IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 13, Issue 4 Ver. II (Jul – Aug 2018), PP 29-38
www.iosrjournals.org

Ultra Performance Liquid Chromatographic Seperation And Quantification Of Baclofen And And Its Potential Impurities In A Injection Formulation

Venkata Narasimha Rao G[a,*], Muralee Krishna[b], Ranjith Kumar Reddy[b], Ravi Kumar Bellam[c]

[a]*Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, A.P, India*
[b]*Formulations Research and Development Centre, Glenmark Pharmaceuticals Ltd, Taloja, Mumbai, India;*
[c]*VIGNAN University-Vadlamudi, Guntur (Dist.), Andhra Pradesh-522213, India.*
Corresponding Author: Venkata Narasimha Rao Ga

Abstract: *This study explores theapplication ofreversed phaseliquid chromatography (UPLC) for the simultaneous quantification of Baclofen and its potential impurities in a pre-filled syringe. The separation of impurities from Baclofen was achieved on Acquity BEHC18 100 x 2.1,1.7 µm, using a simple inexpensive buffer (potassium phosphate, 0.01 M), a mixture of acetonitrile and methanol as the mobile phase. The mobile phase was pumped at a flow rate of 0.3 mL min^{-1} using a simple linear gradient elution mode. The sample components were detected by UV-VIS detector at 225 nm. The method can resolveBaclofen and Pharmacopeia (USP and Ph. Eur) impurities in a single chromatographic injection within 8 minutes of run time. The method is validated as per the current ICH guidelines for method Validation for parameters like Precision, Linearity, Accuracy, and Robustness.A linear correlation of 0.999 was achieved between the analytes concentration and detector response between LOQ to 150% of the target specifications. The method is linear, accurate and precise between LOQ and 150% of the target concentration.*

Keywords: *UPLC, Baclofen Injection formulation, Potential impurities, Method Development and Validation.*

Date of Submission: 04-07-2018 Date of acceptance: 23-07-2018

I. Introduction

Baclofen 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.Its chemical name is 4- amino- 3-(4- chlorophenyl) butanoic acid, with a molecular weight of 213.66. Its structural formula is shown in figure 1. Baclofen is a white to off-white, crystalline powder and slightly soluble in water, very slightly soluble in methanol, and insoluble in chloroform [1]. Baclofen Injection (Intrathecal) is a sterile, pyrogen-free, isotonic solution free of antioxidants, preservatives or other potentially neurotoxic additives indicated only for intrathecal administration. The drug is stable in solution at 37° C. Each milliliter of Baclofen Injection (Intrathecal) contains baclofen 50 mcg, 500 mcg or 2000 mcg and sodium chloride 8.8 mg in Water for Injection having pH range between 5.5-6.8. Each ampule is intended for single use only [2]. The major degradation impurity of Baclofen is USP Baclofen related compound A (Ph. Eur impurity A). In Ph. Eur, monograph additionally impurity B is also listed [3-4]. The chemical structures and nature of Impurity A and Impurity B are shown in table 1.

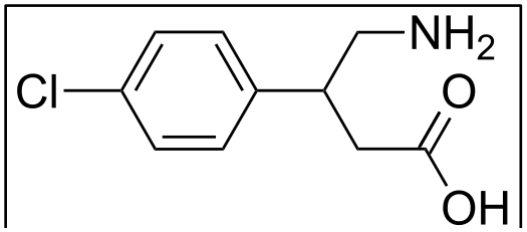
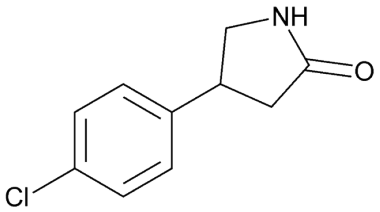
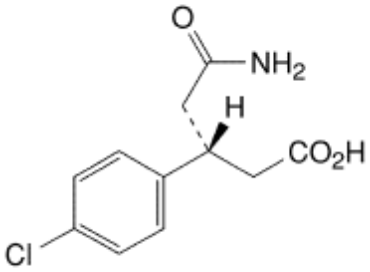


Figure 1. Chemical Structure of Baclofen

A thorough literature studies shows no pharmacopoeia methods on Baclofen injection formulation. However, the API monographs and monographs for tablets, oral solution are available in European Pharmacopoeia and United States Pharmacopoeia.

Table 1. Names, Chemical Structures, and Nature of Baclofen Impurities

Name	Chemical structure	Nature
USP Baclofen Related Compound A Impurity A (Ph. Eur)		Degradation Impurity
Impurity B (Ph. Eur)		Process Impurity

Few literatures are available on the pharmacodynamics, pharmacokinetics, and plasma studies [5-12]. Baclofen monographs (API, Tablets. Oral Solutions) are official in United States Pharmacopoeia and European Pharmacopeia (API) [3,4, 13-14]. These monographs contain methods for the determination of assay of Baclofen by HPLC. These methods uses monobasic potassium phosphate in combination with sodium pentanesulphonate as the buffer solution. The pH used in the assay method of Baclofen tablets was 3.0, while for oral suspension was 3.5. The separation was carried using a C18 column (250 mm x 4.6 mm, 5µm) at 35 ºC. The flow rate was 0.8 mL min^{-1}. Maximum run time in the methods was 35 minutes using a linear gradient programme. The detection was carried at 225nm. Official monographs for Baclofen injection are not available. Few HPLC and LC/MS methods were reported in the literature for the determination of Baclofen in biological samples [15-20].

One Rp-HPLC method was reported by Rajesh M et.al for the determination of Baclofen in bulk drug and pharmaceutical formulations [21]. This method uses ammonium acetate as the buffer solution (pH 5.0), premixed with methanol in the ratio 40:60 v/v. The column used in the study was a reverse phase column (150 mm x 4.6 mm, 5µm). The detection wavelength was 220 nm and the mobile phase flow rate was 1.0 mL min^{-1}. The retention time of Baclofen peak was 5.3 minutes with a total run time of 10 minutes. The method lacks discussion on the specificity with respect to the impurities of Baclofen.

One Rp-HPLC method was reported by Saroj Kumar Raul et.al for the estimation of Baclofen in bulk and pharmaceutical dosage forms [22]. In this method Waters X-bridge C18 column was used. The mobile phase consisted of phosphate buffer and acetonitrile mixed in the ration 80: 20 (v/v) and the detection wavelength was at 219 nm. Baclofen eluted at 3.8 minutes and the total run time is 10 minutes. The method lacks discussion on specificity of the method with respect to the specified impurities of Baclofen.

So far to our knowledge there is no UPLC method available for the simultaneous quantification of Baclofen and its two potential impurities in an injection formulation.

II. Materials And Methods

2.1 Chemicals, Reagents, Standards, and Instruments

The HPLC grade acetonitrile, and methanol were procured from J.T.Baker. The buffer salt Potassium Phosphate (KH_2PO_4 A.R. grade) used in the preparation of mobile phase were obtained from Merck, India. HPLC grade ortho phosphoric acid used to adjust the pH of the mobile phase buffer, was obtained from Fischer Scientific Ltd. The samples used in the study were market samples. The reference standards and impurity standards were procured from USP and custom manufacturer.

An integrated HPLC system is from Waters Corporation, Milford, USA, and equipped with a Waters photodiode array detector (PDA). Data collection and analysis were performed using the Empower software 2pro (Waters Corporation). Balances used for weighing the reference standards and samples were from Mettler

Toledo. The columns *Acquity BEH C18 100 x 2.1, 1.7 µm*, was procured from Waters India. Class A glassware used in conducting the experiments and validations was from Duran and Borosil, India.

2.2. Chromatographic Conditions

The separation of principal peak (Baclofen) and its potential impurities was achieved on a *Acquity BEH C18* column having dimensions of *100 mm x 2.1mm* I.D and a particle size of 1.7 µm. The mobile phase consists of a buffer solution (KH_2PO_4, 0.02 M, pH 3.2) and a mixture of acetonitrile and methanol (50:50 v/v) pumped into the chromatographic system using a linear gradient program (Gradient Programme-1) at a flow rate of 0.3 mL/ min. The chromatographic column was maintained at a temperature of 40°C throughout the run. The sample size was 1 µL. The detection wavelength chosen as 225 nm based on lambda maxima depicted by active compound and individual impurities.

Gradient Programme-1

Time (min)	**Mobile phase A (Buffer pH 3.20)**	**Mobile phase B (Acetonitrile: Methanol) (50:50)**
0	68	32
1.1	68	32
3.2	48	52
6.0	40	60
6.5	68	32
8.0	68	32

2.2 Standard and Sample Preparations

2.2.1 Diluent Preparation

A mixture of mobile phase A and mobile phase in the ratio 50:50 v/v was used as the diluent, for the preparation of standards, system suitability solutions, and sample solution.

2.2.2 ***Preparation of Baclofen working standard stock solution*****:**

Accurately weighed and transferred 50 mg of Baclofenworking standard into 100 mL volumetric flask. Added 25 mL of the diluent and sonicated for 5 minutes to dissolve the contents. Made up to the mark with the diluent (500µgmL^{-1}).

2.2.3 Preparation of impurity mixed stock solution (50 µgmL^{-1})**:**

Accurately weighed and transferred 2.5 mg of each impurity (A and B) of Baclofeninto 50 mL volumetric flask. Added 10 mL of the diluent and sonicated for 5 minutes to dissolve the contents. Made up to the mark with the diluent.

2.2.4 Preparation of Standard Solution for assay determination of Baclofen (200 µgmL^{-1})

Pipette out 4 mL of the working standard stock solution into 10 mL volumetric flask. Add 5 mL of the diluent, swirl to mix and make up to the mark with the diluent to obtain a concentration of 200 µgmL^{-1}.

2.2.5 Preparation of Baclofen Standard Solution for impurities determination (0.4 µgmL^{-1})

Pipette out 2 mL of the working standard stock solution into 100 mL volumetric flask. Add 20 mL of the diluent, swirl to mix and make up to the mark. Further dilute 2 mL of the above solution into 20 mL with the diluent to obtain a concentration of 0.4 µgmL^{-1}.

2.2.6 Preparation of Sample Solution

Transfer the contents of at least 10 or sufficient prefilled Baclofen injection solution into a clean dry beaker. Pipette out a 10 mL of solution containing to 20 mL volumetric flask. Added 10 mL of the diluent and sonicated for 5 minutes to dissolve the contents. Made up to the mark with the diluent (500 µgmL^{-1}). Pipette out 4 mL of the working standard stock solution into 10 mL volumetric flask. Add 5 mL of the diluent, swirl to mix and make up to the mark with the diluent to obtain a concentration of 200 µgmL^{-1}.

2.2.7 Preparation of sample solution spiked with impurity mixture

Pipette 2 mL of the sample into 10 mL volumetric flask. Added 2mL of the diluent and sonicated for 5 minutes to mix the contents. Add 0.4 mL of the impurity mixed stock solution using a micro pipette, and diluted up to the mark with the diluent. Mixed well for get a final concentration of 1% of target sample concentration.

2.2.8 Preparation of Placebo Solution:

Transferred placebo equivalent to 10 mg of Baclofen into 20 mL volumetric flask. Added 10 mL of the diluent and sonicated for 5minutes to mix the contents. Make up to the mark with the diluent.

III. Results And Discussion

3.1 Method development and optimization

The detection wavelength was determined by injecting a detectable concentration of Baclofen and individual impurity standard into the chromatographic system having photodiode array(PDA) detector. Baclofen sodiumand its impurities exhibits maximum absorbance at 225 nm. Hence the detection wavelength was selected as 225 nm.

3.1.1 Selection of Chromatographic Conditions

Based on the structure of Baclofen/impurities and functional groups present, the method development was initiated using the principles of reversed phase chromatography(RPC). RPC is the first choice for neutral, acid, and basic molecules having molecular weight less than 2000 Da [23].Based on the reported pKa (Strongest acidic, pKa=3.89) , Strongest basic, pKa=9.79) of the molecule [1], a simple mono basic phosphate buffer (KH_2PO_4, 0.02M) with pH 6.0 was chosen and initial trials were conducted using methanol as the isocratic mobile phase (50:50) v/v.The stationary for separating the impurities and principal peak was a reversed phase octadesylsilyl column 150 mm x 4.6 mm, 5µm, at 30 ºC, and The injection volume was chosen as 2 µL to keep minimum possible load so as to achieve repeatable Gaussian peak. The detection wavelength was 225 nm.

Method Development and Optimisation

With above chromatographic conditions, impurity mixed solution was injected into the chromatographic system. The inference was Baclofenwas eluted within 20 minutes of run time. Theone of the two impurities namely USP impurity related compound A was found at the retention time of 19 minutes. The impurity B was either merged or did not elute under these chromatographic conditions. A gradient programme was introduced with varying concentration of organic mobile phase. There was no significant impact on the selectivity. Further methanol was replaced with acetonitrile and with the same gradient programme impurity mixed solution was injected into the chromatographic system. Impurity was eluted and was merged partially with Impurity A.

Further different attempts were made with other buffer solutions like Na_2HPO_4 (0.01M, pH 7.2), sodium perchlorate (0.05 M, pH 5.6). The impurities were not separated from each other and the baseline not proper. Now again $KH_2PO_4$was used by reducing the pH to 4.0, and with acetonitrile in gradient (T/%B 0/10, 20/45, 35/55, 45/55, 50/10, 60/10) elution mode. Selectivity was significantly effected with Baclofen eluted at about 1.5 minutes, impurity B at 2.5 minutes, and impurity A at 4 minutes.Further the baseline was optimised with several optimisation trials with pH (3.5, 3.2, 3.0, 2.9), gradient programme. Optimum separation and baseline was obtained at pH 3.2. The final optimized method parameters are described in the section 2.2. The optimized chromatogram obtained is shown in figure 2.

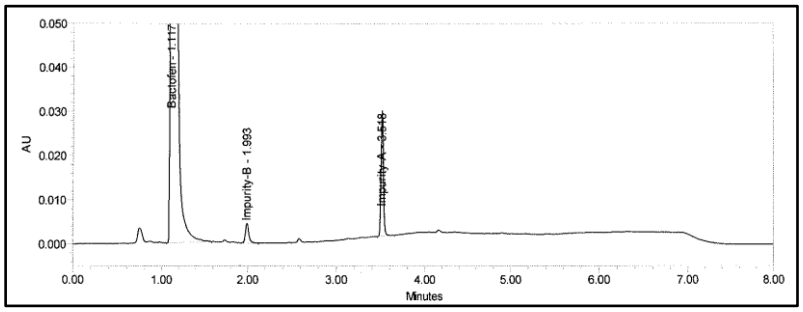


Figure 2. Chromatogram obtained from Optimized parameters

IV. Method Validation

The developed and optimized UPLC method was taken up for validation. The method validation was carried out in accordance with the validation guidelines (Q2R1) recommended by ICH [20]. The developed HPLC method was validated with respect to system suitability, specificity, accuracy, precision, linearity, limit of detection, limit of quantitation and robustness. Each validation parameter is explained in detailed in the sections from 4.1 to 4.8.

4.1 System Suitability and System Precision

The system suitability and system precision for the intended analysis were evaluated from replicate injections of standard solution respectively. The system suitability parameters like USP plate counts, USP tailing factor, USP resolution and %RSD for Baclofen peak were determined and evaluated (Table2). The data shows Baclofen and its specified impurities were well resolved, and the area of the Baclofenpeak was precise with and RSD < 1.0%.

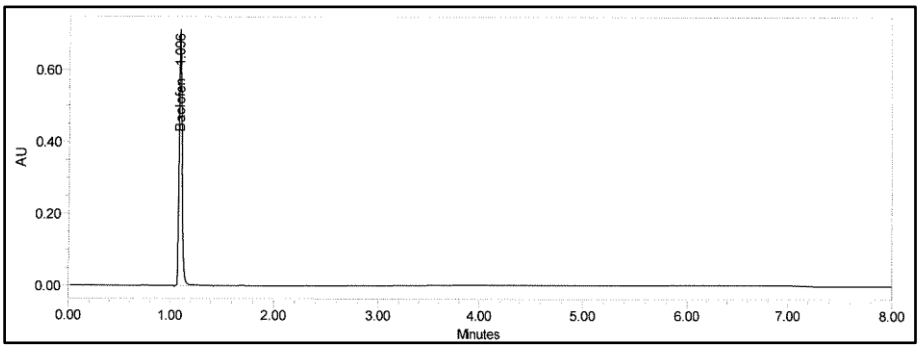


Table 2. Results of System Suitability and System Precision

Peak Name	Retention Time(min)	USP Plate Counts	USP Resolution	USP Tailing	%RSD *	System Precision %RSD **
Baclofen	1.117	3899	-	1.4	0.9	0.8
Impurity B	1.993	4509	13.6	1.24	-	
Impurity A	3.518	7464	25.6	1.05		

* *Data from five replicate injections*
** *Data from six replicate injections*

4.2 Specificity

4.2.1 Diluent interference

The diluent was injected as such into the chromatograph to assess its interference (if any) at the retention time of Baclofen and its potential impurities. No interference is found at the retention time of Baclofen and its impurities (Figure3).

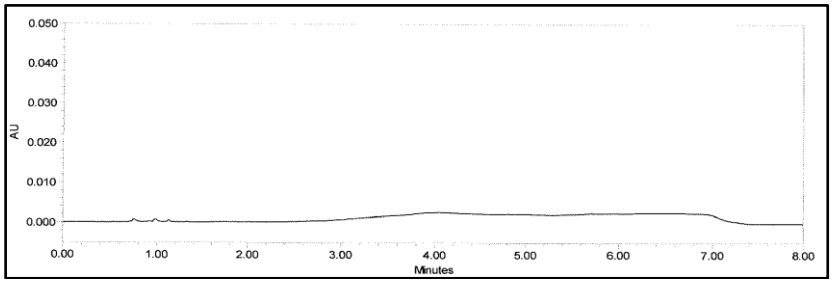


Figure 3. Typical Chromatogram of Diluent

4.2.2 Placebo interference

The placebo solution was injected as such into the chromatograph to assess its interference (if any) at the retention time of Baclofen and its potential impurities. No interference is found at the retention time of Baclofen and its impurities (Figure4).

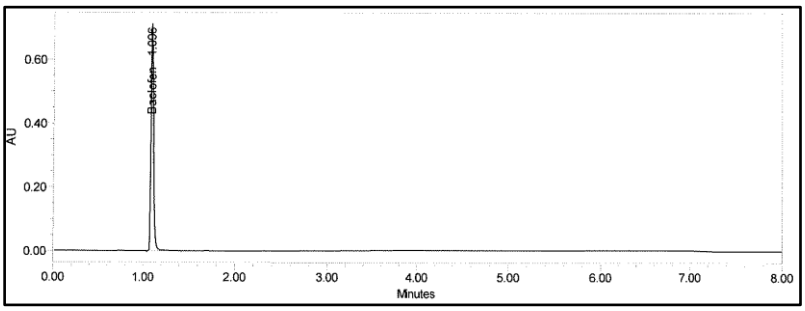


Figure4. Typical Chromatogram of Placebo

4.2.3 Interference due to specified impurities of Baclofen

To assess the interference due the specified impurities of Baclofen, a sample solution spiked with small portion (1%) of listed impurities (as mentioned in the Table1) was injected into the chromatographic system. The Baclofenpeak was evaluated for the homogeneity by measuring the purity angle and purity threshold using the empower software. Assay of Baclofen Sodium in presence of the specified impurity mixture was calculated and compared with that of the unspikedsample (Table 3).Assay of the sample spiked with impurities was found 97.9% and that of pure sample was 98.1%. The absolute difference (0.2) is very small and indicates that the assay of Baclofen was unaffected in presence of its impurities. The purity data indicate that purity angle is less than the purity threshold for all three samples, which concludes that Baclofenpeak is homogenous, and the new method is analyte specific.

Table3. Comparison of Assay Results and Peak Purity Results of Sample Spiked with Impurity and Unspiked Sample

Sample Details	% Assay	Purity Angle	Purity Threshold	Purity Flag*
Unspiked sample-1	97.8	0.453	0.665	No
Unspiked sample-2	97.9	0.235	0.946	
Unspiked sample-3	97.9	0.454	0.684	
%Average	97.9	NA		
Spiked sample-1	98.1	0.345	0.674	
Spiked sample-2	98.3	0.346	0.696	
Spiked sample-3	97.9	0.239	0.435	
%Average assay	98.1	NA		
Difference between average assays of unspiked and spiked sample				
0.2				
**Purity flag 'No' indicates, peak is homogenous (Empower software)* *Peak is homogeneous if purity angle is less than purity threshold*				

4.2.4 Interference from degradation products

To assess the interference due to the degradation products, Baclofen Sodium for injection and placebo solutions were exposed to various stress conditions. The stressed and neutralized samples were then diluted with the diluent to obtain a concentration of about 200 µg mL^{-1}. The obtained solutions were chromatographed as per the optimized methodology. All chromatograms were processed by using the Empower 2pro software. The homogeneity of Baclofenpeak and specified impurities from the stressed samples was evaluated from the purity angle and peak threshold data using the PDA detector. The stress parameters, stress conditions, %degradation in each stress parameter are listed in Table 4.The data of stress study indicate that degradation was observed in acid hydrolysis (7.5%), base hydrolysis (6.9%), oxidation (1.7%), andthermal condition (0.24%). The purity angle was less than the purity threshold in all the stress conditions which indicates that Baclofen peakand observed specified impurity are homogeneous in presence of its degradation impurities and the method is stability indicating.

Table 4. Results of Stress Study and Peak Purity Data for Baclofen

Parameter	Stress Conditions	%Assay of Degraded Sample A	%Degradation w.r.t. Control B *	Purity Angle	Purity Threshold
Control sample	No exposure	97.8	NA	0.453	0.656
Acid hydrolysis	2 mL of 5N HCl for 5 hours at room temperature	90.3	7.5	0.345	0.634
Base hydrolysis	2 mL of 5N HCl for 5 hours at room temperature	90.9	6.9	0.453	0.753
Oxidation	1 mL of 30% H_2O_2 for 5 hours at room temperature	96.1	1.7	0.342	0.664
Thermal degradation	65ºC for 72 hours	97.6	0.24	0.289	0.547
Photolytic degradation(UV)	200-Watt hours / m^2	97.8	0.05	0.363	0.555
Photolytic degradation (light)	1.2 Million lux hours	97.7	0.08	0.366	0.735

**B= (97.8- A)/97.8*100*

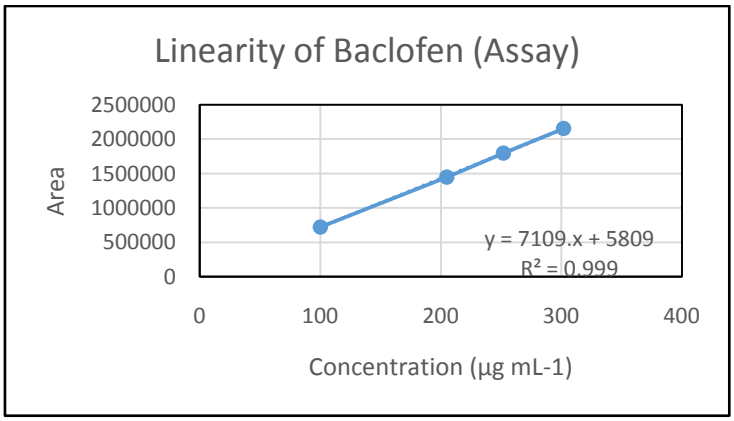
4.3 Linearity

Linearity of detector response was demonstrated from LOQ to 150% of target specificationsof the impurity.Using minimum six calibration levels (50%, 100%, 125% and 150%) with respect to sample concentration (200 µg mL^{-1}) for assay determination. The linearity solutions were prepared from a standard stock solution by appropriate dilutions. Each solution was chromatographed, and area response was recorded. The data was evaluated by using linear regression method. The correlation coefficient (R^2) was found greater

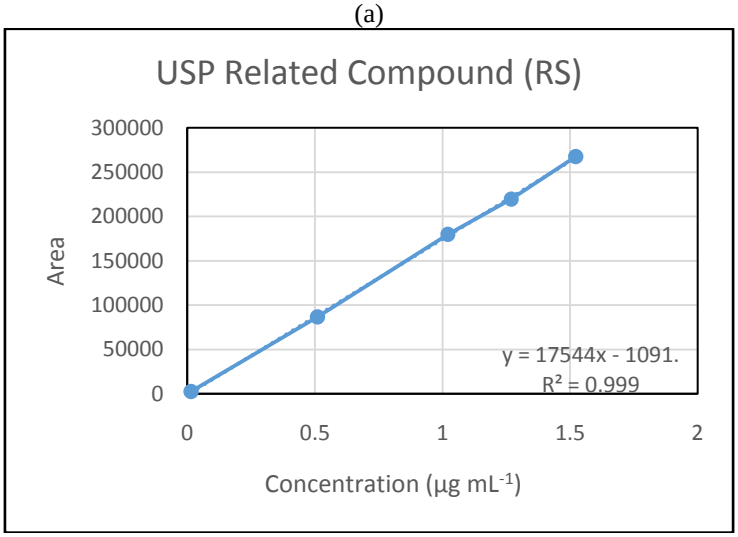
than 0.998 for all specified impurities and for Baclofen peak (Table 5). A linearity graph is plotted between the concentration and areas (Figure 11).This indicates an excellent linear relationship between the concentrations and obtained peak areas by the proposed method.

Table 5. Summary of Regression Parameters

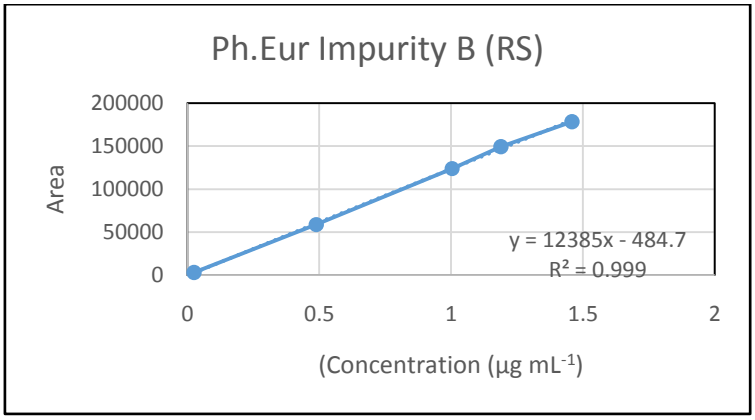
S. No.	Parameter	Obtained Values		
		Baclofen	**Imp A**	**Imp B**
1	Correlation coefficient(R^2)	0.9997	0.9997	0.9994
2	Slope	7109.1	175440	123858
3	Y-Intercept	5809	-1091.5	- 1091.5
4	Residual sum of squares	1893840989	23759045	13904956



(a)



(b)



(c)

Figure 11. Calibration plot for (a) Baclofen (b) USP related compound A (c) Ph.Eur impurity B

4.4 Accuracy

The accuracy of the analytical procedure expresses the degree of the closeness of the obtained results to the true values. A study of accuracy (recovery) was performed on known amount of placebo by spiking active pharmaceutical ingredient and impurities at specification level. Samples were prepared as per the proposed method at different levels i.e., LOQ, 50%, 100%, and 150% of target analyte concentration in triplicate (n=3) for impurities. For assay, the amount of Baclofen was spiked at levels, 50%, 100%, and 150% into the placebo. Each preparation was injected in duplicate (n=2) into the chromatographic system. From the average of the two areas obtained, calculated the amount of Baclofenand each individual impurity recovered from the placebo for each recovery levels (Table 6). Mean and relative standard deviation of all three recovery levels were calculated and assessed for accuracy of the method. The % Mean recovery and % RSD for each recovery level are tabulated in table 6. These results show that method is capable of extract the impurities and active compound from the placebo precisely.

Table 6. Results of accuracy experiment

Name of analyte Peak	**% Mean Recovery and %RSD**							
	LOQ		**50%**		**100%**		**150%**	
Baclofen Sodium	-		98.8	0.5	98.9	0.6	98.6	0.2
USP Related Compound A	96.0	1.2	97.2	1.0	96.1	1.4	96.1	1.1
Ph. Eur Impurity B	95.6	1.8	95.8	1.6	95.2	1.7	96.0	1.4

4.5 BPrecision

Method Precision and Intermediate Precision (Ruggedness)

Precision or repeatability of the test method was evaluated by analyzing six samples of the same batch as per the proposed method for assay determination. For impurities determination the method precision was carried by spiking the individual impurities (A and B) at their specification limit of 0.5%. Intermediate precision or ruggedness of the method was performed by analyzing six samples of the same batch as per the proposed method on another day, using another column and system. A comparison of system suitability parameters is made between the method precision and intermediate precision and are tabulated in Table 7-8. The average assay obtained in M.P and I.P are 97.8 and 97.9 respectively. The %RSD of 12 precision results is 0.65 which shows the method is precise for the determination of Baclofen assay. The %RSD (n=12) for impurity A was 3.54, and that of impurity B was 2.76 (Acceptance criteria =NMT5% RSD), indicating that the method is also precise for the determination of impurities.

Table 7. Comparison of System Suitability Parameters

System suitability Parameters	**Results**					
	Method Precision			**Intermediate Precision**		
	I	**II**	**II**	**I**	**II**	**III**
USP tailing	1.40	1.05	1.24	1.39	1.05	1.26
USP plate count	3899	7464	4509	40231	7946	4395
USP resolution	-	25.6	13.6	-	25.4	13.5
% RSD of five standard injections	0.9	-		1.0	-	
Retention Times	1.117	3.517	1.993	1.121	3.520	1.998

I-Baclofen, II- USP Related Compound A, III- Ph. Eur Impurity B

Table 8. Comparison of Method Precision and Intermediate Precision

Sample No	Method Precision			Intermediate Precision		
	I	II	III	I	II	III
1	98.4	0.49	0.45	97.4	0.51	0.47
2	97.2	0.48	0.44	97.5	0.49	0.43
3	97.4	0.47	0.46	97.6	0.48	0.43
4	97.0	0.48	0.44	98	0.5	0.44
5	98.4	0.49	0.45	98.3	0.52	0.44
6	98.2	0.47	0.45	98.8	0.48	0.46
Mean (n=6)	97.8	0.48	0.45	97.9	0.50	0.45
% RSD (n=6)	0.65	2.54	1.68	0.55	3.29	3.69
% RSD (n=12) (M.P/I.P)	**0.65**	**3.54**	**2.76**	-		

I-Baclofen, II- USP Related Compound A, III- Ph. Eur Impurity B

4.6 Range

The range of the analytical method falls between 50 to 150% of Baclofen concentration in the sample for assay and LOQ to 150% of specification levels for impurities determination, in which it has been demonstrated to have a suitable level of precision, Accuracy, and Linearity.

4.7 Limit of Detection and Quantification

This limit was defined as the lowest concentration level that provided a peak area with signal to noise ration higher than 3:1 for detection and 10:1 for quantification. The limit of detection (LOD) and limit of quantification (LOQ) were estimated from the signal to noise ratio (Table 9).

Table 9. Results of LOD and LOQ

Name of analyte Peak	µg mL^{-1}			
	LOD	**LOQ**	**S/N-LOD**	**S/N-LOQ**
USP Related Compound A	0.006	0.015	4	11
Ph. Eur Impurity B	0.009	0.025	3	10

4.8 Robustness

Robustness study was performed on the chromatographic parameters which are susceptible to change during the preparation of solution, handling, and operation of the instrument. The parameters studied are mobile phase flow rate, column oven temperature, and mobile phase pH. The mobile phase flow rate was studied at ±10% from the optimized flow rate. The column temperature on was studied at ±5 °C. The variation in mobile pH was studied at ±0.2 units. The system suitability parameters like USP resolution (between baclofen and Ph.Eur impurity B) , USP Plate counts and relative standard deviation were evaluated (Table 10).

Table 10. Results of Robustness/Ruggedness Experiment

S. No.	Condition	Actual Condition	Altered Condition	USP Resolution	USP Plates	%RSD
1	**Control**	**--**	**--**	**13.6**	**3899**	**0.90**
2	Flow (in mL min^{-1})	0.3	0.27	14.0	3890	0.86
			0.33	13.7	4003	0.88
3	Column oven temperature (°C)	40	35	14.2	3896	0.90
			45	13.6	4010	0.84
4	Mobile pH	3.2	3.0	13.8	3999	0.89
			3.4	13.9	4005	0.91

The above data indicates that USP resolution, is slightly effected with change in flow rate and column oven temperature. USP plate counts and %RSD are not significantly impacted due to the deliberate changes made to the chromatographic conditions.

V. Conclusions

A rapid, simple, sensitive, accurate and reliable RP-UPLC method was developed and validated for the determination of Baclofen inan injection formulation.In this method Baclofen Sodium and its impurities were quantified simultaneously in single chromatographic run with high degree of accuracy and precision. The stability indicating power of the method was established through stress studies. All the degradationproducts

formed during stress studies were wellseparated from the analyte peak which is evident from the peak purity data. The method discusses the nature of the molecule under stress conditions where it was shown that the compound sensitive towards acid and base hydrolysis followed by oxidation.The forced degradation studies indicate that the molecule is stable to thermal exposure. The validation data meets the acceptance criteria for the validation parameters as per the current ICH guidelines. The method can be successfully employed for

References

[1]. Baclofen; Drug Bank, Extracted from https://www.drugbank.ca/drugs/DB00181
[2]. Baclofen; rxlist; Extracted from https://www.rxlist.com/baclofen-injection-drug.htm.
[3]. Baclofen monograph; USP
[4]. Baclofen monograph; Ph.Eur
[5]. Müller H, Zierski J, Dralle D, Krauss D, Mutschler E. Pharmacokinetics of intrathecal baclofen. InLocal-spinal therapy of spasticity 1988 (pp. 223-226). Springer, Berlin, Heidelberg.
[6]. Shellenberger MK, Groves L, Shah J, Novack GD. A controlled pharmacokinetic evaluation of tizanidine and baclofen at steady state. Drug metabolism and disposition. 1999 Feb 1;27(2):201-4.
[7]. Wuis EW, Dirks MJ, Vree TB, Van der Kleijn E. Pharmacokinetics of baclofen in spastic patients receiving multiple oral doses. Pharmaceutisch Weekblad. 1990 Apr 1;12(2):71-4.
[8]. Wuis EW, Dirks RJ, Vree TB, van der Kleyn E. High-performance liquid chromatographic analysis of baclofen in plasma and urine of man after precolumn extraction and derivatization with o-phthaldialdehyde. Journal of Chromatography B: Biomedical Sciences and Applications. 1985 Jan 1; 337:341-50.
[9]. Millerioux L, Brault M, Gualano V, Mignot A. High-performance liquid chromatographic determination of baclofen in human plasma. Journal of chromatography A. 1996 Apr 5; 729(1-2):309-14.
[10]. Flärdh M, Jacobson BM. Sensitive method for the determination of baclofen in plasma by means of solid-phase extraction and liquid chromatography–tandem mass spectrometry. Journal of Chromatography A. 1999 Jun 18; 846(1-2):169-73.
[11]. Tosunoğlu S, Ersoy L. Determination of baclofen in human plasma and urine by high-performance liquid chromatography with fluorescence detection. Analyst. 1995 Jan 1; 120(2):373-5.
[12]. Kochak G, Honc F. Improved gas—liquid chromatographic method for the determination of baclofen in plasma and urine. Journal of Chromatography B: Biomedical Sciences and Applications. 1984 Jan 1; 310:319-26.
[13]. Baclofen tablets; United States Pharmacopoeia; USP41–NF36 Page 445.
[14]. Baclofen Compounded Oral Suspension; United States Pharmacopoeia; USP41–NF36 Page 444.
[15]. Spahn H, Krauβ D, Mutschler E. Enantiospecific high-performance liquid chromatographic (HPLC) determination of baclofen and its fluoro analogue in biological material. Pharmaceutical research. 1988 Feb 1;5(2):107-12.
[16]. Tosunoğlu S, Ersoy L. Determination of baclofen in human plasma and urine by high-performance liquid chromatography with fluorescence detection. Analyst. 1995 Jan 1;120(2):373-5.
[17]. Hefnawy MM, Aboul-Enein HY. Enantioselective high-performance liquid chromatographic method for the determination of baclofen in human plasma. Talanta. 2003 Dec 4;61(5):667-73.
[18]. Zhu Z, Neirinck L. Chiral separation and determination of R-(−)-and S-(+)-baclofen in human plasma by high-performance liquid chromatography. Journal of Chromatography B. 2003 Mar 5;785(2):277-83.
[19]. Wuis EW, Dirks RJ, Vree TB, van der Kleyn E. High-performance liquid chromatographic analysis of baclofen in plasma and urine of man after precolumn extraction and derivatization with o-phthaldialdehyde. Journal of Chromatography B: Biomedical Sciences and Applications. 1985 Jan 1;337:341-50.
[20]. Flärdh M, Jacobson BM. Sensitive method for the determination of baclofen in plasma by means of solid-phase extraction and liquid chromatography–tandem mass spectrometry. Journal of Chromatography A. 1999 Jun 18;846(1-2):169-73.
[21]. Rajesh M, Manzoor Ahmed, Maanasa Rajan BN; A Validated Rp-Hplc Method For The Estimation Of Baclofen In Bulk Drug And Pharmaceutical Formulations, Indian Journal of Research in Pharmacy and Biotechnology; Mar-Apr 2013 Vol 1(2); Page 216.
[22]. Saroj Kumar Raul, B. V. V. Ravi kumar,Ajaya kumar Pattnaik and Prabhat Kumar Sahoo; Rp-hplc method development and validation For the estimation of baclofen in bulk and Pharmaceutical dosage forms; Int. J. Chem. Sci.: 11(1), 2013, 390-398.
[23]. McCalley DV, Brereton RG. High-performance liquid chromatography of basic compounds: Problems, possible solutions and tests of reversed-phase columns. Journal of Chromatography A. 1998 Dec 18; 828(1):407-20.

IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) is UGC approved Journal with Sl. No. 5012, Journal no. 49063.

Venkata Narasimha Rao Ga. " Ultra Performance Liquid Chromatographic Seperation And Quantification Of Baclofen And And Its Potential Impurities In A Injection Formulation." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 13.4 (2018): 29-38.