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NOTE

PURIFICATION OF ^{3}H-DIHYDROALPRENOLOL BY TWO DIMENSIONAL THIN LAYER CHROMATOGRAPHY

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SUMMARY

A two dimensional thin-layer chromatographic method developed for the purification and analysis of (-)-[^{3}H]dihydroalprenolol, by using an acidic mobile phase (butanol/water/acetic acid 25:10:4, v/v) in one direction and a basic eluent (chloroform/acetone/triethylamine 50:40:10, v/v) in another direction.

Key words: two dimensional thin-layer chromatography, (-)-[^{3}H]dihydroalprenolol

(-)-[^{3}H]Dihydroalprenolol ([^{3}H]DHA) is a competitive beta-adrenergic antagonist which has been successfully used as a radioligand for direct studies of beta-adrenergic receptors [1]. The radioligand is not stable due to self-radiolysis on storage, and the presence of even a small percentage of radioactive impurity might bias the interpretation of data, therefore purification of the radioligand has to be performed before its application in binding studies. As a method for this purification two dimensional thin-layer chromatography (2D TLC) was chosen.

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The two following eluents were selected [2,3]:

- butanol/water/acetic acid (25:10:4, v/v) and
- chloroform/acetone/triethylamine (50:40:10 v/v),

with retention factors R_f of [^{3}H]DHA being 0.50 and 0.63, respectively. After the TLC plate development in the acidic system (first direction) the plate was air dried and development was made in the second direction using the basic eluent. The position of the [^{3}H]DHA ligand was approximately determined from the position of the marker spots (cold alprenolol) under a UV lamp. The exact position of the ligand was found by scanning the chromatograms for ^{3}H-radioactivity. For evaluation of the efficiency of the developed method the plate was cut into small squares and the tritium in each part was determined by liquid scintillation counting. Fig. 1 illustrates the results obtained. The fraction of tritium found in the spot corresponding to the position of [^{3}H]DHA was 71.6%.

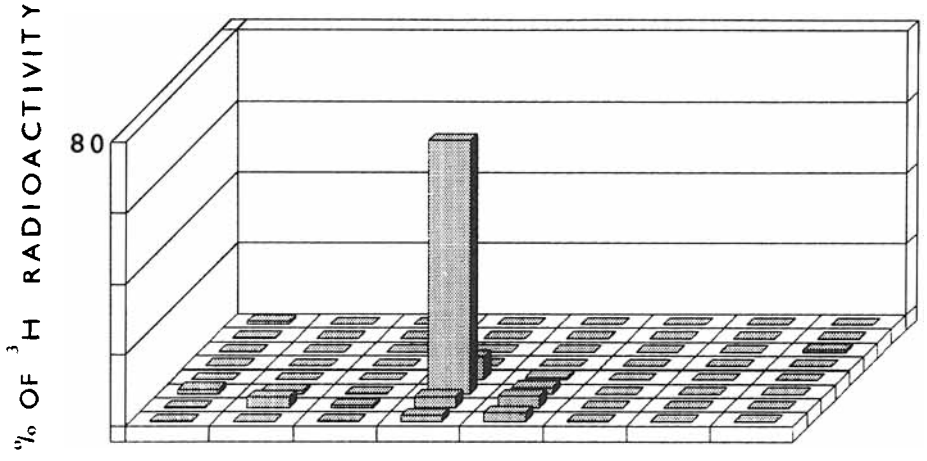

Fig. 1 Distribution of ^{3}H-radioactivity on the chromatoplate. (Horizontal plan of the picture is equal to the area of 8x8 cm.)

EXPERIMENTAL

Materials

(-)-[^{3}H]Dihydroalprenolol was purchased from the Biological Research Center, as a solution in 96% ethanol with specific radioactivity 2.74 TBq/mmol and radiochemical concentration 37 MBq/ml. (-)-Alprenolol (d-tartrate salt) used was a product of Sigma Chemical Co., USA. TLC plates of Silufol UV_{254} were purchased from Kavalier, Sazava, Czechoslovakia. Solvents and other chemicals were from Lachema, Brno, Czechoslovakia. Liquid scintillation cocktail SLD 31 is the product of Spolana, Neratovice, Czechoslovakia. Water-ethanolic KOH solution is a mixture of 320g of solid KOH, 400 ml of 96% C_2H_5OH and 1200 ml of distilled H_2O.

Method

In analytical 2D TLC the cold marker (alprenolol, ~5ug) and ^{3}H-ligand (1 x 10^6 dpm) were spotted onto the plate (12x12 cm). Thin-layer chromatography after the first direction in the acidic solvent was followed by the second, perpendicular direction in the basic solvent. Between the individual developments the plate was dried by air. The position of the analyte can be determined, as described above, under a UV lamp. The exact localization of the spot of ^{3}H-ligand was established by the measurement of ^{3}H using a radiochromatographic reader Tesla NZQ 901 (Tesla, Litovel, Czechoslovakia). Radioactivity was measured by liquid scintillation counting in each part of the plate by cutting the chromatographic plate into small squares (1 cm^2) and digesting them with 0.2 ml of the water-ethanol KOH solution.

In preparative 2D TLC the amount of spotted ligand was usually 10^7-10^8 dpm without cold marker alprenolol. The spot of pure radio-ligand of a diameter of about 1 cm, was located

by the ^{3}H radioactivity scanner, cut out, scraped and eluted with 96% ethanol.

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