Flow Injection Spectrophotometric Determination of Baclofen in Pharmaceutical Formulation Using Prussian Blue Reaction

Ghadah Fadhil
Department of Chemistry, College of Science, University of Baghdad, Baghdad-Iraq.
E-mail: ghadaalhakim@yahoo.com

Abstract

A simple, rapid and sensitive spectrophotometric method for the determination of baclofen in pharmaceutical preparations were developed by combining a spectrophotometric detector with Flow injection analysis. This method based on the oxidation of iron (II) to iron (III) by baclofen and formation of complex between iron (III) and potassium hexacyano ferrate (II) in acidic solution. The absorbance from prussian blue dye is recorded at 700 nm. Chemical and physical parameters of this system were investigated. The linearity of baclofen is ranged from (0.05-25) mmol.L^{-1} with correlation coefficient r = 0.9972. The limit of detection (S/N=3) was equaled 0.01 mmol.L^{-1} the L.O.Q was 1.05 mmol.L^{-1} and a relative standard deviation for 6 replicate determinations of baclofen in 10 mmol.L^{-1} solution was 1.3 % using 100 µL sample volume. The proposed method was successfully applied to the determination of baclofen in pharmaceutical formulation with recovery 99%. Using paired t-test it was shown that there was no significant difference between the proposed method and official method on that basis the proposed method can be accepted as an alternative analytical method. [DOI: 10.22401/JNUS.20.1.03]

Keywords: Baclofen (BCF), Flow injection analysis, Spectrophotometric, Prussian blue (PB).

Introduction

Baclofen (BCF) (4-amino-3-p-chlorophenyl butyric acid) Fig.(1) is a chemical analogue of γ-amino butyric acid (GABA) and is widely used as a skeletal muscle relaxant in the treatment of spastic disorders[1], which is widely used in the treatment spastcity resulting from multiple sclerosis, muscle spasms, muscular rigidity and spinal cord injuries, where pain persist predominantly, in such cases the quick onset of action is of prime importance[2].

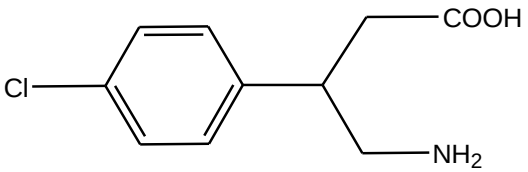


Fig.(1): Chemical structure of Baclofen.

Baclofen is official drug in B.P and U.S.P[3,4]. Baclofen is available in oral and intravenous formulation. Though the conventional oral tablets are widely used, they suffer from a few practical drawbacks such as it is non-suitability when quick onset of action is required[5]. In efficacy in treatment of different diseases it is important to determine the amounts of Baclofen in the tablets. Several methods reported for determination of Baclofen biological fluids and pharmaceutical formulation, based on RP-HPLC with UV detection[6], RF-HPLC - PDA for simultaneous of Baclofen and tiganidine[7], HPLC with fluorescence detection[8], LC-mass spectrometry [9] and uv-spectrometry[10]. There is a little study to make determination of Baclofen by colorimetric method by spectrophotometry[11-13].

Prussian blue (PB) is obtained by the addition of Fe(III) salt to a solution of $[Fe(CN)_6]^{4-}$. Turn bults blue (TB) is formed by the addition of Fe(II) salt to a solution of $[Fe(CN)_6]^{3-}$. It's known appreciated that TB and PB are the same because of the rapidity of electron exchange through a Fe-CN-Fe linkage. The exact hue depends on the method of prepration, which dictate the energy of the transfer of electrons from Fe(II) to Fe(III)[14], this work was applied to determination of paracetamol using merging zone-FIA[15].

The work conducted in this research relies on the use of baclofen (Lioresal) as an oxidant for the prepared Fe(II) ion to form Fe(III), then the formed Fe(III) meets with $[Fe(CN)_6]^{4-}$ to form prussian blue (PB) which is $Fe_4[Fe(CN)_6]_3$ that is blue in color, then passes

through flow cell where it irradiated with 700nm LED at variable intensity using photo silicon diode as detector[15].

Experimental

Reagents and chemicals

Chemicals

All chemicals used were of analytical reagent grade. Deionizer water was used throughout this work.

BCF stock standard solution, $C_{10}H_{12}ClNO_2$, 213.67 g.mol^{-1}, Syria, 100 mmol.L^{-1} was prepared by dissolving 2.1367 g/100 mL distilled water. Iron (II)(100 mmol.L^{-1}) was prepared by dissolving 13.901 g in 500 ml distilled water. A stock (100 mmol.L^{-1}) solution of potassium hexacyano ferrous (II) $K_4[Fe(CN)_6]$ (Fluka AG) was prepared by dissolving 18.3925 g in 500 mL distilled water. Nitric acid (1 mol.L^{-1}), dilute 128 mL of 70% HNO_3 with water to 2 L, sulphuric acid (1 mol.L^{-1}), dilute 11mL/2L of 96% H_2SO_4, HCl (1 mol.L^{-1}) dilute 176.50 mL/2L of 35% HCl.

The flow system used for the determination of BCF, shown schematically in Fig.(2). A peristaltic pump: three channels (Ismatec Switzerland). The drug solution was injected through the six-way injection valve (IDEX corporation, USA) with sample loop (0.7 mm i.d., Teflon, variable lengths), the source (5mm O.D, low current, Round LED, red light), the detector (Siemens, silicon photodiode) and the amplification unit[15]. Gragh x-t recorder (1023-kompen sogragh, Siemens, Germany).

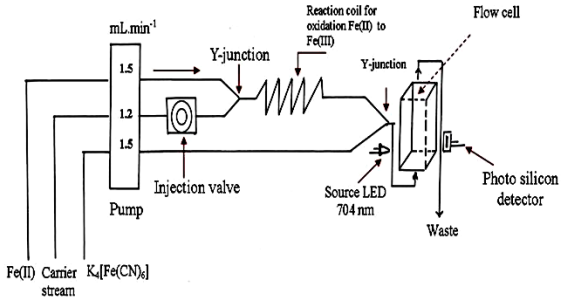
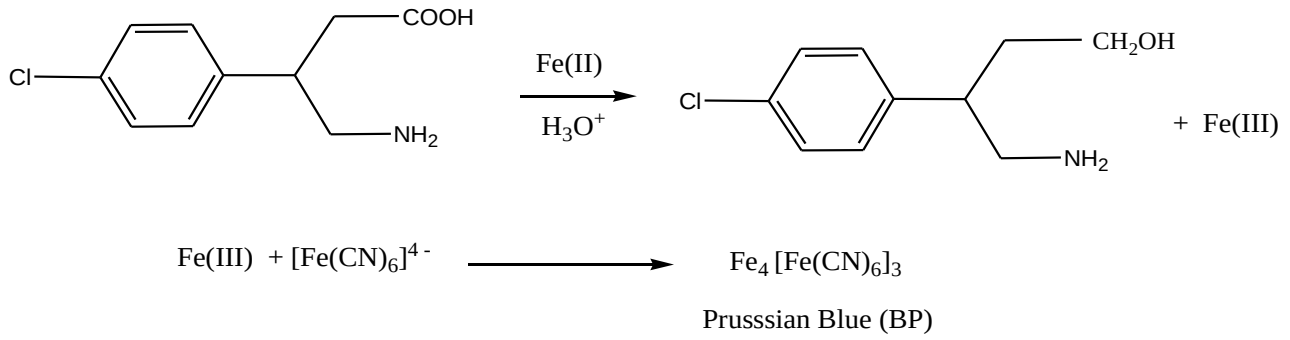


Fig.(2): Schematic diagram of flow injection analysis system for the determination of BCF.

Methodology

The whole manifold system for BCF determination via: BCF-Fe(II)-$[Fe(CN)_6]^{4-}$-H_3O^+ system shown in Fig.(2). The manifold system is composed of three lines: First line supplied with Fe(II) ion (5 mmol.L^{-1}) at 1.5 mL.min^{-1}, while the second represented the carrier stream (acidic medium) leading to the injection valve which allows the use of 100 µL and a flow rate of 1.2 mL.min^{-1}. Both line meet at Y-injection; with an outlet for reactant product Fe(II), which is loaded to loop (100cm) for completion of reaction and oxidation of Fe(II) to Fe(III) by BCF. The third line supplied with $K_4[Fe(CN)_6]$(1mmol.L^{-1}) at 1.5mL. min^{-1} flow rate. Both out coming lines meet at the Y-injection for completion of reaction between produced Fe(III) and $[Fe(CN)_6]^{4-}$ to form colorful blue complex, $Fe_4[Fe(CN)_6]_3$ then passes through flow cell where it irradiated with light emitting diode having 704 nm with a variable intensity monitored and a photo silicon diode as a detector[15] and shown in Fig.(2). UV spectra were measured with an uv-vis (CARY 100 conc.) spectrophotometer (Japan). A proposed mechanism of oxidation of Fe(II) by BCF in acidic medium to Fe(III) is presented in (scheme-1), that was proved practically and spectroscopically.



Scheme (1): Proposed mechanism of reaction between BCF and Fe(II) in acidic medium.

Results and discussion

Spectroscopic study of P.B complex

Using preliminary experimental concentrations of the chemicals used in the main reaction where as following : Fe(II) (8 mmol.L^{-1}), K_4 [Fe(CN)$_6$] (5mmol.L^{-1}) and BCF (10mmol.L^{-1}) in 0.5 mol.L^{-1} HCl. Fig.(3) shows the various spectrum obtained for (a) BCF, (b) Fe(II)-$[Fe(CN)_6]^{4-}$-H_3O^+ system and (c) BCF-Fe(III)-$[Fe(CN)_6]^{4-}$-H_3O^+ system. It shows the disappearance of both absorption maxima of BCF and response from Fe(II)- $[Fe(CN)_6]^{4-}$-H_3O^+ system. It might be attributed to the total consumption of both reactant and reaction of BCF with Fe(II) in acidic medium to form Fe(III) then reacted it with $K_4[Fe(CN)_6]$ to form blue complex (P.B) which gave λ_{max} at 700 nm.

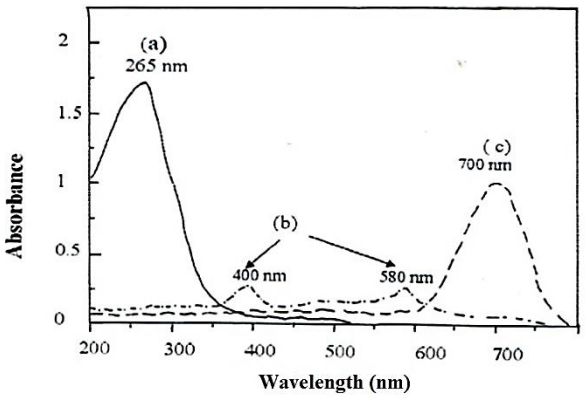


Fig.(3): Absorbance spectra for the determination of BCF.

a: Absorbance spectra(—) for BCF.
b: Absorbance spectra(—·—·) for Fe(II)-$[Fe(CN)6]^{-4}$—$H3O^+$.
c: Absorbance spectra(— —) for BCF - Fe(II)-$[Fe(CN)6]^{-4}$ — $H3O^+$ system .

Optimization of experimental conditions

Chemical variable

Effect of Fe(II) concentration:

Series 0-15 mmol.L^{-1} of Fe(II) were prepared at concentration of potassium hexacyano ferrate(II) of 3 mmol.L^{-1} and using a preliminary concentration of BCF 10 mmol.L^{-1} in 0.05 mol.L^{-1} H_2SO_4 as a carrier stream. Fig.(4) shows that 5 mmol.L^{-1} of Fe(II) is a suitable optimum concentration while at a concentration above this, distortion of the peak is observed, which might be due to small particles that may adhere on the glass wall.

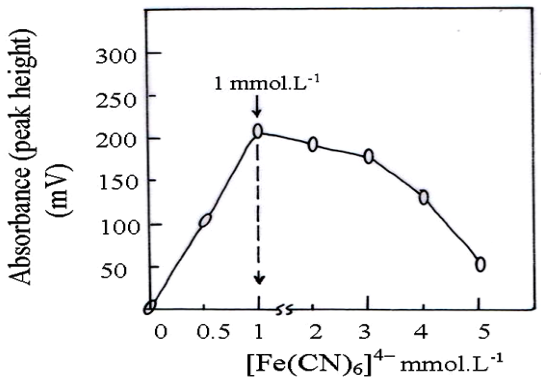


Fig.(4): Effect of variation of iron(II) concentration on absorbance for P.B complex, [BCF]: 10 mmol.L^{-1}, 100 µL.

Effect of variation of hexacyano iron(II) concentration on the absorbance of the prussion blue formation

A series (0–5) mmol.L^{-1} of hexacyano iron(II) solutions were prepared at optimum constant concentration of Fe(II) of 5 mmol.L^{-1} and using a chosen concentration of BCF 10 mmol.L^{-1}. Fig.(5) shows that 1mmol.L^{-1} of $K_4[Fe(CN)_6]$ is a suitable optimum concentration while at a concentration above this a wide broad peaks were obtained, this might be

attributed to inner filter effect due to probably precipitated particulate of the complex.

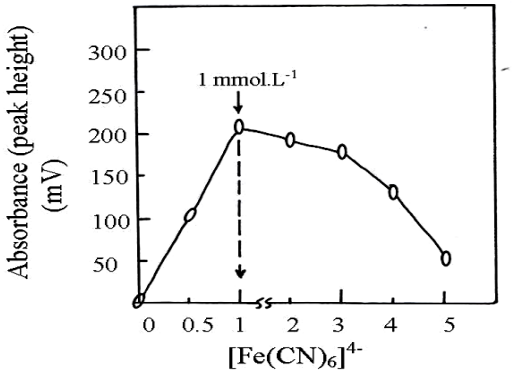


Fig.(5): Effect of variation of $[Fe(CN)_6]^{-4}$ concentration on determination of BCF (10 mmol.L^{-1}), 100 µL.

Effect of acidic medium on the absorbance of the prussion blue formation

The oxidation of Fe(II) with BCF was studied in different acidic media (nitric, hydrochloric and sulphuric acid) at a series of concentration (0.01- 0.1) mol.L^{-1} in addition to the aqueous phase medium. The results are depicted in Fig.(6). In which that nitric acid increases absorbance which might be explained as it is a powerful oxidizing agent in addition to BCF drug effect. While using sulphuric acid absorbance is increases which might be explained to yielding immediately the coloured species on the basis of the outcome of the results obtained from this section, sulphuric acid medium with concentration (0.01) mol.L^{-1} was chosen as optimum medium to conduct the reaction pattern.

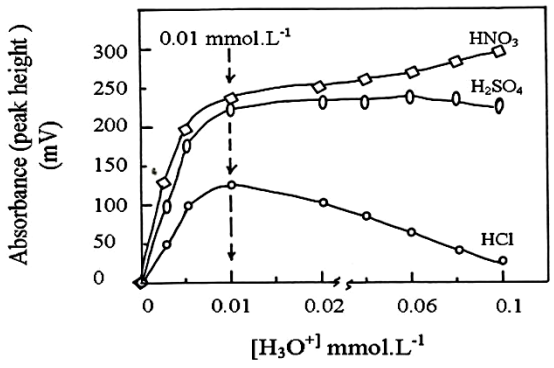


Fig.(6): Effect of type and concentration of acidic (HNO_3, H_2SO_4, HCl) on absorbance of P.B complex for determination of BCF (10 mmol.L^{-1}), 100 µL.

Physical parameters

Flow rate effect

Using optimum concentration of the reactant, Fe(II) (5 mmol.L^{-1}), $K_4[Fe(CN)_6]$ (1mmol.L^{-1}) and using a chosen concentration (10 mmol.L^{-1}) of BCF with the injected sample volume of 100 µL at a variable flow rate as tabulated in table 1and Fig.(7). The results shown that the best flow rate for both lines of Fe(II) and $K_4[Fe(CN)_6]$ is (1.5 mL.min^{-1}) and (1.2 mL.min^{-1}) for the carrier stream.

Table (1)
Effect of the variation of flow rate on absorbance of P.B complex.

Peristaltic pump speed	Flow rate (mL.min^{-1})			Absorbance (mv) n=3
	Fe(II)	$[Fe(CN)_6]^{-4}$	carrier stream	
5	0.9	0.9	0.7	80
10	1	1	0.9	100
15	1.3	1.3	1.0	190
20	1.5	1.5	1.2	230
25	1.9	1.9	1.6	225
30	2.3	2.3	1.9	175

It was noticed that at low flow rate there is an increase in dilution and dispersion, while at higher speed due to the departure of the reactant from flow cell prior to the completion of reaction.

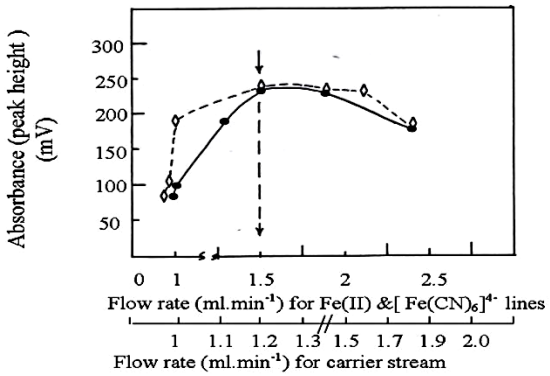


Fig.(7): Flow rate variation effect on the absorbance at optimum concentration parameters, 100 µL (sample volume), [BCF]: 10 mmol.L^{-1} .

Effect of sample volume

Using the optimum parameters achieved in previous section. Variable sample volume (80, 100, 150, 200 µL) were injected. The data obtained were plotted as shown in Fig.(8)

showing that the optimum sample volume is (100 µL) given a high of response and regular. Using larger volume i.e. > 100 µL it gave a slight higher response and irregular.

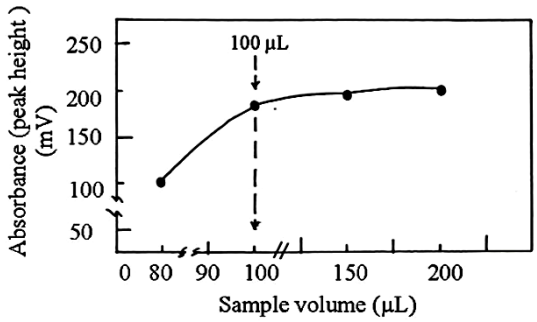


Fig.(8): Effect of variation of sample volume on absorbance of P.B complex, [BCF]: 10 mmol.L^{-1} .

Variation of absorbance versus concentration of BCF

Various concentrations (0.01–50) mmol.L^{-1} BCF were prepared using the parameters established above. Each measurement were repeated three times. The absorbance of the average peak height (mv) was plotted against the concentration of BCF (Figure 9). A straight line graph from (0.05–25) mmol.L^{-1} of BCF was obtained. Above 25 mmol.L^{-1} the value for correlation coefficient will deviated from linearity. The obtained results were tabulated in Table (2).

Table (2)
Summery of calibration graph results for the determination of BCF.

Practical measured concentration mmol.L^{-1}	**calibration graph concentration mmol.L^{-1}**	**Linear regression equation y^(mv)= a+b[BCF] mmol.L^{-1}**	**r r^2 %**	**$t_{tab.}$**	**$t_{cal.}=\frac{\lvert r\rvert\sqrt{n-2}}{\sqrt{1-r2}}$ at 95 %**
0.01 – 50	0.05 – 25	20.40+ 19.12[BCF] mmol.L^{-1}	0.9972 99.46 %	2.201≪ 44.83	

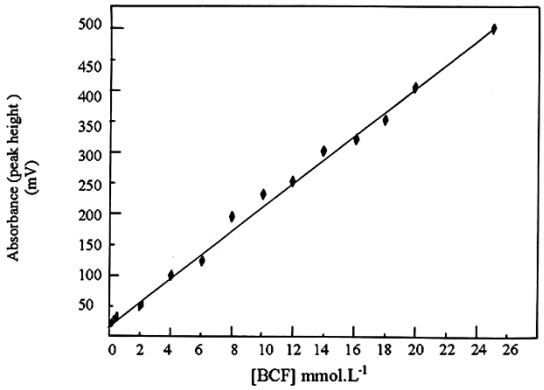


Fig.(9): Effect of variation of BCF concentration on absorbance at optimum conditions: BCF (variation of conc.) – Fe(II) (5 mmol.L^{-1}) – $[Fe(CN)_6]^{-4}$ (1 mmol.L^{-1}) – H_3O^+ (H_2SO_4(0.01 mol.L^{-1})).

Limit of detection

Detection limit of BCF was conducted through three methods as tabulated in Table(3) at injected sample volume of 100 µL, also L.O.Q was reported.

Table (3)
Limit of detection of quantitation for BCF at optimum parameter.

General dilution for the minimum concentration	Based on the slope $X=\frac{3SB}{slope}$	Linear equation $y^{(mv)}=y_B+3S_B$	L.O.Q= $y^{=}y_B+10S_B$
10 µmol.L^{-1}	16 µmol.L^{-1}	0.29 mmol.L^{-1}	1.05 mmol.L^{-1}

x= value of L.O.D based on slope.
s_B= standard deviation of blank solution.
y_B= response for the blank solution.

Repeatability

The work has been conducted in this project was characterized by high precision with good repeatability since the relative standard deviation (RSD) was very small. Table (4) shows the repeatability for the result obtained for the variable concentration of BCF. The response profile at concentration 4, 10 mmol.L^{-1} of six successive injected sample measurements as shown in Fig.(10).

Table (4)
Repeatability for the determination BCF using P.B complex.

[BCF] mmol.L^{-1}	n	Mean $\bar{y}_i$ (mv)	σ_{n-1}	RSD %
4	6	100	0.98	0.98
10	6	230	1.28	0.56
20	5	400	2.32	0.58

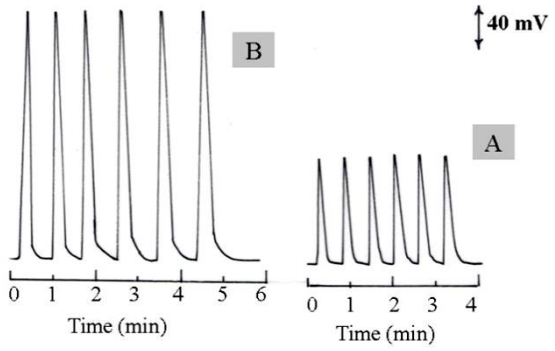


Fig.(10): Successive repeatable measurement for BCF; A: 4 mmol.L^{-1}, B: 10 mmol.L^{-1}.

Analysis of pharmaceutical preparation

The proposed method achieved in this work was used for the analysis of BCF in pharmaceutical preparation and was compared with the official method[4]. Thirteen tablets were weight, crushed and grinded. Tablets containing 10 mg of BCF were weighted 3.3813 g (213.6 mg active ingredient) (10 mmol.L^{-1}) from pharmaceutical preparation, dissolved in a little water, filtration to get ride of undissolved materials and completed the volume to 100 mL with distilled water. 10 mL was drown to each of five 25 mL volumetric flask followed by the addition of gradual volumes of standard BCF (0,1, 1.5, 2, 2.5)mL of 100 mmol.L^{-1}. Flask no.1 is the sample. The measurement were conducted by proposed method and the results were mathematically treated for the standard addition method. The results were tabulated in Table (5) at confidence interval 95%, paired t-test[16] was used as shown in Table (6). The obtained results indication that there was no significant different between developed method (FIA-spectrophotometer) with official method at 95% confidence level because $t_{cal} < t_{tab}$

Table (5)
Results for the determination BCF in pharmaceutical preparation using proposed method.

Pharmaceutical Formulation, content, country	Weight of tablets (g) $\overline{W} \pm t_{0.025,\infty} \frac{\sigma n-1}{\sqrt{n}}$ at 95%	Based on theoretical content (mg)	Practical content (mg)	Efficiency %
Lioraz(10 mg) Razi labs, Syria	0.15825 ± 0.0029	10 ± 0.183	9.9 ± 0.28	99 %

Table (6)
Paired t-test results for the proposed method with official method[4] for the determination of BCF in pharmaceutical preparation.

Proposed method ($\bar{x}$)	official method (μ)	σ_{n-1}	n	$\lvert t_{cal}\rvert = \frac{(\bar{x}-\mu)\sqrt{n}}{\sigma n-1}$	t_{tab} at 95 %, n-1
9.9	10 mg	1.78	3	1-0.0971= 0.097 ≪ 4.303	

Conclusion

The work presented in this research shows the capability of accepting an alternative method for the analysis and determination of BCF using the formation of Prussian blue with good repeatability and accepting linear dynamic range.

Acknowledgements

The author is grateful to Prof. Dr. Nagam S. Turkey Al-Awadie for providing her continuous support throughout the work.

References

[1] Seal Sc. Martindale, "The complete drug reference", 33rd Ed., pharmaceutical press, London, 643, 1999.

[2] Tripathi. K. D., "Essentials of medical pharmacology", 5th Ed., New Delhi, Jaypee brother's medical publishers, 316-318, 2003.

[3] British pharmacopoeia, "Her Majesty's stationery office", London, vol-1, 202, 2005.

[4] US pharmacopoeia NF, vol-23, 207, 2004.

[5] Sean, C., Sweetman, Martinal, "The complete Drug reference", 34th Ed., 1386-1388, 2005.

[6] Abu MR., "Simple and rapid RP-HPLC determination of baclofen in human plasma with UV detection": application to a pharmacokinetic study, J.chromatogr B Biomed Sci Appl., 487,107-115, 1989.

[7] Nalluri B.N., Sushmitha K., Sunandana B. and Babu P., "Development and validation of RP-HPLC-PDA method for simultaneous estimation of baclofen and tizanidine in bulk and dosage forms", J. of applied pharmaceutical science, 2(7), 111-116, 2012.

[8] Sedat T. and Lale E., "Determination of baclofen in human plasma and urine by HPLC with fluorescence detection", Analyst, 120, 373-375, 1995.

[9] Miksa I.R. and Poppenga R.H., "Direct and rapid determination of baclofen (Lioresal) and Carisoprodol (Soma) in bovine serum by liquid chromatography-Mass spectrometry", J.Anal.Toxical, 27 (5), 275-283, 2003.

[10] Islam T., Ferdous S., Jain P. and Reza H.M.; "Method development and validation of baclofen mouth dissolving tablets by UV spectroscopy", Europ.J. Appl.Sci.,5(1),7-11, 2013.

[11] Hosseinimehr S.J., Pourmorad F., Moshtaghi E. and Amini M., "Colorimeteric determination of baclofen with ninhydrin reagent and compare with Hplc method in tablet", Asian J.Chem., 22(1), 522-526, 2010.

[12] Elbashir A.A., Ahmed A.A., Ahmed S.M.A. and Aboul-Enein H.Y., "1,2-Naphthoquinone-4-sulphonic acid sodium salt (NQS) as an analytical reagent for the determination of pharmaceutical amine by spectrophotometry", Applied spectroscopy Reviews ,47, 219-232, 2012.

[13] Ahmed M., Rajesh M., Sathish K.S.A. and Maanasa Rajan B.N., "Zero order and

first order derivative spectrophotometric methods for determination of baclofen in pharmaceutical formulation", Int. J. Chem Tech Res., 3(2), 933-937, 2011.

[14] Ludi A., "Prussian blue, an inorganic evergreen", Journal of chemical education 58, 1013, 1981.

[15] Shakir I.M.A., Turkei N.S. and Al-Awady M.J., "New approach for the online determination of paracetamol using merging zone-continuous flow injection analysis via home made photometric based on 704 nm LED and a photo silicon mini detector", National Journal of chemistry, 39, 455-468, 2010.

[16] Miller J.C. and Miller J.N., "Statistics for Anal. Chem"., 2nd Ed., John wily and sons., New York , 1997.

الخلاصة

طورت طريقة طيفية بسيطة وسريعة وحساسة لتقدير عقار باكلوفين في المستحضرات الصيدلانية من خلال اقتران تقنية التحليل بالحقن الجرياني مع المتحسس الطيفي. استندت الطريقة على اكسدة الحديد (II) الى الحديد (III) بواسطة باكلوفين وتكوين معقد بين الحديد (III) والفيروسيانيد البوتاسيوم في الوسط الحامضي سجلت الامتصاصية للزرقة البروسية (prussian blue) عند 700 نانوميتر. تم دراسة الظروف الفيزيائية والكيميائية للتفاعل. مدى الخطية لمنحني المعايرة لتقدير الباكلوفين يمتد (0.05 – 25) مللي مول. لتر$^{-1}$ مع معامل ارتباط r= 0.9972 حدود الكشف (S/N=3) تساوي 10 مايكرومول.لتر$^{-1}$ وحد التقدير الكمي L.O.Q 1.05 مللي مول.لتر$^{-1}$ والانحراف القياسي النسبي المئوي لتكرار 6 مرات لتركيز 10 مللي مول.لتر$^{-1}$ من الباكلوفين هو 1.3% لانموذج محقن بحجم 100 مايكرولتر. طبقت الطريقة بنجاح لتقدير باكلوفين في أحد المستحضرات الصيدلانية مع معامل تقدير (الاستردادية) يساوي 99%. استخدم اختبار t– المزدوج وتبين أنه لايوجد فرق جوهري بين الطريقة المقترحة والطريقة الرسمية وبالامكان استخدام الطريقة المقترحة كطريقة تحليلية بديلة.