Kurzmitteilungen:

Spectrophotometric Determination of Baclofen

Spektrophotometrische Bestimmung von Baclofen

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Baclofen [γ-amino-β-(p-chlorophenyl)butyric acid] is a potent orally active antispasmodic agent in humans[1]. It was determined in biological materials by GLC[2], GLC-MS[3-4], HPLC[5-7], TLC[8], and by fluorimetry[9]. Some other methods have been reported especially for the quantitation of baclofen in pharmaceutical dosage forms such as TLC, UV and visible spectrophotometry[10-15]. Here a new spectrophotometric method based on the formation of a yellow coloured derivative of baclofen with 2,4-dinitrofluorobenzene (FDNB) was developed.

Experimental Part

Materials: Baclofen and its tablets (Lioresal® 10 and 25 mg) were generous gifts of Ciba-Geigy, Istanbul, Turkey. - FDNB was purchased from Aldrich, Steinheim, W.Germany. - All the other chemicals were obtained from commercial sources and were of analytical grade.

Test Solutions

5.0×10^{-4} M aqueous baclofen and 6.0×10^{-2} M methanolic FDNB solutions were prepared daily.

Apparatus

m.p. were determinded with a Büchi apparatus. - Beckman B and Carl Zeiss PMQ II spectrophotometers were used. - IR spectra: Perkin Elmer 577 instrument. - ^{1}H-NMR- and Mass spectra were obtained using a Bruker WM 250 (250 MHz) and a Varian Mat CH 7 A (80 eV) spectrometers, respectively.

Assay Procedure

0.5-6.0 ml of aqueous stock baclofen solution were pipeted into a 10 ml glass stoppered reaction vial. 0.5 ml methanolic FDNB solution and 100 mg (1 mM) solid $NaHCO_3$ were added. The final volume was made up to 8.0 ml with distilled water and heated at 60 °C for 45 min in a thermostated water-bath. After cooling the solution was transferred into a 10 ml volumetric flask and filled up to volume with distilled water. 2.0 ml of this solution were diluted to 10 ml with ethanol containing 3% HCl. The absorbance of the resulting solution was measured at 355 nm against a blank prepared similarly. The calibration graph was prepared by plotting the concentrations of the pure derivative of baclofen (μg/ml) against the absorbances. The quantity of baclofen in the tablets was calculated from the regression equation of the calibration graph.

Analysis of Tablets

20 tablets each claimed to contain 10 or 25 mg substance were ground to a fine powder. A quantity of powder equivalent to about 10 or 25 mg of baclofen was weighed and transferred into a 100 ml calibrated flask and 50 ml distilled water were added. The mixture was shaken mechanically for 30 min. The volume was adjusted to 100 ml with distilled water, then the mixture was filtered. The first 10 ml were rejected. 2.0 ml of filtrate were worked up as described above.

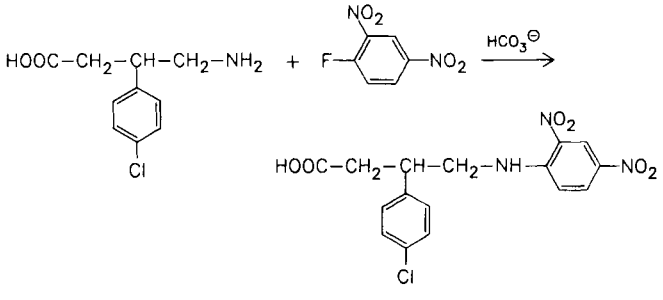
Synthesis of the Derivative

0.5 mM baclofen was dissolved in 1 ml of distilled water. 5 mM FDNB solution in 5 ml ethanol and 300 mg solid $NaHCO_3$ was added. The mixture was heated at 60 °C for 45 min and acidified to pH 3.0 with 2 N HCl. After staying overnight the precipitated derivative and the excess of reagent were filtered off. The reaction product was purified by preparative layer chromatography (silicagel G) using chloroform-methanol (9:1) as the solvent (hRf=33). - m.p. 210-212 °C ($CHCl_3$:MeOH: 9:1), yield 160 mg (84%), yellow crystals. $C_{16}H_{14}ClN_3O_6$ (379.8) calcd. C 50.6 H 3.71 N 11.1 found C 50.4 H 3.62 N 10.8. - IR(KBr): 3330 (OH-bonded), 1715 (C=O), 1615 (C=C, arom.), 1580 and 1330 cm^{-1} (C-NO_2). - ^{1}H-NMR ($CDCl_3$);δ(ppm)=1.85 (broad S;1H,NH*), 2.95 (d;2H,$\underline{H}_2$C-CO), 3.15-4.00 (2 m,3H,N-C$\underline{H}_2$-C$\underline{H}$-), 5.79 (broad S;1H,COOH*), 7.20-9.05 (m,Ar,7H). - MS(FD):379 $M^{+\cdot}$ (2.5), 298 (1.5), 252 (1), 196 (100), 183 (17), 150 (10), 141 (24), 77 (18).

*Exchangeable with D_2O

Results and Discussion

The reaction between baclofen and FDNB proceeds quantitatively in aqueous ethanolic or methanolic medium.



Other solvents such as propanol, isopropanol, acetonitrile, dimethylformamide gave poorly reproducible results. The structure of the derivative was confirmed by spectroscopic methods as γ-2,4-dinitrophenylamino-β-(p-chlorophenyl)butyric acid. In order to obtain the optimum amount of the reagent, the reaction was carried out using various

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substance/reagent ratios. Best results were obtained at a 10 fold excess of reagent. At room temp. the reaction rate was slow. The reaction was complete at 60 °C in 45 min. Higher temp. gave different by-products (TLC controls).

No separation of the derivative is necessary, the addition of ethanolic HCl eliminates the interference of the hydrolysis products of FDNB[16]. The solution is highly stable at light and at room temp. No remarkable absorbance changes were observed within 24 h, contrary to other methods such as a colorimetric one[14] using 2,4,6-trinitrobenzenesulfonic acid as derivatizating reagent in which the final solution was decomposed within 30 min.

The absorption spectrum of the derivative shows a maximum at 355 nm with $\varepsilon=2.6 \times 10^4 l \cdot mol^{-1} \cdot cm^{-1}$. *Sandell* sensitivity was calculated as 0.015 $\mu g \cdot cm^{-2}$. *Beer's* law was obeyed between 1-15 $\mu g \cdot ml^{-1}$ of baclofen. A linear equation of

$$y = 0.0634 x + 0.0424$$

with $r = 0.9992$ was calculated.

The reproducibility of the method was determinated by analysing ten replicate samples each containing $10\mu g \cdot ml^{-1}$ of baclofen; at this concentration level the rel. standard deviation was calculated to be 0.22% (n=10).

The present method was also applied to the assay of baclofen in tablets and the results were compared with those of the UV spectrophotometric method[11-12] (Table 1). The mean values found by both methods were compared by t-test at the 95% confidence level. There was a significant difference between the two methods. The comparison of the precision of both methods was made by F-test at the 95% confidence level: there was no significant difference between them. The average recoveries of the present method and the UV spectrophotometric method were 100.05 ± 0.05% (S.D) and 100.73 ± 0.06% (S.D), respectively. The analysis of Lioresal® tablets (25 mg) with the UV method gave higher results than declared. It is expected that the tablet excipients contain some UV-absorbing components.

As a result the present method is highly specific and sensitive. It can be easily used for the assay of baclofen in pharmaceutical preparations.

Table 1: Comparison of the both methods using t and F tests (Lioresal® tablet, 10 mg)

Method		Mean recovery,% (n=10;p=0.05; t=2.10)	Standard dev.,% (n=10;p=0.05; F=3.18)
FDNB	method ...	100.05	0.05
UV	method ...	100.73	0.06
		t=2.64*	F=1.67

* Significant difference

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