Asian Journal of Chemistry Vol. 22, No. 1 (2010), 522-526

Colorimetric Determination of Baclofen with Ninhydrin Reagent and Compare with HPLC Method in Tablet

SEYED JALAL HOSSEINIMEHR*, FERESHTEH POURMORAD,
EHSAN MOSHTAGHI and MOHSEN AMINI†
Department of Medicinal Chemistry, Faculty of Pharmacy,
18 Kilometer Khazar Boulevard, Mazandaran University of Medical Sciences, Sari, Iran
Tel/Fax: (98)(151)3261244; E-mail: sjhosseinim@yahoo.com; sjhosseinim@mazums.ac.ir

A simple and sensitive spectrophotometeric method has been developed for the determination of baclofen in tablet. Baclofen reacts with ninhydrin reagent in aqueous solution producing a coloured product. In this study, the different experimental parameters affecting the development and stability of the colour were carefully studied and optimized. The modified HPLC with ion pair method was used for quantification of baclofen in the same tablets that used with spectrophotometery. The absorbance-concentration plot is linear over the range 0.4-1.4 mg/mL with correlation coefficient of 0.995. The molar absorptive was 283.36 mol/L cm. The validity of the described procedures was assessed to show that this method had good validity at different times. Statistical analysis of the results has been carried out with F-test and T-test showed that it has high accuracy with HPLC method, but it has not good precision compared to HPLC method.

Key Words: Baclofen, Colorimetry, Spectrophotomery, Ninhydrin.

INTRODUCTION

Baclofen (4-amino-3-*p*-chlorophenylbutyric acid) (Fig. 1) is a chemical analogue of γ-aminobutyric acid (GABA) and is widely used as a skeletal muscle relaxant in the treatment of spastic disorders[1,2]. 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 in biological fluids and pharmaceutical formulation, based on HPLC[3,4] mass spectroscopy[5,6] capillary electrophrosis[7] and polarography[8]. There is a little study to make determination of baclofen by colorimetric method by spectrophotometry[9]. Baclofen has NH_2 group that reacts with ninhyrdin to produce a intensive colour with good accuracy and precise[10].

This study suggests simple and sensitive colorimetric procedure for the determination of baclofen in tablet based on reaction of -NH_2 group with ninhydrin reagent was carried out and then accuracy of this method compared with HPLC.

†Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

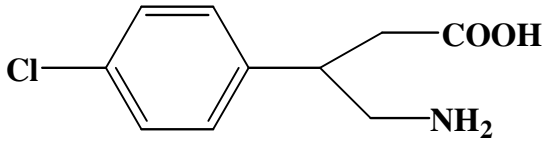

Fig. 1. Structural formula of baclofen

EXPERIMENTAL

Baclofen (standard substance) was from Chemi Darou Ind. Co. (Iran) and ninhydrin, sodium pentane sulfonate (HPLC grade) and HPLC grade methanol were provided by Merck Co. (Germany). Baclofen tablets (containing 10 mg of baclofen) were obtained from the pharmaceutical company in Iran (Zahravi).

A digital double beam spectrophotometer (Perkin-Elmer EZ201, USA) was used for conventional measurements. All pH measurements were done with a digital pH meter model Metrohm 744 (Switzerland). The HPLC system consisted of a model K-1001 solvent delivery system equipped with a Rheodyne injection valve (20 µL sample loop inserted) and a UV-Vis spectrophotometric detector model K-2600 set at 230 nm (all from Knauer Assoc., Germany). The analysis was performed using a ODS-C_{18} column (250 mm × 4.6 mm i.d., 5 µm particle size, Shim-pack VP-ODS) and the corresponding guard column (5 mm × 4.6 mm i.d., 5 µm particle size) according to USP with ion pair method. All solvent were filtered and degassed before entering the column. The mobile phase was acetic acid (0.3 N)-methanol (62.5:45 v/v) containing 2 mL of sodium pentane sulfonate (0.36 N) as ion pair. The mobile phase flow rate was 1.0 mL/min and all the measurements were done at ambient temperature.

Solution: A freshly prepared 1 mg/mL aqueous solution of baclofen (standard substance) was used as the stock solution that diluted with the distilled water to obtain the working standard solution ranging from 0.4-1.2 mg/mL. A ninhydrin stock solution (2 mg/mL) was prepared by dissolving ninhydrin in water.

Baclofen determination: Ninhydrin's reagent method

Calibration curve: 0.5 mL aliquots of drug solution ranging from 0.4 to 1.4 mg/mL working standard solution was added to 0.5 ninhydrin stock solutions in tube and mixed well. The solution was allowed to heat on a water bath at 95 ± 5 ºC for 10 min. After cooling, 2 mL of distilled water was added to tube. The absorbance was measured at 570 nm against a blank prepared simultaneously without ninhydrin solution.

Assay: Twenty tablets (10 mg) were grinded to fine powder and 10 mg of powder equal to pure baclofen was weighted and transferred to 10 mL volumetric flask and dissolved in double distilled water, under stirring. This solution was filtered and the procedure described for the calibration curve was followed. Five series were used and each experiment repeats three times.

HPLC method: The suitable quantities of standard baclofen were dissolved in mobile phase to obtain a solution having known concentration of 4 mg/mL. Twenty tablets (10 mg) were grinded to fine powder and 40 mg of powder equal to pure baclofen was weighted and transfer to 10 mL volumetric flask. This solution was filtered and benzoic acid was used as internal standard at 0.47 mg/mL. The equal volume (10 µL) of the standard drug solution and the assay preparation were separately injected into the column, recorded the chromatograms and then measured the responses for the obtained peaks. Each experiment was repeated three times for the standard drug solution and the tablets.

RESULTS AND DISCUSSION

Baclofen exhibited a low UV absorption at 265 nm. The reaction between baclofen produced UV absorption at 570 nm. To optimize the condition, a number of parameters such as temperature, time, reagent concentration and solvent were investigated. Baclofen was capable to react with ninhydrin only at higher temperature. Maximum colour was obtained by heating on water bath at 95 ± 5 ºC for 10 min. 0.5 mL of 0.2 g % of ninhydrin reagent was found optimum to maximize the colour intensity. Water solution had good results in comparison to methanol or phosphate buffer.

Under the described condition, the calibration graph was linear in the concentration range of 0.4 to 1.4 mg/mL of baclofen. The linear regression equation was Y = 0.7539X - 0.1161. Where X is the concentration of absorbent in mg/mL and correlation coefficient (R^2) 0.9955 (n = 6) indicating good linearity. The results of analysis showed that detection limit was 0.0035 mg/mL. The E_{max} was calculated to be 283.36 L/cm mol.

Inter-day precision was determined by analyzing three calibration curves with quality control samples on three different days. Inter-day assay precision was determined by analyzing five replicates of quality control samples extracted in the same batch. The results of inter-day and intar-day precision for baclofen in tablets by percentage recovery were between 100 and 102 % and there was not observed any significantly difference between analyzing days.

Application in a dosage form: For establishing the specificity of this method for tablets, five series each of 20 tablets powdered and 40 mg equal baclofen weighted and then quantity of baclofen determined with spectrophotometry based reaction to ninhyrdin. The mean quantity and recovery were found to be 10.11 mg and 101 ± 0.8 % per each tablet, respectively. The accuracy and reliability of this method was compared with HPLC method. Quantification of the same tablets that assayed with ninnhydrin reagent was determined by HPLC method. The mean quantity and recovery were found to be 9.83 ± 0.06 mg and 98.27 ± 0.56 % per each tablet respectively. The performance of the ninhydrin method was judged by calculating the student's t- and F-values. The analytical results obtained from this investigation and comparison of student's t- and F-values with these methods is showed in Table-1.

TABLE-1
CONTENT UNIFORMITY OF BACLOFEN IN ZAHRAVI TABLETS WITH TWO METHODS, EACH TABLET CONTAINING 10 mg BACLOFEN

	Ninhydrin method		HPLC method	
	Found* (mg)	Recovery (%)	Found* (mg)	Recovery (%)
1	10.073	100.73	9.75	97.5
2	10.20	101.96	9.87	98.75
3	10.033	100.32	9.9	99
4	10.033	100.32	9.8	98
5	10.21	102.864	9.83	98.31
Mean ± SD	10.11±0.078	101.2±1.1	9.83±0.06	98.31±0.59
RSD (%)	0.77		0.61	
T	6.30** (2.036)			
F	1.69** (6.390)			

*Found represents the mean of three analysis.
**The figures in parenthesis are the tabulated values of t and F at p = 0.05.

The application of simple and sensitive analytical method for quantification of drug in formulations is important for achieving good treatment of diseases. In order to determine the accuracy and precision of baclofen in tablets, ninhydrin reagent was used. In this study we observed that baclofen reacted to ninhydrin and produced a intensive violet colour that has a wavelength at 570 nm. Abdellatef and Khalil[10] showed that gabapentin [(aminomethyl)cyclo-hexaneacetic acid)] reacted with ninhydrin reagent *via* oxidative deamination of the primary amino group followed by the condensation of the reduced ninhydrin to form the coloured purple reaction product. Baclofen is structurally similar to gabapentin, but baclofen has aromatic ring with a two carbon space to amino group. The proposed mechanism of reaction is showed in Fig. 2, in this condition free amine group react with active group in ninhydrin. This reaction was performed in heat condition. This proposed mechanism is similar to reaction gabapentin with ninhydrin[10]. The proposed methods for determination of baclofen were applied to commercial tablets. Quantification of baclofen was not observed any significantly in different days in tablets and this assay is reproducible. The amounts of baclofen in the same tablets that assayed with ninhydrin were determined by HPLC method. At 95 % confidence level, the calculated F-value do not exceed the theoretical values, therefore, there is no accurately significant difference between the ninhydrin and HPLC methods. But the calculated t-value showed a significantly difference between colorimetric and HPLC method, therefore, precise of theses method is different. There are several HPLC method described determination of baclofen in human plasma. These techniques were separation and quantification of baclofen enantiomers in plasma[4,6]. Jain *et al.*[8] showed that baclofen can chelate with zinc and form complex to determined with polarographic method. Present results showed ninhydrin method has accurate same to HPLC, but it is not for precise in determination of baclofen in tablets. It should be noticed that the amounts of baclofen was lower than 10 mg in tablets that assayed by HPLC method and percentage of recovery was less than 100 %.

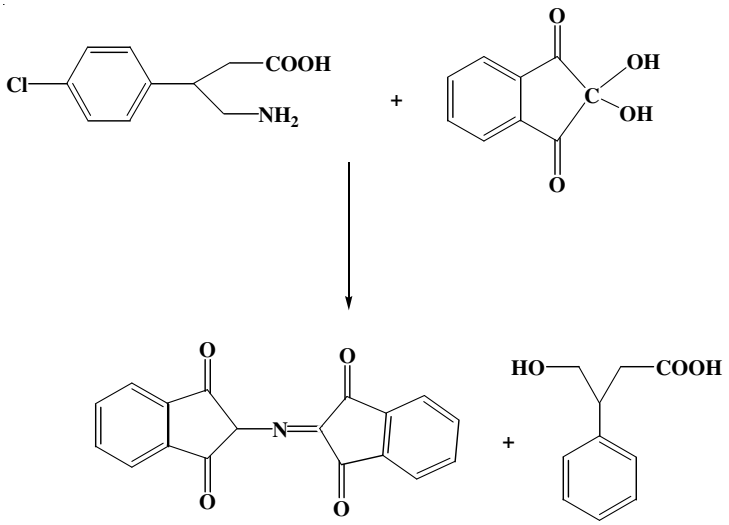

Fig. 2. Suggested reaction pathway between baclofen and ninhydrin reagent under hot condition

Conclusion

In conclusion, the ninhydrin reagent was used spectrophotometry for determination of baclofen in tablets and compare with HPLC method this method is simple and sensitive. The accuracy of this method was not different with HPLC method.

ACKNOWLEDGEMENT

This research based and presented in Ph.D. Thesis of Mr. Ehsan Moshtaghi for receiving of Pharmacy Doctor Degree in Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.

REFERENCES

1. A. Dario, R. Pisani, S. Sangiorgi, A. Soragna, M. Reguzzoni, M. Protasoni, F. Pessina, R. Fesce, A. Peres and G. Tomei, *Eur. J. Pharmacol.*, **550**, 33 (2007).
2. D.E. Hansel, C.R. W. Hansel, M.K. Shindle, E.M. Reinhardt, L.V. Madden, E.B. Levey, M.V. Johnston and A.H. Hoon Jr., *Pediatr. Neurol.*, **29**, 203 (2003).
3. Z. Zhu and L. Neirinck, *J. Chromatogr. B*, **785**, 277 (2003).
4. M. Hefnawy and H.Y. Aboul-Enein, *Talanta*, **61**, 667 (2003).
5. R. Goda, N. Murayama, Y. Fujimaki and K. Sudo, *J. Chromatogr. B*, **801**, 257 (2004).
6. M. Flardh and B.M. Jacobson, *J. Chromatogr. A*, **846**, 169 (1999).
7. S.Y. Chang, N.-Y. Zheng and C.-S. Chen, *Int. J. Appl. Sci. Eng.*, **2**, 277 (2004).
8. S. Jain, N.K. Jain and K.S. Pitre, *J. Pharm. Biomed. Anal.*, **31**, 1035 (2003).
9. H.M. Saleh, M.M. El-Henawee, G.H. Ragab and S.S.A. El-Hay, *Spectrochim. Acta*, **67A**, 1284 (2007).
10. H.E. Abdellatef and H.M. Khalil, *J. Pharm. Biomed. Anal.*, **31**, 209 (2003).

(*Received*: 13 February 2009; *Accepted*: 11 September 2009) AJC-7865