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Note

Pitfalls in assessing the radiopurity of tritiated steroids by high-performance liquid chromatography

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High-performance liquid chromatography (HPLC) is commonly adopted when checking the purity of a radiolabelled compound. If the radiolabelled compound is of high specific activity, it is usual, for economical reasons, to chromatograph an aliquot of the radiolabelled compound with the corresponding unlabelled ("cold") compound, which acts as a marker. The mixture of the "cold" and "hot" compound is chromatographed and all fractions of the eluent are collected, including the band corresponding to the elution of the "cold" compound. The purity of the radiolabelled compound is usually expressed as the radioactivity collected in this band as a fraction of the total radioactivity analysed.

Two fundamental assumptions are made when using this method to express radiopurity. It is assumed first, that the system used is capable of separating all impurities from the pure labelled compound, and second that the pure labelled compound co-chromatographs with the corresponding "cold" compound.

In this paper we report an observation that indicates that the second assumption can be incorrect with tritiated prednisolone when using a Hypersil MOS HPLC system.

EXPERIMENTAL

About 2 μCi of [6,7-^{3}H]prednisolone (46 Ci/mmol) (Amersham International, Great Britain) was added to 100 μl of methanol containing about 200 ng of "cold" prednisolone (Sigma, Great Britain). An aliquot of this mixture (50 μl) was chromatographed on a Hypersil MOS (C_8) 25 cm × 4.6 mm column (5 μm) (Phase Separations, Great Britain), which was eluted with isopropanol–acetic acid–water (15:1:84) at a flow-rate of 2 ml/min (Altex Model 110) (Anachem, Great Britain). Prednisolone was detected by a fixed-wavelength detector (Waters Model 440) at 254 nm, which was connected to a chart recorder (10 mV) set at 1 mm/sec. Fractions of eluent were collected in plastic vials, at 5-sec intervals, starting from the point of injection until 1 min after "colds" prednisolone had been completely eluted from the column. The radioactivity in each vial was measured by scintillation counting (LKB Wallace, Great Britain) after the addition of 3 ml of scintillant to each vial (Rialuma Lumac, The Netherlands).

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RESULTS AND DISCUSSION

The radioactivity results obtained are illustrated in Fig. 1 in the form of a histogram. Superimposed on this is the absorbance of prednisolone recorded on the chart recorder. It is obvious that the time at which maximum radioactivity is eluted from the column does not correspond with the time at which the maximum absorbance of prednisolone occurs. This discrepancy in retention times cannot be due to the lag time between the recording of the peak and the emergence of the effluent containing prednisolone, for two reasons. First, the presence of such a lag time would result in elution of the radioactivity after, rather than before, the recording of the absorbance of the "cold" parent compound. Second, when a check was made on the duration of the lag time, it was found to be negligible.

This phenomenon, known as isotopic fractionation and reviewed by Klein[1], has been observed for other compounds such as cortisone[2], aldosterone[2], testosterone[3], mianserin[4] and folic acid[5]. In chromatographic studies where both the tritium- and carbon-14-labelled isotopes of the same compound have been studied, the tritiated compound has been found to be more hydrophilic than the parent compound[2,4,5]. Klein[1] found that the adsorption distribution coefficient, a measure of the ability of a molecule to partiton between the stationary phase and the eluent, varies appreciably with the nature and the position of the label in the molecule. It would appear from our observations and those of others that the adsorption distribution coefficient of the tritiated compound is less than that of the corresponding "cold" or carbon-14-labelled compound. The underlying mechanism producing this difference in the adsorption distribution coefficient is still unclear.

In light of our observations, the assumption widely made in biochemistry and pharmacology, that the radiotracer behaves identically with the corresponding "cold" compound, must be treated with caution.

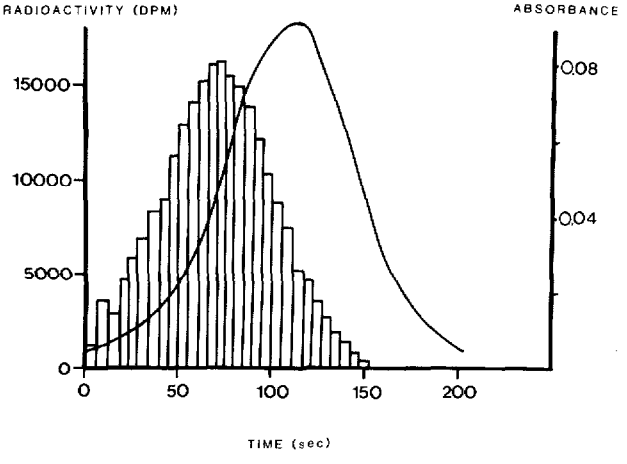

Fig. 1. Elution of [^{3}H]prednisolone and "cold" prednisolone on a Hypersil MOS column. The time recorded is the time from first elution of radioactivity (25 min).

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