2-26-76 5. ~~Precolum~~
Purge of Analytical Column after Elution of Addact

- Column was purged with 80% MeOH - several strong peaks came out x0.10

total Vol = 13.0 ml
100 µl → 1122 cpm

⇒ 1.459×10^5 counts collected

Conclusions on Peak 1 isolation:

1. only 12% of the hydrolysate was recovered as semipurified peak 1 — this is much lower than expected — and I know that more than 70% of the hydrolysate radioactiv. was present as peak 1
2. Peak 1 probably was there, but it didn't stick to precolumn.

8-2-76 Peak 1 Isolation - 2nd Stage of Purification

- The sample is at 4 ml level in 10% MeOH
- add 4 ml H_2O ∴ conc. MeOH now 5%
- load on precolumn
- Put precolumn in Micromeritics and purge with 22% strong eluent (80% MeOH) = 17.6% MeOH
- Peak 1 eluted at 26 min - most of it was collected in a pear-shaped flask.

Volume: 8.7 ml
25 µl (µpipette): 6,152 cpm
50 µl ("): 12,621
25 µl (Hamilton Syr: 6213
} 250 cpm/µl

$$\frac{2.175 \times 10^6 \text{ cpm}}{0.3} = 7.25 \times 10^6 \text{ dpm}$$

0.3 ~ efficiency

$$\frac{7.25 \times 10^6 \text{ dpm}}{\left(2.2 \times 10^6 \frac{\text{dpm}}{\mu\text{Ci}}\right)} = 3.3 \mu\text{Ci of AFB, bound to DNA}$$

SA AFB, $= 0.027 \frac{\mu M}{\mu Ci}$

$\left(0.027 \frac{\mu M}{\mu Ci}\right)(3.3 \mu Ci) = 0.0891 \mu M$ of AFB,

$= 27.8 \mu g$ of aflatoxin

0.0891 μM of adduct (MW ~ 476)

$= 42.41 \mu g$ of adduct

8-3-1 : 500μl , 42 μg of adduct → 0.0840 μg/μl

⇒ 10.5 μg adduct in 8.3.2a,b

⇒ 2.1 μg/ml = 2.1 ng/μl

3-76 Purification of Peak 1 (Cont)

$(250\ cpm/\mu l)(8.7 \times 10^3\ \mu l) = 2.175 \times 10^6\ cpm$
in total sample — in whole sample

Calculation of Amound of Adduct Isolated:

$2.175 \times 10^6\ cpm \longrightarrow 27.8\ \mu g$ aflatoxin

$\longrightarrow 42.4\ \mu g$ of Adduct

- Rotovap the sample (8.7 ml) to dryness at 50°C (~30 min) – residue was visible — may have been a salt
- add 100 x 5 (500 μl) 10% MeOH – scrape with micropipette to suspend/dissolve all of residue

didn't all go in → all of residue

500
– 275 — remainder
225

↓ supports contention that there was Ca^{++} salt

500 μl ≡ Sample 8-3-1

845 [illegible] ng/μl

- 125 μl in 1 ml volum. — Sample 8-3-2a
- 125 μl in 1 ml volumet. — Sample 8-3-2b
- 25 μl (μpipette) 2120 ng ↓ 10% MeOH 10 ml — 212 ng/ml = 0.212 ng/μl — Sample 8-3-4 STANDARD
  → some [illegible] this sample

8-4-76 Count 5 μl of 8-3-2a: 4394 cpm

⇒ 4.39×10^5 cpm in sample (? poor solubility)
([illegible] ~20% recovery)

5875 cpm — 5 µl

→ 4.99×10^5 cpm in total sample

$\frac{5 \times 10^5 \text{ cpm}}{0.3 \text{ (ml)}} = 16.7 \times 10^5$ dpm

$\frac{16.7 \times 10^5 \text{ dpm}}{2.2 \times 10^6 \frac{\text{dpm}}{\mu\text{Ci}}} = 0.75 \mu\text{Ci}$

$(0.75 \mu\text{Ci})(6.027 \frac{\mu\text{M}}{\mu\text{Ci}}) = 0.0203 \mu\text{M}$

$\times 312$

$= 6.32$ µg AFB$_1$

$\times 476$

$= 9.66$ µg adduct

8-4-76 1. Inject 50 µl 8-3-4
There were (3) earlier-eluting peaks in the chromatogram. One was at ~15 min and had a very large A_{254}. This sample had some debris in it, so the original will be injected.

2. Inject 5 µl 8-3-1 - the A_{254} contamination was lower but it was still significant, especially since this sample ~~was~~ is destined for MS.

Final Purification of Adduct

Conditions: 10% MeOH to 35% of 80% in 10 min, then hold at 35%. Adduct elutes at 25 min.

Inject: 100 µl, 140 µl, 140 µl, 125 µl } entire sample

- Collect peak 1 (~8 ml)
- add ~ 8 ml MeOH
- reduce at 40° quickly (15 min) with vac. evaporator (rotary) with vac. pump.
- when ~ 0.3 ml - transfer from 50 to 10 ml pear-shaped flask (wash w/ MeOH)
- reduce further to ~ 200 µl
- reduce further with N_2 (TFE tube)
- final vol est. = 125 µl } 425 µl
- add 300 µl MeOH

note that no mention was made of a solid ppt forming

count 5 µl (Hamilton 10 µl syr.) → 23,500 counts/4 min

SAMPLE 8-4-1

⇒ Total sample has 9.7 µg of adduct - which is ~ 1/4 what was expected (but ~ what I started with this morning)

$\frac{9.7\,\mu g}{.425\,ml} = 22.7\ \mu g/ml \cdot ng/\mu l$ conc.