standard was kindly gifted by Torrent Pharmaceuticals Ltd., Ahmedabad, Gujarat. The synthetic mixture containing 50 mg TAP and 8 mg LOR was prepared in the laboratory. HPLC grade methanol (Merck Ltd., Mumbai, India), HPLC grade acetonitrile (Finar Chemicals Ltd.,Mumbai, India) were used in the study. The water for RP-HPLC was prepared by triple glass distillation and filtered through a nylon 0.45 µm – 47 mm membrane filter. Nylon 0.45 µm – 47 mm membrane filter (Gelman Laboratory, Mumbai, India) and Whatman filter paper no. 41. (Whatman International Ltd., England) were used in the study.

PREPARATION OF SOLUTIONS & REAGENTS:

Preparation of TAP and LOR Standard Stock Solutions

An accurately weighed quantity of both tapentadol hydrochloride and lornoxicam standard (5 mg) were weighed and transferred to 100 ml separate volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution having concentration 50 µg/ml for both of the drugs.

Preparation of Synthetic mixture

Tapentadol hydrochloride (50 mg) and Lornoxicam (8 mg) were taken and then both the drug were mixed with Starch, Lactose, Magnesium Stearate and Talc. Total 100 mg of mixture was prepared.

Preparation of Sample Solution

The powder of synthetic mixture equivalent to 50 mg of TAP and 8 mg of LOR transferred to 100 mL volumetric flask. 50 mL methanol was added and sonicated for 20 min. The solution was filtered through whatman filter paper No. 41 and the volume was adjusted up to the mark with methanol (HPLC Grade) to prepare 50 µg/mL of TAP and 8 µg/mL of LOR. From the above solution, 0.5mL was taken and was transferred to 10 mL volumetric flask and volume was adjusted up to the mark to obtain solution having 25 µg/mL of TAP and 8 µg/mL LOR. An aliquot (20 µl) of sample solution was injected under the operating chromatographic condition and responses were recorded.

Preparation of 0.02M Phosphate buffer solution (pH 3.5)

Phosphate buffer (0.02 M KH_2PO_4, pH 3.5) was prepared by dissolving accurately weighed 0.272 g of potassium dihydrogen phosphate in 100 ml HPLC-grade water and the pH adjusted to 3.5 by diluted ortho-phosphoric acid.

Preparation of Calibration Curve

Aliquots equivalent to 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 ml standard solution of TAP and 0.4, 0.8, 1.2, 1.6, 2.0 and 2.4 ml standard solution of LOR were transferred into a series of 10 ml volumetric flasks separately and volume was adjusted to the mark with solvent (Methanol) to get concentrations 5, 10, 15, 20, 25 and 30 µg/ml of TAP and 2, 4, 6, 8, 10 and 12 µg/ml of LOR. 20 µl of each of the solution were injected into HPLC system and analyzed. Calibration curve was obtained by plotting respective peak area against concentration in µg/ml and the regression equation was computed.

Selection of detection wavelength

By preparing appropriate solution concentration of each drug (TAP and LOR) both drugs were scanned over the range of 200 to 400 nm in spectrum mode. While studying the overlay spectra, it was observed that these 2 drugs show optimum absorbance at 275 nm. This wavelength was used for detection of TAP and LOR.

Chromatographic Condition

Stationary phase: C18 column (150 mm x 4.6 mm i.d., 5 µm particle size) was used at ambient temperature (40 °C).

Mobile Phase: Phosphate buffer pH 3.5 : Acetonitrile [65 : 35, v/v/v]

Flow rate: 1.0 mL/min

Injection volume: 20 µL

Detection: The elution was monitored at 275 nm using PDA detector.

METHOD VALIDATION

The method was validated in compliance with ICH guidelines [15].

Accuracy (recovery study)
To study the accuracy of the proposed method, recovery studies were carried out by standard addition method at three different levels (75%, 100%, and 125%). A known amount of drug was added to reanalyzed sample powder and percentage recoveries were calculated.

Method Precision (Repeatability)
The precision of the instrument was checked by repeated injection (n = 6) of standard solutions of TAP (10 µg/ml) and LOR (4 µg/ml) under the same chromatographic condition and measurement of peak area, retention time and tailing factor. The low %RSD values (less than 2%) indicates that proposed method is repeatable.

Intermediate Precision (Reproducibility)
The intra-day and inter-day precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of TAP (5, 10, 15 µg/ml) and LOR (2, 4, 6 µg/ml). The result was reported in terms of relative standard deviation (% RSD).

Limit of Detection and Limit of Quantification
LOD and LOQ of drugs can be calculated using the following equations designated by International Conference on Harmonization (ICH) guidelines. [15]
$LOD = 3.3 \times \sigma/S$
$LOQ = 10 \times \sigma/S$
Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Robustness
The robustness was studied by analyzing the same samples of TAP and LOR by deliberate variations in the method parameters. The change in the responses of TAP and LOR were noted. Robustness of the method was studied by changing the composition of mobile phase by ± 2 % of organic solvent, flow rate by ± 0.2 ml/min and column oven temperature by 40 ± 2oC. The parameters used in system suitability test were asymmetry of the chromatographic peak, tailing factor and theoretical plates, as %RSD of peak area for replicate injections.

RESULTS AND DISCUSSION

A RP-HPLC method was developed and validated for the determination of TAP and LOR in combined synthetic mixture on a C18 column (150 mm x 4.6 mm i.d., 5 µm particle size) with variable wavelength detection at 275 nm. The retention times of TAP and LOR were 2.244 and 4.918 min, respectively. Linear correlation was obtained between area and concentration of TAP and LOR in the concentration range of 5-35 µg/ml and 2-12 µg/ml respectively for both drugs. The low RSD value of intra-day (0.30-0.47 % for TAP and 0.28-0.70 % for LOR) and inter-day (0.42-0.62 % for LOR and 0.80-0.90 % for LOR) at 275nm, reveal that proposed method is precise. The limit of detection (LOD) and limit of quantification (LOQ) for TAP and LOR were found to be 0.11 and 0.33 µg/ml and 0.04 and 0.13 µg/ml, respectively. These data show that method is sensitive for the determination of TAP and LOR. The recovery experiment was performed by the standard addition method. The mean recoveries were 99.75 ± 0.52 and 98.07 ± 0.49 for TAP and LOR, respectively (Table 2). The results of recovery studies indicate that the proposed method is highly accurate. The proposed validated method was successfully applied to determine TAP and LOR in combined synthetic mixture. No interference of the excipients with the retention time of drugs appeared; hence the proposed method is applicable for the routine simultaneous estimation of TAP and LOR.

CONCLUSION

Rapid separation of TAP and LOR was successfully attained with a relatively short retention time, provides good resolution, good peak shape, gives reliable and highly reproducible results on C18 HPLC column. In this proposed RP-HPLC method, the linearity was observed in the concentration range of 5-35 µg/ml and 2-12 µg/ml respectively for TAP and LOR with co-efficient of correlation, (r^2) =0.998 and (r^2) =0.999 for TAP and LOR, respectively at 275 nm. The results of the analysis of combined synthetic mixture by the proposed method are highly reproducible and reliable. The method can be used for the routine analysis of the TAP and LOR in combined synthetic mixture without any interference of excipients.

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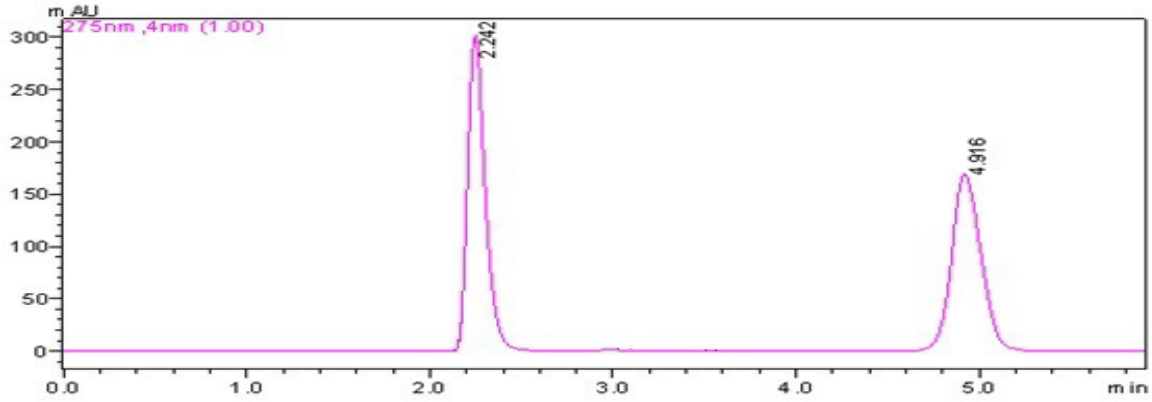

Figure 1 Chromatogram of TAP (50µg/ml) and LOR (8µg/ml) at 275 nm

Figure 1: Chromatogram of TAP (50 µg/ml) and LOR (8µg/ml)

PARAMETERS	TAP	LOR
Concentration range (µg/ml)	5-35	2-12
Regression equation (y = mx + c) Slope (m) Intercept (c)	y = 3412.18x + 10863.5 3412 10863.5	y = 7671.9x + 6430.38 7671.9 6430.38
Correlation Coefficient (r^2)	0.998	0.999
Accuracy (Recovery ± S.D.) (n = 3)	99.75 ± 0.52	98.07 ± 0.49
Method precision (Repeatability) (% RSD, n= 6)	0.916	0.782
Intraday (n = 3) (% RSD)	0.30-0.47	0.28-0.70
Interday(n = 3) (% RSD)	0.42-0.62	0.80-0.90
LOD(µg/ml)	0.11	0.04
LOQ (µg/ml)	0.33	0.13
Assay ± S. D. (n = 3)	100.69 ± 0.85	101.46 ± 0.37

Table 1: Data Showing Linearity and Precision of the Developed Method

Drug	Level	Amount taken (µg/ml)	Amount added (%)	% Mean recovery ± S.D. (n = 3)
TAP	I	10	75	100.18 ± 0.55
	II	10	100	99.67 ± 0.77
	III	10	125	99.42 ± 0.26
LOR	I	1.6	75	96.33 ± 0.59
	II	1.6	100	98.45 ± 0.51
	III	1.6	125	99.44 ± 0.38

Table 2: Recovery Data

Drug	Label Claim (mg)	Amount Found (mg)	% Label claim ± S.D. (n=3)
TAP	50	50.34	100.69 ± 0.85
LOR	8	8.10	101.46 ± 0.37

Table 3: Results of Analysis of Tablet Dosage Forms Containing TAP and LOR

Parameters	TAP ± % CV (n=6)	LOR ± % CV (n=6)
Retention time (min)	2.244 ± 0.16	4.918 ± 0.24
Tailing factor	1.032 ±1.262	1.388 ± 1.274
Theoretical plates	2275 ± 1.698	4059 ± 1.846
Resolution	5.441 ± 1.367	

Table 4: System suitability test parameters for the proposed RP-HPLC method

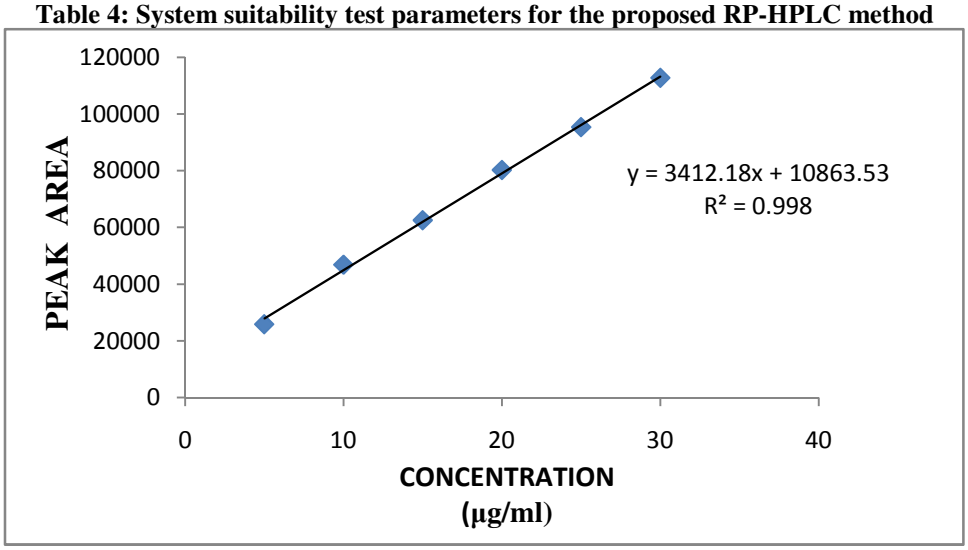

Figure 2 Linearity curve of TAP (5-35 µg/ml)

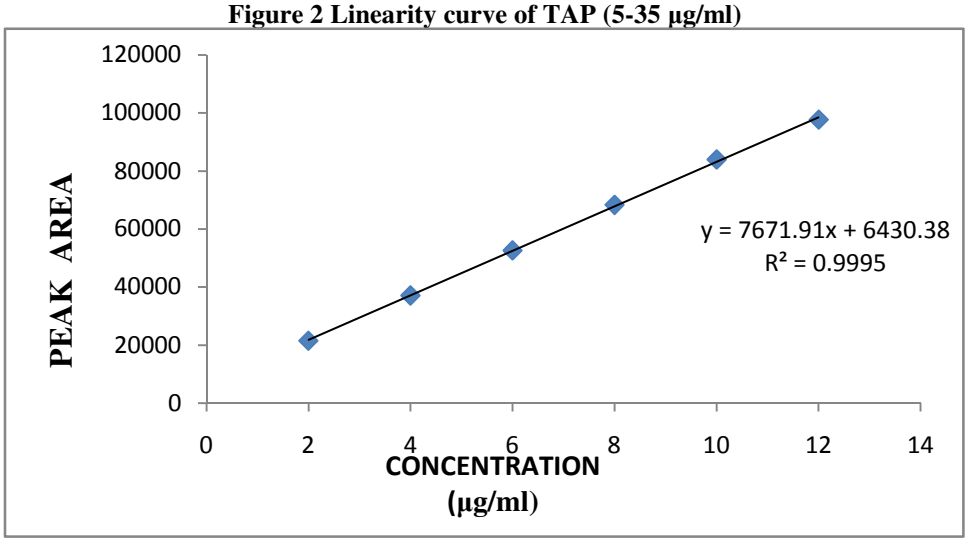

Figure 3 Linearity curve of LOR (2-12 µg/ml)

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