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REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR SIMULTANEOUS ESTIMATION OF BACLOFEN AND TRAMADOL IN BULK AND PHARMACEUTICAL DOSAGE FORM

J. Shireesha, P. Aravinda Reddy

Mother Teresa College of Pharmacy, N.F.C Nagar, Ghatkesar, Medchal, Telangana

Abstract:

The present research work describes a novel, simple, accurate, sensitive, rapid reversed-phase liquid chromatographic method for simultaneous estimation of Baclofen and Tramadol in bulk & pharmaceutical formulations. The chromatographic separation was achieved on WATERS HPLC with PDA detector and column Inertsil ODS, phosphate buffer and methanol as mobile phase at a flow rate of 1.0ml/min. The detection was carried out at 256 nm. The retention time of Baclofen and Tramadol was found to be 2.5 and 3.9. %Recoveries obtained for Baclofen and Tramadol were 99.85% & 100.5%.The %RSD below 2.0 shows the high precision of proposed method. The method was validated for precision, Recovery, Specify Detection & Quantification limits in accordance with ICH guidelines.

KEYWORDS: Baclofen, Tramadol, RP-HPLC, Validation

Corresponding author:

P. Aravinda Reddy,

Mother Teresa College of Pharmacy,

N.F.C Nagar, Ghatkesar, Medchal, Telangana.

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INTRODUCTION:

Baclofen is a gamma-amino-butyric acid (GABA) derivative used as a skeletal muscle relaxant. Baclofen stimulates GABA-B receptors leading to decreased frequency and amplitude of muscle spasms. It is especially useful in treating muscle spasticity associated with spinal cord injury. It appears to act primarily at the spinal cord level by inhibiting spinal polysynaptic afferent pathways and, to a lesser extent, monosynaptic afferent pathways.Tramadol is a narcotic analgesic proposed for moderate to severe pain. It may be habituating.it is a centrally-acting analgesic, exists as a racemic mixture of the trans isomer, with important differences in binding, activity, and metabolism associated with the two enantiomers.

MATERIALS AND METHODS:

Chemicals and Reagents: Spironolactone and Hydrochlorothiazide were obtained as a gift sample from Sun Pharma, Methanol and Acetonitrile from Merck, Potassium dihydrogen phosphate from finer chemicals.

Equipment and Chromatographic Conditions:

The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, PDA detector Analysis was carried out at 256 nm with column Phosphate Buffer (pH-7:3): Methanol (70:30% v/v), dimensions at 35^0C temperature. The optimized mobile phase consists of. Flow rate was maintained at 1 ml/min and run time for 8 min.

Preparation of mobile phase:

Accurately measured 700 ml (70%) of HPLC Methanol and 300 ml of Acetonitrile (30%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filter.

Baclofen & Tramadol standard & sample solution preparation:

Preparation Standard Solution:

A 10 ml & 100 ml volumetric flask was filled with 10 mg of Baclofen & 10 mg of Tramadol. Approximately 7 ml of diluent was added & it was sonicated to completely dissolve it. After that, the same solvent was used to modify the volume.

Pipette 3ml & 0.3ml of the above stock solutions were pipette out into a 10ml volumetric flask and diluted up to the mark with diluent.

Preparation Sample Solution:

10 tablets should be weighed, crushed & transferred into a 10 mL flask. Add around 7 mL of diluent, sonicate to dissolve it fully & then same solvent used to make up the desired level. This is equivalent to 10 mg of Baclofen & tramadol.

Pipette 3 ml of Baclofen & tramadol of the above solution into a 10ml flask & dilute up to the required quantity with diluent.

Procedure:

In the chromatographic system, inject 20 µL of the standard & sample. Measure areas for Baclofen & Tramadol peaks.

METHOD:

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

SYSTEM SUITABILITY:

The theoretical plates for the Baclofen & tramadol, there should be at least 2000 peaks in the standard solution and the tailing factor for the peaks caused by these drugs should not be greater than 2.0.

Assay of pharmaceutical formulation:

The proposed validated method was successfully applied to determine Baclofen & tramadol in their pharmaceutical dosage form.

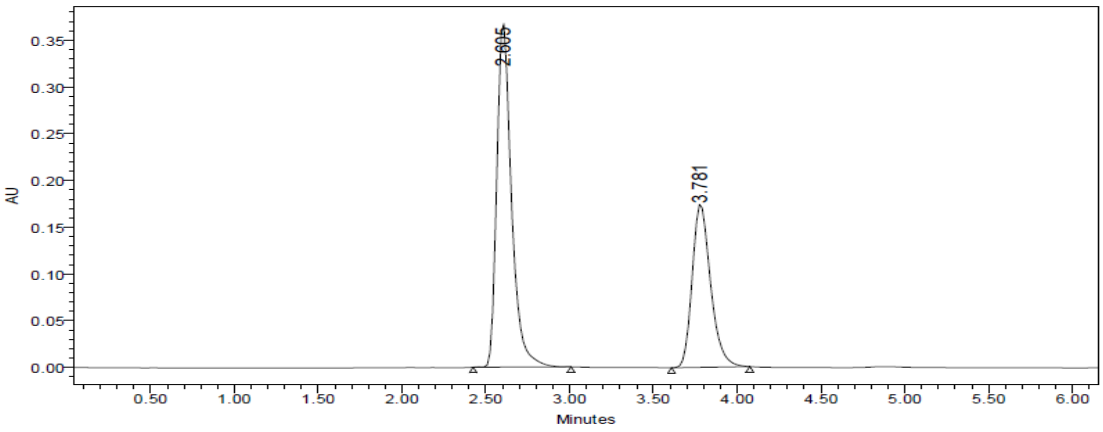

Figure no-1: Optimized Baclofen & Tramadol Chromatogram

SYSTEM SUITABILITY:

To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 8 minutes to equilibrate the column at ambient temperature.

Table no.1: system suitability results for Baclofen & Tramadol

S. No	Name	Retention time	Area	Height	resolution	tailing	plate count
I	**Baclofen**	2.5	124495	213638	8.0	1.2	4680.5
II	**Tramadol**	3.9	1308505	154566	6 0	1.3	6088.4

ACCURACY:

The percentage recovery was computed after sample solutions were made at various concentrations (50%, 100% & 150%).

Table 2 : Accuracy results for Baclofen and Tramadol

Name	%Conc	Area	Amount Added	Amount Found	% Recovery	Mean Recovery
Baclofen	50	656662.4	5.0	5.037	100.8	99.85
	100	1304260	10.0	10.0	100.	
	150	1854598	14.5	14.218	98.8	
Tramadol	50	65796	5.3	5.35	100.7	100.5
	100	124348	10	10.09	100.1	
	150	177938	14.1	14.46	99.72	

LINEARITY:

The chromatograms below illustrate the linearity range, which was determined to be between 100 and 500 µg/ml for Baclofen & 5 and 25µg/ml for Tramadol.

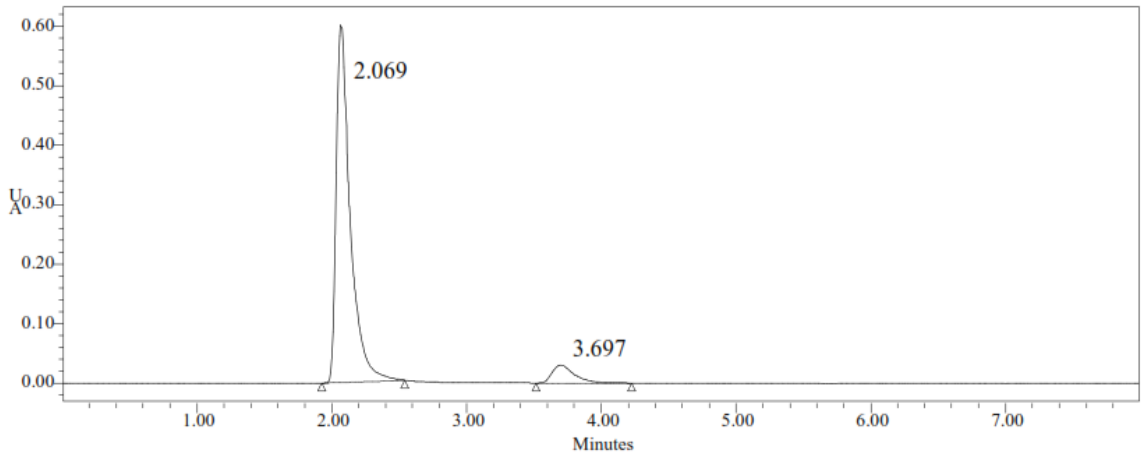

Figure no 28: Chromatogram linearity conc-100µg/ml of Baclofen & 5 µg/ml of Tramadol

Table -18 Analytical performance parameters of Baclofen & Tramadol

Parameters	Baclofen	Tramadol
Slope	66612	12498
Intercept	53625	50302
Correlation coefficient (R^2)	0.999	0.999

The obtained correlation coefficient, 0.999, falls within the acceptable range. The range of 10% to 50% for Baclofen and 5% to 25% for Tramadol was found to be linear.

LIMIT OF DETECTION FOR BACLOFEN & TRAMOADOL

The signal to noise ratio was assessed after the sample with the lowest concentration was prepared in relation to the baseline noise.

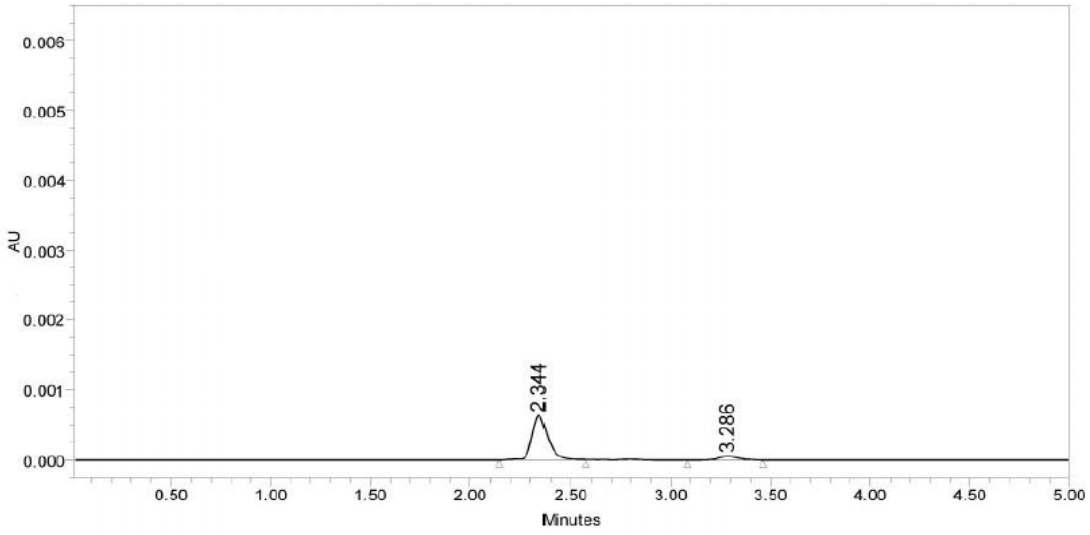

Figure no : Baclofen & Tramadol showing LOD

Table-3 Results of LOD

Name	Baseline noise	Signal obtained	S/N ratio
Baclofen	51	150	2.9
Tramadol	51	154	3

The sample with the less concentration was prepared in relation to the baseline noise & the signal to noise ratio was assessed.

Table-4 Changes in the Mobile Phase's Organic Composition for Baclofen

S. No.	Name	Change in the Mobile Phase	System Suitability	
			Plate Count	Tailing
I	Baclofen	10% less	4508.4	1.3
II		*Actual	4673.4	1.4
III		10% more	4318.1	1.3
I	Tramadol	10% less	4508.4	1.3
II		*Actual	4673.4	1.4
III		10% more	4318.1	1.3

It was observed that the percentage RSD for the mobile phase fluctuation and flow rate change was less than 1, falling within the acceptable range. Thus, the approach is reliable.

CONCLUSION:

One of the most advanced analytical tools available today is HPLC. RP-HPLC was used to estimate the amounts of Baclofen & Tramadol. The pH 3.0 phosphate buffer & methanol as the mobile phase were tuned using a 70:30% v/v mixture of methanol and phosphate buffer. As the stationary phase, inertsil C18 column C18 and comparable chemically bound to porous silica particles were employed. A UV detector set to 260 nm was used for the detection. Chromatography was performed on the solutions at a steady 0.8 ml/min flow rate. Baclofen and Tramadol were found to have linearity ranges of 100–500 mg/ml for Baclofen and 5–25 mg/ml for Tramadol. For both drugs, the linear regression coefficient was less than 0.999.

Accuracy & precision of the procedure are indicated by the % RSD readings being less than 2%. Tramadol and Baclofen recovery rates range

from 98 to 102%. It was observed that LOD & LOQ were within the range.

The validation parameters obtained results that satisfied USP and ICH standards. It concluded that the approach was straightforward, precise, accurate, and linear. With a high degree of accuracy & precision, the approach was shown to have a good applicability in routine laboratory analysis.

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