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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF BACLOFEN AND TRAMADOL IN ITS BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Baclofen and Tramadol was done by RP-HPLC. The Phosphate buffer was pH3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/v. Inertsil C_{18} column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Baclofen and Tramadol were found to be from 100-500 µg/ml of Baclofen and 1-5µg/ml of Tramadol. Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Baclofen and Tramadol. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

KEYWORDS: *Inertsil C_{18}, Baclofen and Tramadol, RP-HPLC.*

INTRODUCTION

The chromatographywas discovered by Russian Chemist and botanist *Micheal Tswett* (1872-1919) who first used the term chromatography (colour writing derived from Greek for colour – Chroma, and write – graphein) to describe his work on the separation of coloured plant pigments into bands on a column of chalk and other material such as polysaccharides, sucrose and insulin [1].

"*Chromatography is a method in which the components of a mixture are separated on an adsorbent column in a flowing system*".

The adsorbent material, or stationary phase, first described by Russian scientist named Tswett in 1906, has taken many forms over the years, including paper, thin layers of solids attached to glass plates, immobilized liquids, gels, and solid particles packed in columns. The flowing component of the system, or mobile phase, is either a liquid or a gas. Concurrent with development of the different adsorbent materials has been the development of methods more specific to particular classes of analytes. In general, however, the trend in development of chromatography has been toward faster, more efficient [2].

"In his early papers of Tswett (1906) stated that chromatography is a method in which the component of a mixture are separated on an adsorbent column in a flowing system. Chromatography has progressed considerably from Tswett's time and now includes a number of variations on the basic separation process".

"Chromatography is a physical method of separation in which the component to be separated are distributed between two phases of which in stationary while other moves in a definite direction (IUPAC)" [3].

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Chromatographic Process: [4]

Chromatographic separations are based on a forced transport of the liquid (mobile phase) carrying the analyte mixture through the porous media and the differences in the interactions at analytes with the surface of this porous media resulting in different migration times for a mixture components. In the above definition the presence of two different phases is stated and consequently there is an interface between them. One of these phases provides the analyte transport and is usually referred to as the mobile phase, and the other phase is immobile and is typically referred to as the stationary phase. A mixture of components, usually called analytes, are dispersed in the mobile phase at the molecular level allowing for their uniform transport and interactions with the mobile and stationary phases. High surface area of the interface between mobile and stationary phases is essential for space discrimination of different components in the mixture. Analyte molecules undergo multiple phase transitions between mobile phase and adsorbent surface. Average residence time of the molecule on the stationary phase surface is dependent on the interaction energy. For different molecules with very small interaction energy difference the presence of significant surface is critical since the higher the number of phase transitions that analyte molecules undergo while moving through the chromatographic column, the higher the difference in their retention. The nature of the stationary and the mobile phases, together with the mode of the transport through the column, is the basis for the classification of chromatographic methods.

Types of Chromatography:

The mobile phase could be either a liquid or a gas, and accordingly we can subdivide chromatography into Liquid Chromatography (LC) or Gas Chromatography (GC). Apart from these methods, there are two other modes that use a liquid mobile phase, but the nature of its transport through the porous stationary phase is in the form of either (a) capillary forces, as in planar chromatography (also called Thin-Layer Chromatography, TLC), or (b) electro osmotic flow, as in the case of Capillary Electro Chromatography(CEC) [7].

Methods in Chromatography: [5]

1. According to nature of stationary and mobile phase:

- Solid- Liquid chromatography
- Liquid-Liquid chromatography
- Gas- Solid chromatography
- Gas -Liquid chromatography

2. According to principle of separation:

A. Adsorption chromatography:

- Gas Solid chromatography
- Thin layer chromatography
- Column chromatography
- High performance liquid chromatography
- Affinity phase chromatography
- Hydrophobic Interaction chromatography (HIC)

B. Partition chromatography:

- Gas liquid chromatography
- Paper partition chromatography
- Column partition chromatography

3. Based on modes of chromatography:

- Normal phase chromatography
- Reversed phase chromatography

4. Other types of chromatography:

- Size exclusion chromatography (SEC)
- Gel permeation chromatography
- Gel chromatography
- Gel Filtration
- Gel permeation chromatography
- Ion exchange chromatography
- Chiral chromatography

High Performance Liquid Chromatography (HPLC): [6]

The acronym *HPLC*, coined by the Late Prof. Csaba Horvath for his 1970 Pittconpaper, originally indicated the fact that high pressure was used to generate the flow required for liquid chromatography in packed columns. In the beginning, pumps only had a pressure capability of 500psi [35bars]. This was called *high pressure liquid chromatography*, or HPLC. The early 1970s saw a tremendous leap in technology. These new HPLC instruments could develop up to 6,000psi [400bars] of pressure, and incorporated improved injectors, detectors, and columns. With continued advances in performance during this time [smaller particles, even higher pressure], the acronym HPLC remained the same, but the name was changed to high performance liquid chromatography [8-10].

High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantitative the compounds that are present in any sample that can be dissolved in a liquid. Today, compounds in trace concentrations as low as *parts per trillion* (ppt) may easily be identified. HPLC can be, and has been, applied to just about any sample, such as pharmaceuticals, food, nutraceuticals, cosmetics, environmental matrices, forensic samples, and industrial chemicals [11].

MATERIALS AND METHODS

Materials:

Baclofen & Tramadol KH_2PO_4, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid

Methodology:

HPLC Method Development:

Optimization of Column: The method was performed with various columns like C18 column, hypersil column, lichrosorb, and inertsil ODS column. Inertsil ODS (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 0.8ml/min flow.

Method Validation:

Precision: Accurately weigh and transfer 25mg of baclofen and tramadol working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 3 ml of baclofen & tramadol of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Intermediate Precision/Ruggedness: To evaluate the intermediate precision (also known as Ruggedness) of the method,
Precision was performed on different day by using different make column of same dimensions.

Accuracy:

Preparation of Standard stock solution:

Accurately weigh and transfer 10 mg of baclofen and tramadol 10mg of working standard into a 10mL& 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 3ml& 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Linearity: Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of baclofen and tramadol (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Limit of Detection:

Limit of Detection: (For baclofen): Accurately weigh and transfer 10 mg of baclofen working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Limit of Detection: (For tramadol): Accurately weigh and transfer 10mg of tramadol working standard into a 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Limit of Quantification:

Limit of Quantification (for baclofen): Accurately weigh and transfer 10 mg of baclofen working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Limit of Quantification (for tramadol): Accurately weigh and transfer 10mg of tramadol working standard into a 100mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

a) The flow rate was varied at 0.8ml/min to 1.2ml/min:

Standard solution 300ppm of baclofen & 3ppm of tramadol was prepared and analysed using the varied flow rates along with method flow rate.

On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.

* Results for actual flow (1.0ml/min) have been considered from Assay standard.

b). The Organic composition in the Mobile phase was varied from 50% to 50%:

Standard solution 300µg/ml of Setraline & 3µg/ml of tramadol was prepared and analysed using the varied Mobile phase

composition along with the actual mobile phase composition in the method.

On evaluation of the above results, it can be concluded that the variation in 10%.

Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by change in the Mobile phase ±10.

* Results for actual Mobile phase composition (55:45 Methanol: Buffer (ph-2.8) has been considered from Accuracy stand.

RESULTS

Table No. 1: Optimized chromatographic conditions for simultaneous estimations of Baclofen and Tramadol by RP-HPLC method

Instrument used	Waters HPLC with auto sampler and PAD ordetector
Column	Inertsil ODS (4.6 x 150mm, 5µm)
Column temperature	Ambient
Buffer	6.8 grams of potassium dihydrogenortho phosphate in 1000 ml water pH adjusted with orthophaosparic acid
pH	3.0
Wavelength	260 nm
Mobile phase ratio	30% buffer 70% Methanol
Flow rate	0.8 min/ml
Auto sampler temperature	Ambient
Injection volume	10µl
Run time	10 minutes

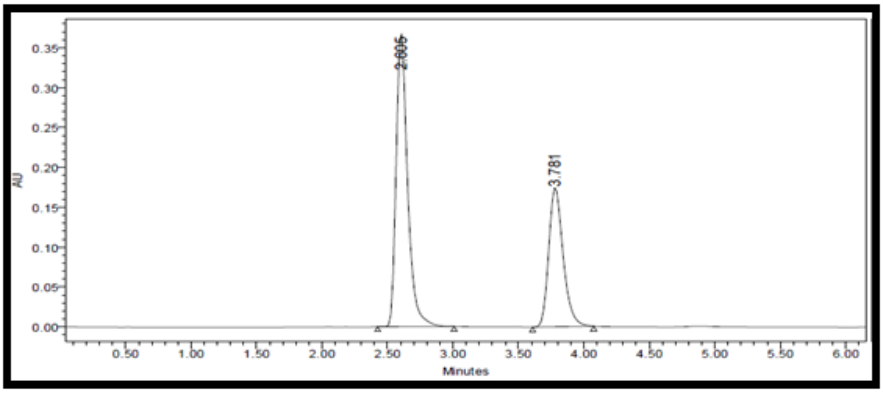

Fig. 1: Chromatogram of Trail-6

Observation: The separation of two analytical peaks was good. The plate count also above 2000, tailing factor below 2, and the resolution is above 2. The condition is taken as optimized method.

Validation Parameters:

System Suitability:

Table No. 2: Results of system suitability parameters for Baclofen and Tramoadol

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	**Baclofen**	2.5	124505	213642		1.2	4673.4
2	**Tramoadol**	3.9	1308495	154566	6 0	1.3	6090.3

Precession:

Table No. 3: Results of method precession for Tramoadol:

Injection	Area
Injection-1	123149
Injection-2	123766
Injection-3	124271
Injection-4	124691
Injection-5	124956
Average	124162.7
Standard Deviation	725.6
%RSD	0.6

Table No. 4: Results of method precession for Baclofen:

Injection	Area
Injection-1	1302729
Injection-2	1302947
Injection-3	1303236
Injection-4	1303977
Injection-5	1309759
Average	1304529.8
Standard Deviation	2961.1
%RSD	0.2

Intermediate Precision:

Table No. 5: Results of Intermediate precision for Baclofen

Injection	Area
Injection-1	1300148
Injection-2	1304520
Injection-3	1305937
Injection-4	1306476
Injection-5	130871
Average	1305070.2
Standard Deviation	3061.8
%RSD	0.2

Table No. 6: Results of Intermediate precision for Tramoadol

Injection	Area
Injection-1	122487
Injection-2	122626
Injection-3	122632
Injection-4	122702
Injection-5	122962
Average	122681.8
Standard Deviation	174.8
%RSD	0.1

Accuracy:

Table No. 7: Accuracy (recovery) data for Baclofen

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	56659.5	5.0	5.036	100.7%	99.84%
100%	1304258	10.0	10.003	100.0%	
150%	1854608	14.4	14.224	98.780%	

Table No. 8: Accuracy (recovery) data for Tramoadol

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	100.51%
100%	124353	10	10.10	100.01%	
150%	177940	14.2	14.45	99.68%	

Linearity:

Table No. 9: Area of different concentration of Baclofen

S.No.	Linearity Level	Concentration	Area
1	**I**	100ppm	668934
2	**II**	200ppm	956781
3	**III**	300ppm	1313873
4	**IV**	400ppm	1563458
5	**V**	500ppm	1867084
Correlation Coefficient			0.999

Table No. 10: Area of different concentration of Tramoadol

S.No.	Linearity Level	Concentration	Area
1	I	1ppm	66510
2	II	2ppm	94701
3	III	3ppm	124802
4	IV	4ppm	152731
5	V	5ppm	179732
Correlation Coefficient			0.999

Variation in Flow:

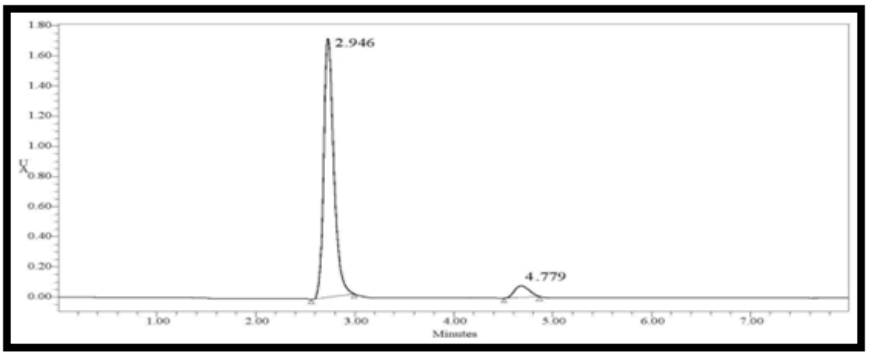

Fig. 2(a): Chromatogram snowing less flow of 0.6ml/min

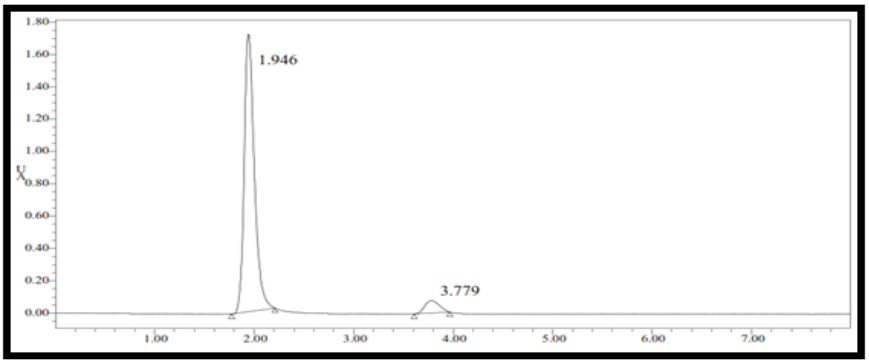

Fig. 3(b): Chromatogram snowing more flow of 1.0ml/min

Table No. 11: Flow Rate (ml/min) data for Baclofen

S. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	5339.9	1.4
2	0.8	4673.4	1.3
3	1.0	5216.0	1.4

Table No. 12: Flow rate (ml/min) data for Tramoadol

S. No.	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	7063.3	1.3
2	1.0	6090.3	1.2
3	1.2	6998.0	1.3

Table No. 13: Change in Organic Composition in the Mobile Phase for Baclofen

S. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4508.4	1.3
2	* Actual	4673.4	1.4
3	10% More	4318.1	1.3

Table No. 14: Change in Organic Composition in the Mobile Phase for Tramoadol

S. No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6387.7	1.2
2	* Actual	6090.3	1.2
3	10% More	6232.5	1.2

SUMMARY AND CONCLUSION

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Baclofen and Tramadol was done by RP-HPLC. The Phosphate buffer was p^H 3.0 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 70:30 % v/ v. Inertsil C_{18} column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 260 nm. The solutions were chromatographed at a constant flow rate of 0.8 ml/min. the linearity range of Baclofen and Tramadol were found to be from 100-500 µg/ml of Baclofen and 1-5µg/ml of Tramadol. Linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Baclofen and tramadol. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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