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*Please reply to The Director*
Your reference

Our reference

Date
7 September 1984

Dear Matt

Sorry for the delay in answering your letter of 6th August. Thanks for the information and samples. Analysis of the samples is in progress and I should have the results by the end of September.

I promised details of our procedures for work up and analysis. These I now enclose.

I note your November dates and will contact you about these later.

Yours sincerely

Tim

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# CDE ANALYSIS OF POLLEN AND ENVIRONMENTAL RESIDUES

Three methods are used depending on instrument availability, matrix and sensitivity required.

1. GC-MS (SIM) in EI mode using HFB derivs (VG 7070 EQ instrument).

2. GC-EC using HFB derivs, all trichothecenes detected as their hydrolysis products T-2 tetraol, scirpentriol, DON, NIV, 3,4,7,15-tetraol, verrucarol.

3. GC-MS (SIM) in -ve CI mode using PFP derivs (Finnegan 4500 quadrupole) [PFP derivs are used because MV & T-2 tetraol $(HFB)_4$ derivs give $M^-$ ions beyond the 1000 amu mass range of the instrument. The VG 7070 EQ give rather poor sensitivity in -ve CI mode].

Methods 1 and 2 are frequently used in parallel. GC-EC was subject to too much interference when used to detect T-2 & DAS but for pollen matrixes it has been shown to be a reliable method for detecting the polyhydroxytrichothecenes. For other matrixes such as leaves GC-EC is subject to too much interference and GC-MS is used. Any positive by GC-EC would need to be confirmed by GC-MS. Best limits of detection are obtained with method 3 especially for polyhydroxytrichothecenes such as DON, NIV, T-2 tetraol, but is not routinely used because of non-availability of the instrument. Negative CI also has the advantages that it is less prone to interference from background. Method 3 is used mainly for blood and urine analysis where lack of detection ranging from 0.1-5 ppb can be obtained.

R M BLACK
CDE Porton Down
6/9/84

# COE TRICHOTHECENE ANALYSIS

## POLLEN ANALYSES – METHODS 1 & 2

POLLEN (0.1 – 10 mg)

1. 3ml 90% MeOH, tumble 1h
2. 2ml 90% MeOH, tumble 15min.

↓

90% MeOH EXTRACT

1. Defat 3x 5ml petrol-ether 60-80°
2. Concentrate on rotary evaporator

↓

DRY RESIDUE

1. Dissolve in 1ml $CH_2Cl_2$ (x2)
   (rinse flask with Sep-Pak eluate)

↓

FLORISIL SEP-PAK

1. Elute 5ml $CHCl_3$/MeOH (7:3)
   [High MeOH content required for nivalenol]

↓

ELUATE → 1. Concentrate under $N_2$ → **GC-MS**

1. Concentrate under $N_2$
2. 1ml 1% NaOH/MeOH, 24h, ambient temp.
3. Neutralise with 0.3g Amberlite IR-120
4. Filter, rinse with MeOH (5x 2ml)
5. Concentrate on rotary evaporator

↓

DRY RESIDUE

1. Dissolve in 2ml water
   (rinse flask with Sep-Pak eluate)

↓

$C_{18}$ SEP-PAK

1. Rinse 2ml $H_2O$
2. Elute 2ml 40% MeOH

↓

ELUATE

1. Concentrate on rotary evaporator

↓

DRY RESIDUE

1. 3x 1ml MeOH
2. Concentrate under $N_2$

↓

REACTI-VIAL

↓

METHOD 1

GC-MS CONDITIONS (HFB derivs, EI)

DERIVATIZATION - as for GC-EC

GC CONDITIONS

COLUMN : 25m BPI, 0.25mm dia
INJECTOR TEMP : 260°
OVEN TEMP : 160° for 1min, 160–275° at 10°/min, 275° for 5min
TRANSFER LINE : 260°
CARRIER : He, 15psi
SPLIT 1:1, 0.5 µl injected

MS CONDITIONS

INSTRUMENT : VG 70-70EQ
MODE : SIR, low resolution, EI

Two groups of 10 ions monitored, A 5 min 15 sec → 9 min 60 sec
B 9 min 48 sec → 16 min 30 sec

IONS MONITORED, RETENTION TIMES

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| A | NIV | 1096, 1077 | $7^{38}$ | B | DAS | 502, 674 | $11^{35}$ |
| | OON | 884, 865 | $8^{37}$ | | 15-ACOON | 730, 688 | $10^{22}$ |
| | sc-triol | 870, 855 | $8^{27}$ | | HT-2 | 655, 672 | $12^{59}$ |
| | T-2 tetraol | 869, 868 | $8^{45}$ | | T-2 | 519, 501 | $15^{28}$ |
| | FUS-X | 962 923 | $9^{33}$ | | 15-MAS | 656, 625 | $9^{56}$ |

Higher resolution may help for extracts of leaves where considerable background occurs.

Detection limit usually around 0.5ng – 5ng per sample depending on the background.

METHOD 2

GC-EC CONDITIONS

DERIVATIZATION

1. To dry residue in 1ml Reacti-vial add 200 µl toluene / $CH_3CN$ (95:5)
2. Add 20 µl HFBI (Undiluted)
3. Vortex 30 sec
4. Heat at 60°, 2h, cool.
5. Add 500 µl 5% $NaHCO_3$
6. Vortex 2 min.
7. Remove aqueous layer
8. Vortex 30 secs with 500 µl water (x2), remove aqueous layer.
9. Inject 0.5 µl of toluene soln.

GC CONDITIONS

INSTRUMENT : PYE 304
COLUMN : 25m BPI, 0.25 dia.
INJECTOR TEMP : 260°
DETECTOR TEMP : 275°
OVEN TEMP : 170° 5min, 170-190° 5°/min ; 190° 5min ;
190 - 250° 6°/min ; 250° 5 min.
CARRIER : $H_2$, 9 psi
MAKE-UP : $N_2$, 10 psi (total flow 20 ml/min)
SPLIT FLOW : 10 ml/min
SEPTUM PURGE : 3 ml/min.

RETENTION TIMES :

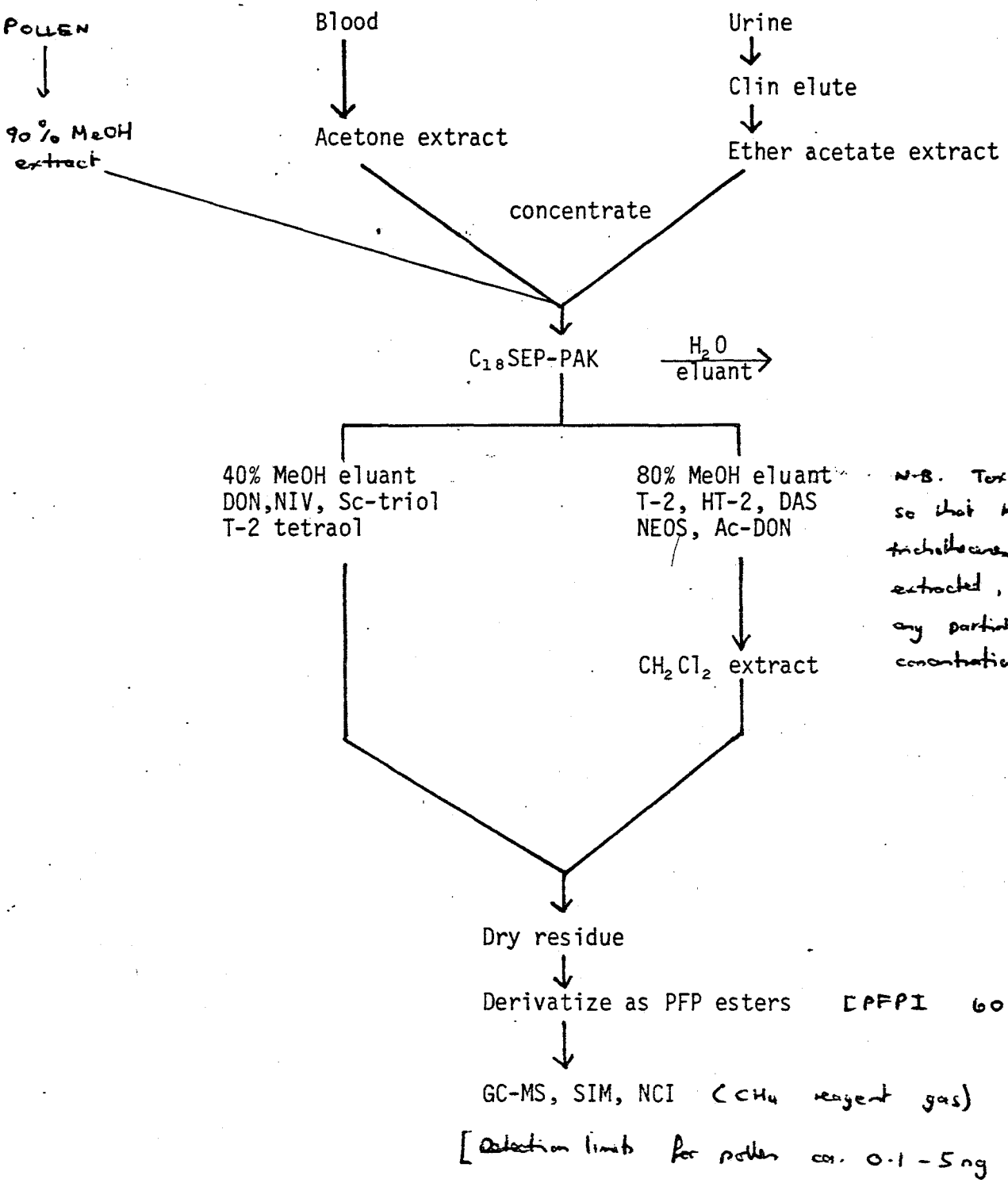
| | |
|---|---|
| T-2 tetrol | 14.8 min |
| scirpentriol | 13.2 min |
| nivalenol | 11.0 min |
| DON | 13.9 min |
| 3,4,7,15-tetrol | 10.7 min |
| verrucarol | 14.6 min. |

Occasional interfering peaks are usually separated using isothermal conditions.

Approx. detection limits : T-2, DAS, NEOS 5 ng per 1-10 mg pollen
VER-A, ROR-A, NIV, DON 20-25 ng per 1-10 mg "

METHOD 3

# TRICHOTHECENES: DETECTION IN BLOOD, URINE & POLLEN




N.B. Toxins are fractionated so that the extracted trichothecenes can be extracted, thus avoiding any partial hydrolysis on concentration.