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BAKLOFENİN TABLETLERDE FLUORESKAMİN İLE SPEKTROFLUORİMETRİK MİKTAR TAYİNİ*

SPECTROFLUORIMETRIC DETERMINATION OF BACLOFEN IN TABLETS WITH FLUORESCAMINE

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SUMMARY

In this study, a spectrofluorimetric method has been developed for the assay of baclofen. The method depends on the formation of a fluorophore between the drug and fluorescamine. The derivatisation reaction proceeded quantitatively at pH 9.0 and room temperature within 5 min when the molar ratio of reagent to baclofen was 100. The fluorescence intensity was measured at 490 nm using 365 nm excitation filter. Linearity was observed over the concentration range 10-1250 ng/ml of baclofen. The method was applied to the determination of baclofen in tablets and the results were statistically compared with those obtained by the official method.

ÖZET

Bu çalışmada, baklofen için spektrofluorimetrik bir tayin yöntemi geliştirilmiştir. Yöntem, baklofen ile fluoreskamin arasında bir fluorofor oluşmasına dayanmaktadır. Türevlendirme reaksiyonu pH 9.0 da, belirteç/amin mol oranı 100 olduğunda, oda temperatüründe 5 dak içersinde kantitatif olarak yürümektedir. Fluoresans şiddeti, 365 nm eksitasyon filtresi kullanılarak 490 nm de ölçüldü. 10-1250 ng/ml baklofen konsantrasyonları aralığında doğrusallık gözlendi. Yöntem baklofen içeren tabletlere uygulandı ve sonuçlar, farmakope yöntemi ile elde edilen sonuçlarla istatistiksel olarak kıyaslandı.

INTRODUCTION

Baclofen, γ-amino-β-(p-chlorophenyl)butyric acid has been commonly used for controlling muscle spasticity. Various methods including UV (1) and visible (2) spectrophotometry, spectrofluorimetry (3), GC (4) and HPLC (5) have been reported for the assay of this drug in both tablets and biological materials.

Fluorescamine, 4-phenylspiro[furan-2(3H),1-phthalan]-3,3'-dione reacts rapidly with primary amines to give highly fluorescent pyrrolinone derivatives (6-8).

This study presents a simple and sensitive spectrofluorimetric method for the determination of baclofen using this reagent.

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EXPERIMENTAL

Instrument : A Zeiss PMQ II spectrophotometer equipped with ZFM 4 fluorescence attachment and St 41 mercury lamp was used. All measurements were carried out at 490 nm using 365 nm filter for excitation.

Chemicals: Baclofen and its tablets (Lioresal®) were kindly provided from Ciba-Geigy, Istanbul, Turkey. Fluorescamine was purchased from Aldrich Chem. Co. Milvaukee, WI,USA. Other chemicals were analytical-reagent grade.

Solutions : a) *Standard solutions* : About 10 mg of baclofen, accurately weighed, was dissolved in 100 ml of water. Standard solutions (0.08-0.4, 0.4-2 and 2-10 $\mu g/ml$) were prepared from this solution by appropriate dilutions with water. b)*Sample solution* : Tablet powder, equivalent to about 15 mg of baclofen was accurately weighed and transferred into a 50 ml calibrated flask, 30 ml of water was added and shaken mechanically for 15 min. The volume was diluted with water, mixed and filtered. A 1 ml volume of the filtrate was adjusted to 50 ml with water in a calibrated flask. c)*Reagent solution* : Fluorescamine was dissolved in acetone to give a concentration of 0.13% (w/v) and protected from light. d)*Reference standard solution* : Prepared by diluting a stock solution of quinone sulphate (10 $\mu g/ml$ in 0.1N H_2SO_4 solution). Measuring system of the instrument was calibrated by using this solution. e)*Buffer solution* : Disodium tetraborate solution (0.025 M) was adjusted to pH 9.0 with 0.1 N NaOH solution.

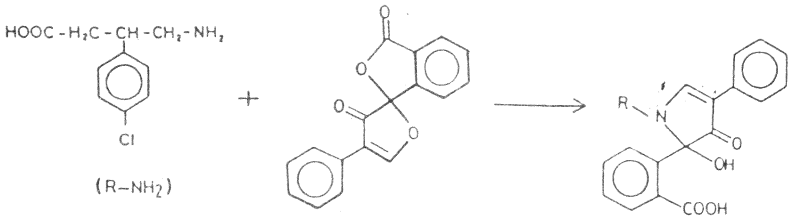
Assay procudure : An aliquot of 0.5 ml of standard and sample solution were placed into a 10 ml glass, stoppered test-tube and 3 ml of buffer solution was added. While vortex-mixing the contents of the tube, 0.5 ml of reagent solution was added rapidly and the mixing was continued for 30 sec. After 15 min the fluorescence intensity was measured at 490 nm while exiting at 365 nm against a blank prepared similarly. The fluorescence intensity of the reference solution, with appropriate concentration, was also measured at the same wavelength combination.

Calibration graphs for three concentration ranges were prepared by plotting the concentration against the relative fluorescence intensity (I_F). The amount of baclofen

in tablets was calculated from the regression equation of the calibration graph over the concentration range 250-1250 ng / ml.

RESULTS AND DISCUSSION

The reaction between baclofen and fluorescamine produced a highly fluorescent derivative (Scheme). Several experimental parameters, effecting the reaction such as pH, amount of the reagent, reaction time were optimised.



Reaction Scheme

In order to obtain the optimum pH the reaction was carried out at different pH ranges. The results shown in Fig.1 indicated that maximum fluorescence was obtained at pH 9.0.

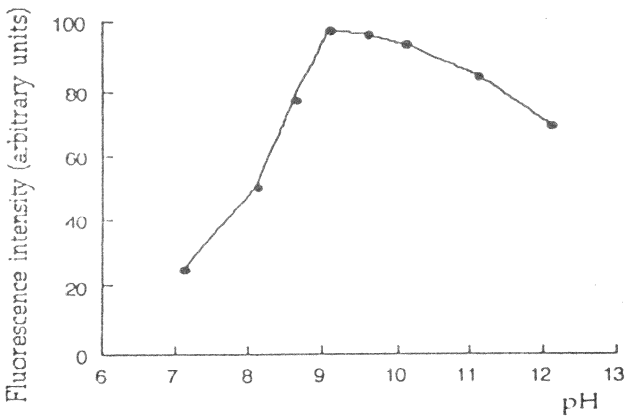


Fig.1 Effect of pH on the reaction of baclofen with fluorescamine

The reagent amount required was examined by changing the mole ratio of fluorescamine to baclofen. A 100 fold molar excess of reagent was found to be necessary to complete the reaction (Fig. 2).

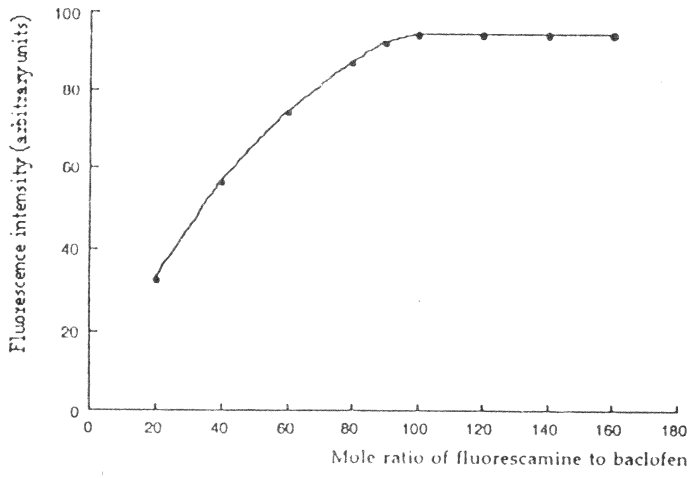


Fig. 2 Effect of reagent concentration on the reaction of baclofen with fluorescamine

The fluorescence intensity reached to a maximum within 5 min and measured at a wavelength combination of 395 and 490 nm. The derivative was stable in reaction medium for at least 30 min in dark.

Under the optimum conditions described above a linear correlation was obtained between I_F and over the concentration range 10-1250 ng/ml. The regression equations were I_F=0.120 C + 0.540 (r=0.9991), I_F =0.490 C + 0.380 (r=0.9998) and I_F=0.348 C+1.021 (r=0.9999), for 10-50, 50-250 and 250-1250 ng/ml concentration ranges respectively.

The present method was also applied to the assay of tablets and the results were compared with those obtained by the official method (2) in terms of t- and F-tests. There is no significant difference between the two methods. Statistical evaluations were shown in Table.

Table Comparison of the results obtained by proposed and official methods for the assay of baclofen in tablets (each tablet contains 10 mg of baclofen)

Statistical value	Proposed method	Official method
Mean	10.15	10.18
Recovery (%)	101.50	101.82
RSD (%)	0.38	0.34
Confidence limits	10.11-10.19	10.14-10.22
t-test of significance*	2.19	
F-test of significance*	2.31	

* n=6 p=0.05 t=2.23 F=5.05

The present method is sensitive and specific. It is also simple, because neither heating nor organic solvent extraction are needed. It can be applied for the routine pharmaceutical analysis.

REFERENCES

1. Kracmar,J., Kracmarova,J.: Pharmazie, 38, 524 (1983).
2. *United States Pharmacopeia, XXI^st Revision, First Supplement, 1985* , United States Pharmacopeial Convention, Inc. 12601, p. 1704.
3. Ersoy,L.: Analyst, 110, 881 (1985).
4. Degen, P.H.,Riess,W.: J. Chromatogr., 117, 399 (1976).
5. Wuis, E.W., Dirks, R.J.M., Vree,T.B., Van der Kleyn,E.: J.Chromatogr., 337, 341 (1985).
6. Udenfriend, S., Stein, S., Böhlen, P., Dairman, W., Leimgruber, W., Weigele,M.: Science, 178, 871 (1972).
7. Nagy, A.F.: J. Pharm. Sci., 68, 249 (1979).
8. İskender, G., Orak, F.: Bull. Hacettepe Fac. Pharm., 5 , 9 (1985) .

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