

3-1-76

HPLC Analysis of Butanol Extract

The previous pages describe an experiment in which it was demonstrated that radioactivity covalently bound to a base was extracted with Butanol. The sample (11-3-1) still has ~ 200 μ l of $BuOH$ in the lower phase. This will be concentrated and analyzed by HPLC.

Experiment

→ 65°C hydrolysate

- warm sample (1) to room T (was stored in freezer).
- remove sample by 50 μ l portions and place in PS vial - yield = 190 μ l
- evaporate $BuOH$ with N_2 - the final volume
- was 30 μ l. Fractions were collected at 24 sec intervals - results are in R. Croy's research notebook.

- Results - nice chromatogram with about 3-4 large peaks of 364 nm - absorbing material. The radioactivity was primarily in 2 of the peaks. Two large 254-abs. peaks were observed.

Conclusions: Generally, the results are somewhat encouraging. ~~A nucleoside~~ base-AFB₁ adducts were separated well from 254 nm - absorbing material. The reason that (2) major adducts ~~were~~ seen could be that there was chemical degradation or that there are actually (2) present - analysis of nucleoside adducts under these conditions shows only a single peak with radioactivity. The rest of the 3H primarily elutes early in the chromatogram and may be unhydrolyzed adducts which are qualitatively different from the late-eluting compound.