# BAKLOFENİN TABLETLERDE 1,2-NAFTOKİNON 4-SÜLFONİK ASİD SODYUM TUZU İLE SPEKTROFOTOMETRİK MİKTAR TAYİNİ

## SPECTROPHOTOMETRIC DETERMINATION OF BACLOFEN IN TABLETS WITH 1,2-NAPHTOQUINONE-4-SULPHONIC ACID SODIUM SALT

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### SUMMARY

A spectrophotometric method was developed for the determination of baclofen alone and in tablets. The method was based on condensation reaction of baclofen with 1,2-naphthoquinone-4-sulphonic acid sodium salt (NQS), at pH 7.5 and 70°C within 20 min., when the molar ratio NQS to baclofen was 4. After the extraction of the derivative with dichloromethane : n-butanol (4:1), absorbance was measured at 455 nm. A linear relationship between absorbance and concentration was obtained over the range of 5-50 $\mu g \cdot ml^{-1}$ of baclofen. The results of the analysis of baclofen tablets so obtained correlated well with those obtained by the offical method at 95% confidence level.

### ÖZET

Baklofenin tek başına ve tabletlerde miktar tayini için spektrofotometrik bir yöntem geliştirilmiştir. Yöntem, baklofen ile 1,2-naftokinon-4-sülfonik asid sodyum tuzu (NQS) arasında pH = 7.5'da, NQS/baklofen mol oranı 4 olduğunda ve 70°C de 20 dak. içerisinde tamamlanan kondensasyon reaksiyonuna dayanmaktadır. Oluşan türevin absorbansı, diklormetan : n-butanol (4:1) ile ekstraksiyondan sonra 455 nm de ölçülmüştür. Absorbans ile konsantrasyon arasında 5-50 $\mu g \cdot ml^{-1}$ konsantrasyon aralığında doğrusal bir ilişki olduğu saptanmıştır. Baklofen tabletlerinin analizinden elde edilen sonuçlar, farmakope yöntemiyle elde edilen sonuçlarla %95 olasılık düzeyinde uygunluk göstermiştir.

## INTRODUCTION

Baclofen [4-amino-3-(p-chlorophenyl) butyric acid], is a centrally acting muscle relaxant which is indicated for the alleviation of sings andsymptoms of spaticity. Various methods including UV (1) and visible

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(2 – 4) spectrophotometry, spectrofluorometry (5), GC (6) and HPLC (7,8) have been reported for the assay of this compound in both pharmaceutical preparations and biological materials.

NQS (1,2-naphthoquinone-4-sulphonic acid sodium salt) has been employed for spectrophotometric (9-12) and spectrofluorometric (13-15) determination of several primary and secondary amines.

In this study, a colorimetric method previously described (3) involving derivatisation of baclofen with NQS was modified. The factors effecting the reaction such as pH, temperature, reaction time, amount of the reagent and solvent extraction were investigated.

## EXPERIMENTAL PART

*Instruments*

A Beckman Model B spectrophotometer with 1 cm path length glass cells and a Kent pH-meter Model 7020 with a combined glass electrode were used.

*Chemicals*

Baclofen and its tablets (Lioresal®) were generous gifts of Ciba-Geigy, Istanbul, Turkey. NQS and other chemicals were obtained from E. Merck, Darmstadt, Germany. All solvents used were analytical grade.

*Solutions*

Stock solution : About 25 mg of baclofen was dissolved in 50 ml of water. Standard solutions were prepared from this solution by appropriate dilutions with water.

Reagent solution : A 2 x 10$^{-2}$ M aqueous NQS solution was prepared freshly and protected against light.

Buffer solution : A 0.1 M potassium dihydrogen phosphate solution was adjusted to pH 7.5 with 0.1 N NaOH solution.

*Assay Procedure*

A 1 ml volume of standard solution containing 50 – 500 µg.ml$^{-1}$ of

baclofen was mixed with 1 ml of pH 7.5 buffer solution in a 10 ml stoppered tube. After addition of 0.5 ml of NQS solution the mixture was heated on a thermostated water bath at 70°C for 20 min. Then the reaction mixture was cooled and acidified with 1.5 ml of 0.1 N HCl. The derivative (baclofen-NQ) was extracted with 3x2 ml of dichloromethane : n-butanol (4:1) and centrifuged. The organic phases were dried over anhydrous sodium sulphate and transferred into a 10 ml calibrated flask, then diluted to volume with the same solvent mixture. The absorbance was measured at 455 nm against a blank prepared similarly. Calibration graph was obtained by plotting the absorbance values versus concentrations. Regression equation of the calibration graph was calculated by the method of least squares.

*Sample Preparation*

Twenty tablets were weighed and finely powdered. An accurately weighed of the powder equivalent to 15 mg of baclofen was transferred into a 50 ml calibrated flask. After adding 25 ml water, the mixture was shaken for 30 min. and diluted to volume with water, mixed and filtered. The first 10 ml portion of the filtrate was discarded. 1 ml of the resulting solution was studied as described above. The quantity of baclofen was calculated by means of the regression equation.

## RESULTS AND DISCUSSION

The reaction between aliphatic primary amino group of baclofen and NQS produced a highly coloured derivative (Scheme).
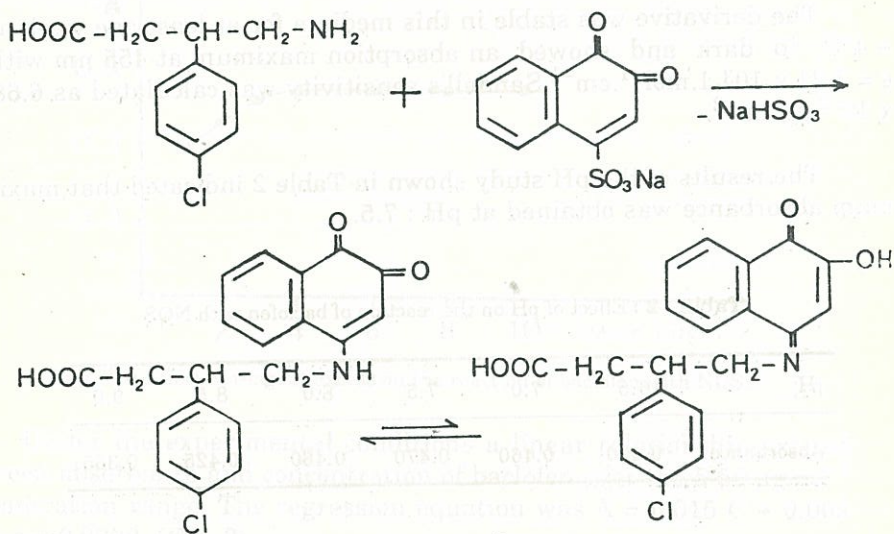


Reaction scheme

This study the effect of various parameters on absorption intensity and the optimum reaction conditions were investigated.

To increase the stability of NQ-derivatives, an organic solvent extraction is necessary and it also eliminates the colour of unreacted reagent and other interferences (9-12). Therefore, we employed an extraction procedure which was avoided in the previous paper (3). As the derivative can be extracted into an organic solvent the method proposed is suitable for determination of baclofen in biological fluids and this subject is now under investigation.

For the extraction, it was found that a mixture of dichloromethane : n-butanol (4:1) was the most suitable organic solvent (Table 1).

Table – 1 : Maximum wavelengths and absorbance values of baclofen – NQ in various organic solvents

| Solvent | $\lambda$max (nm) | A |
|---|---|---|
| Ethyl Acetate | 445 | 0.440 |
| Chloroform | 435 | 0.445 |
| Dichloromethane | 435 | 0.450 |
| Dichloromethane : n - butanol (3:1) | 455 | 0.450 |
| Dichloromethane : n - butanol (4:1) | 455 | 0.465 |
| Benzene : pentanol (3:1) | 450 | 0.390 |

The derivative was stable in this medium for at least one week at + 4°C, in dark and showed an absorption maximum at 455 nm with $\varepsilon = 3.21 \times 10^3$ 1.mol$^{-1}$.cm$^{-1}$. Sandell's sensitivity was calculated as 6.68 $\times 10^{-2}$ µg.cm$^{-2}$.

The results of the pH study shown in Table 2 indicated that maximum absorbance was obtained at pH : 7.5.

Table – 2 : Effect of pH on the reaction of baclofen with NQS

| pH | 6.5 | 7.0 | 7.5 | 8.0 | 8.5 | 9.0 |
|---|---|---|---|---|---|---|
| Absorbance | 0.430 | 0.460 | 0.470 | 0.460 | 0.425 | 0.355 |

The effect of temperature of the colour intensity was studied. In practice full colour was developed when the reaction mixture was incubated at 70°C within 20 min (Fig.1).
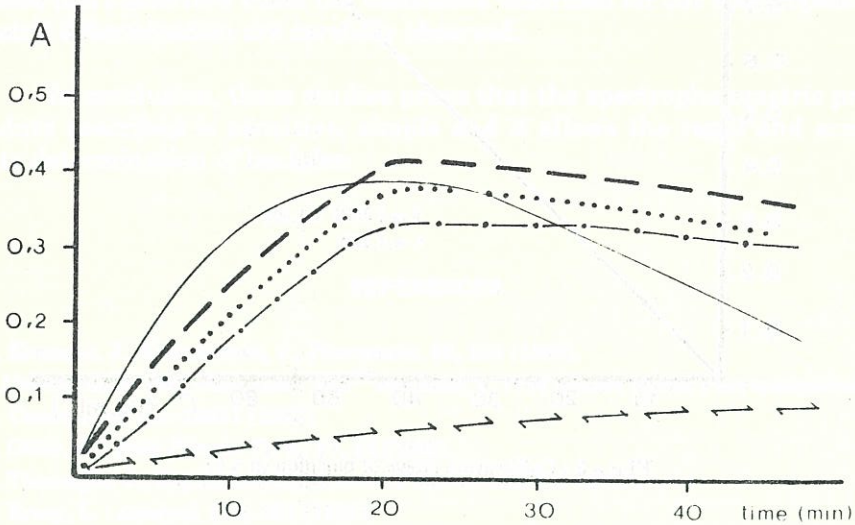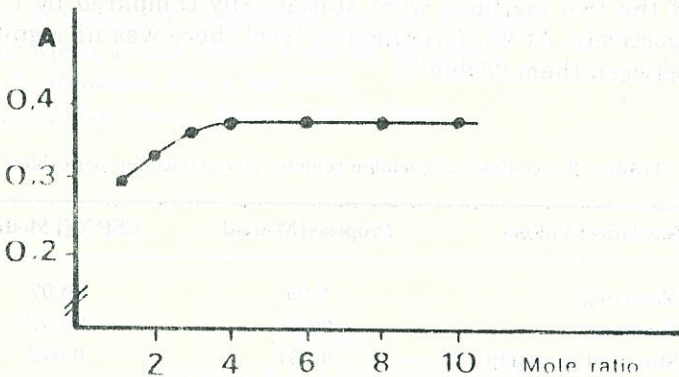


**Fig - 1 :** Effect of heating time on the reaction of bclofen with NQS.
( ⇝ ⇝ room temperature, – . – . 50°C, ...... 60°C, – – – 70°C., —— 80°C)

The NQS amount required was examined by changing the mole ratio of NQS to baclofen from 1 to 10. A 4 fold molar excess of reagent was found to be necessary to complete the reaction (Fig.2).



**Fig - 2 :** Effect of reagent excess on the reaction of baclofen with NQS.

Under the experimental conditions a linear relationship existed between absorbance and concentration of baclofen over the 5-50 $\mu g.ml^{-1}$ concentration range. The regression equation was $A = 0.015\ C + 0.009$ with r = 0.9998. (Fig. 3).
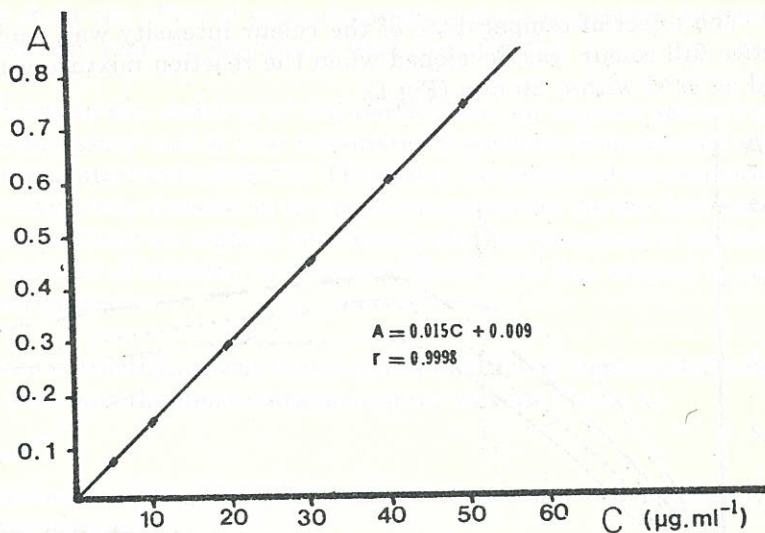
A = 0.015C + 0.009
r = 0.9998

Fi g – 3 : Calibration curve of baclofen (n = 6).

In this study, we revised the calibration curve that of the previous report (3) in terms of linear concentraion range and regression equation.

The method developed has been applied to the determination of baclofen in tablets and the results were compared with those obtained by the official spectrophotometric method (2). The mean values and precisions of the two methods were statistically compared by t - and F - tests respectively. At 95 % confidence level there was no significant difference between them (Table 3).

Table – 3 : Analysis of baclofen tablets (10 mg baclofen per tablet)

| Statistical Values | Proposed Method | USP XXI Method |
|---|---|---|
| Mean (mg) | 9.99 | 10.07 |
| % recovery | 99.85 | 100.65 |
| Standard deviation | 0.067 | 0.072 |
| Relative Standard deviation | 0.67 | 0.72 |
| n | 6 | 6 |
| Confidence limits | 9.92-10.06 | 9.99-10.15 |
| t test of significance | t = 1.72 (p= 0.05 t= 2.23) | |
| F test of significance | F = 1.18 (p= 0.05 F= 5.05) | |

Some tablet additivies such as lactose, magnesium trisilicate, dimethylpolysiloxane, magnesium stearate, starch and carboxymethylcellulose don't interfere when the conditions described for the spectrophotometric determination are carefully observed.

In conclusion, these studies prove that the spectrophotometric procedure described is sensitive, simple and it allows the rapid and accurate determination of baclofen.

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