

ETHOFUMESATE

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NC 8438 / R141

R141

ETHOFUMESATE

RESIDUES

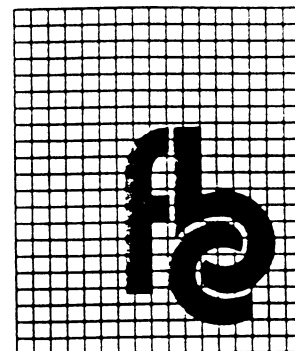
ANALYTICAL METHOD FOR RESIDUES OF ETHOFUMESATE
AND MAJOR METABOLITES IN GRASS AND SUGARBEET
(IMPROVED METHOD).
by
J.D. Manley, M.D. Reeve and P.J. Snowdon.

FBC Report
RESID/85/111
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ANALYTICAL METHOD FOR RESIDUES OF ETHOFUMESATE AND MAJOR
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CHESTERFORD PARK RESEARCH STATION

REPORT AUTHENTICATION

I, the undersigned, hereby declare that the work to which this report refers was performed under my supervision according to the procedures herein described. To the best of my knowledge the study was conducted in compliance with international codes of Good Laboratory Practice and this report provides an accurate record of the results obtained.

..... P. J. Snowden

Study Director

..... 5th March, 1986

Date

CHESTERFORD PARK RESEARCH STATION

ANALYTICAL METHOD FOR RESIDUES OF ETHOFUMESATE AND MAJOR METABOLITES
IN GRASS AND SUGARBEET (IMPROVED METHOD)

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ANALYTICAL METHOD FOR RESIDUES OF ETHOFUMESATE AND MAJOR METABOLITES
IN GRASS AND SUGARBEET (IMPROVED METHOD)

1 SUMMARY OF PERFORMANCE CHARACTERISTICS

1.1 Compounds determined

Ethofumesate; conjugated and non-conjugated residues of the metabolites NC 9607 and NC 8493 (measured as total NC 9607 and total NC 8493).

1.2 Types of sample

Grass and sugarbeet (immature plants, roots and leaves).

1.3 Basis of method

Soxhlet extraction of ethofumesate and non-conjugated metabolites with acetone. Addition of water facilitates the removal of the acetone, leaving an aqueous extract, which, after addition of base, allows separation of ethofumesate by partition into hexane. Clean-up of this component is by silica Sep-pak cartridge prior to determination by gas chromatography (GLC) with flame photometric detection (FPD).

The free metabolites remaining in the aqueous extract (neutralised) are digested with the solids remaining from the soxhlet extraction (to release water soluble conjugates) before acid hydrolysis which liberates free metabolites from conjugation. Subsequent partition of the total metabolites into diethyl ether is followed by clean-up through a florisil Sep-pak cartridge (NC 8493 is acetylated to NC 8906 prior to clean-up) and determination by GLC with FPD.

1.4 Calibration

A curve of the form $y = ax^n$ (where n is typically between 1.5 and 2.0) is applicable over the tested range of 1 to 10 µg/ml ethofumesate, NC 9607 and NC 8493 (measured as NC 8906) i.e. approximately 3 to 30 ng (or equivalent for NC 8493) injected into the GLC.

1.5 Range of application

Recovery determinations have been tested at levels ranging from 0.02 to 50 mg/kg ethofumesate, NC 9607 and NC 8493.

1.6 Recovery efficiency

A mean recovery efficiency of 98.2% was obtained from 45 recovery tests with untreated grass and sugarbeet samples fortified at levels ranging from 0.02 to 50 mg/kg ethofumesate. Mean recovery efficiencies of 76% and 71% were obtained from 41 and 24 tests, respectively, for NC 9607 and NC 8493 at the same fortification levels.

1.7 Precision

The standard deviations for these recovery data were 9.8%, 12% and 14% for ethofumesate, NC 9607 and NC 8493 respectively.

1.8 Limit of determination

In a clean chromatogram, the determination limit for NC 8438, NC 9607 and NC 8493 is estimated as 0.05 mg/kg.

1.9 Time for analysis

Analysis of a batch of 6 samples takes approximately 1 day from extraction to the preparation of the final solution for GLC determination.

2 INTRODUCTION

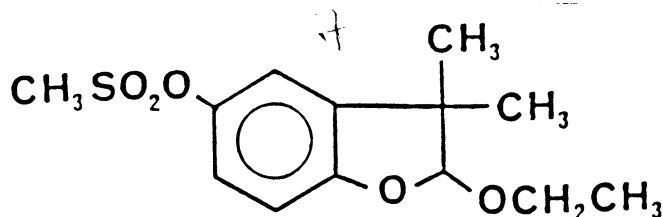
The selective herbicide ethofumesate (NC 8438), 2-ethoxy-2,3-dihydro-3,3-dimethyl benzofuran-5-yl methane sulphonate, has been developed for use mainly on beet and grass crops.

It is therefore of importance to be able to determine residue levels of the active ingredient and its major metabolites occurring in these crops. The current analytical method for the determination of such residues in grass and sugarbeet is described in this report, and is based upon the previously used procedure for grass (1).

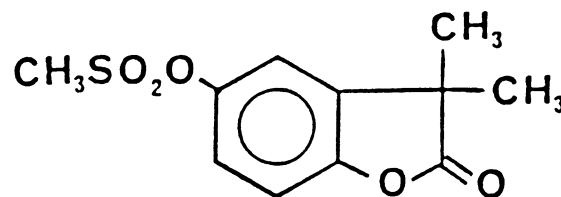
Metabolism studies on ryegrass (2) and sugarbeet (3) have shown that the major metabolites found are conjugated and non-conjugated products of the 2-oxo- and 2-hydroxy- metabolites of ethofumesate, NC 9607 and NC 8493, respectively (Figure 1). Consequently, all of these are included in the analytical method.

Figure 1

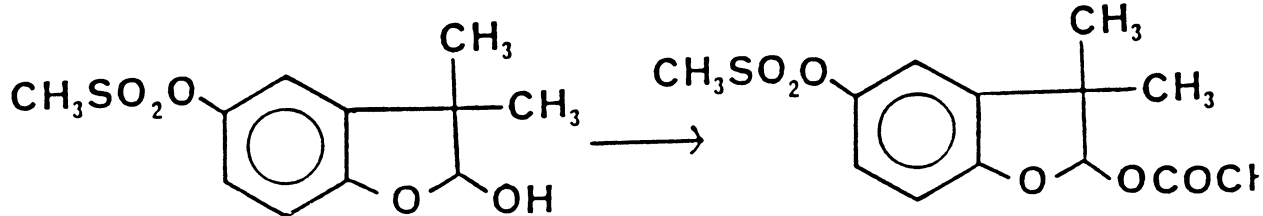
Chemical structures of ethofumesate and non-conjugated NC 9607 and NC 8493



Ethofumesate (NC 8438)
2-ethoxy-2,3-dihydro-3,3-dimethyl benzofuran-5-yl methane sulphonate



NC 9607
2,3-dihydro-3,3-dimethyl-2-oxo-benzofuran-5-yl methane sulphonate



NC 8493
2,3-dihydro-2-hydroxy-3,3-dimethyl benzofuran-5-yl methane sulphonate

NC 8906 (see Section 3)
2-acetoxy-2,3-dihydro-3,3-dimethyl benzofuran-5-yl methane sulphonate

3 PRINCIPLE OF METHOD

The analytical method is based on an earlier procedure (1).

After soxhlet extraction of ethofumesate and non-conjugated residues of NC 9607 and NC 8493 with acetone, water is added which facilitates the removal of the acetone, leaving an aqueous extract. Addition of base allows the partition of ethofumesate into hexane prior to clean-up through a silica Sep-pak cartridge and determination by gas chromatography (GLC) using flame photometric detection (FPD) in the sulphur mode.

The metabolites (free and conjugated NC 9607 and NC 8493) remaining in the aqueous extract (neutralised) and the solids from the earlier soxhlet extraction are then digested together before acid hydrolysis which liberates the free metabolites from conjugation. After partition of the total metabolites into diethyl ether and clean-up by washing with saturated sodium hydrogen carbonate solution, the extracts are further cleaned-up by elution through a florisil Sep-pak cartridge (NC 8493 is acetylated to NC 8906 before florisil clean-up) before determination by GLC with FPD.

4 ANALYTICAL METHOD

4.1 Reagents and apparatus

4.1.1 Reagents

Organic solvents:- Dichloromethane, acetone, toluene, diethyl ether, ethyl acetate, hexane are Fisons Distol Pesticide grade.

Pyridine; Fisons spectrograde

Laboratory reagents:- Concentrated hydrochloric acid (SG 1.18), AR grade; potassium hydroxide, AR grade; acetic anhydride, AR grade; anhydrous sodium sulphate, SLR grade; sodium hydrogen carbonate, SLR grade; Metasil 'A' filter aid.

4.1.2 Standard solutions

Ethofumesate (NC 8438), NC 9607 and NC 8493:- Dissolve the analytical standard (100 mg) in ethyl acetate (100 ml) in a standard volumetric flask to give 1000 µg/ml (ppm) stock solutions. From these prepare working solutions by dilution with ethyl acetate as required.

4-methyl-1,2-phenylenedimethane sulphonate (MPDMS):- Dissolve the compound (100 mg) in ethyl acetate (100 ml) to give 1000 µg/ml (ppm) stock solution, which is then used in the preparation of a more dilute working solution.

All stock solutions should be stored under refrigeration (1°C) when not in use.

4.1.3 Special apparatus

The following items are used in this laboratory.
Alternatives may be acceptable.

Soxhlet extractors (size EX 5/63) with cellulose thimbles
(30 x 100 mm)
Buchi rotary evaporator with water bath at 40°C
Kuderna-Danish evaporators with water bath
Corning hot plate at 95°C
Fisons Fi-monitor to control hot plate
Whirlimix
Ultrasonic bath
Silica Sep-pak cartridge (Waters Associates Ltd.)
Florisil Sep-pak cartridge (Waters Associates Ltd.)
Bibby clips B19, B24, B29

4.2 Gas chromatograph, operating conditions

Instrument : Tracor 550 fitted with flame photometric
detector (FPD) operating in the sulphur mode.

Column : Glass, 1 m x 3 mm i.d., packed with 1.5%
OV 225 on Chromosorb W, acid washed,
DMCS-treated, 80-100 mesh.

Carrier gas : Nitrogen at approximately 30 ml/minute.

Temperatures : Column oven set between 210°C and 220°C
Detector 245°C
Injector 250°C

Retention times : NC 8438 approximately 0.6 to 1.4 minutes
NC 9607 approximately 0.8 to 1.9 minutes
NC 8906 (acetylated NC 8493) approximately
1.0 to 2.9 minutes
MPDMS approximately 1.3 to 3.4 minutes

Injection volume : Approximately 3 µl.

4.3 Preparation of samples

All sample types (frozen) may be suitably prepared for extraction
by chopping in a Hobart food cutter and re-freezing (-20°C) prior
to laboratory analysis.

4.4 Extraction of non-conjugated residues

- 4.4.1 Weigh a subsample of chopped material (25 g) into a cellulose extraction thimble (30 x 100 mm). Fortify at this stage with ethofumesate, NC 9607 and NC 8493 for recovery efficiency tests (see section 5.2).
- 4.4.2 Extract overnight in a soxhlet fitted to a 250 ml round-bottomed flask containing acetone (200 ml) and anti-bumping granules.
- 4.4.3 After cooling allow the thimble to drain before transferring the extract to a 500 ml round-bottomed flask with water (150 ml).

[Retain the extraction thimble and solids for extraction of water soluble conjugates - step 4.7].
- 4.4.4 Evaporate off all the acetone using a Buchi rotary evaporator and reduced pressure to leave the extract in aqueous solution.

4.5 Separation of ethofumesate

- 4.5.1 Transfer the extract into a 500 ml separating funnel. Add 3 M potassium hydroxide (2.5 ml) and shake to mix.
- 4.5.2 Add hexane (100 ml), shake gently and allow to separate. Run the lower (aqueous) layer into the 500 ml round-bottomed flask and decant upper, hexane through a No. 4 filter paper into a Kuderna-Danish evaporator (K-D).
- 4.5.3 Return aqueous phase to separating funnel and repeat step 4.5.2 twice more.

[Retain the aqueous layer for extraction of water soluble conjugates in step 4.7].
- 4.5.4 Reduce contents of K-D to less than 5 ml on a boiling water bath.
- 4.5.5 Take extract just to dryness in a dri-block at 50°C under a stream of nitrogen or air.

4.6 Sep-pak clean-up of ethofumesate

- 4.6.1 Dissolve residue in hexane (3 ml), agitating the extract using an ultrasonic bath. Centrifuge any sediments (if necessary) at 3000 rpm for 3 minutes. Transfer supernatant onto a Sep-pak silica cartridge.
- 4.6.2 Repeat 4.6.1 once more.

- 4.6.3 Elute Sep-pak cartridge with 2 + 3 v/v dichloromethane + hexane (5 ml). Discard this fraction.
 - 4.6.4 Elute any ethofumesate with 9 + 1 v/v dichloromethane + hexane (10 ml). Collect this fraction.
 - 4.6.5 Take the cleaned extract just to dryness in a dri-block at 50°C under stream of nitrogen or air.
 - 4.6.6 Dissolve residue in appropriate volume (usually 0.5 ml) of Marker solution (10 µg/ml of MPDMS in ethyl acetate) prior to injection into GLC.
- 4.7 Extraction and hydrolysis of conjugated metabolites
- 4.7.1 Transfer the solids (step 4.4.3) and aqueous fraction (step 4.5.3) to a 500 ml conical flask with acetone (50 ml). Add 1 M hydrochloric acid (20 ml) to neutralise, then heat on a gently boiling water bath for 1 hour.
 - 4.7.2 Filter warm aqueous digest on a Buchner funnel through a pad of Metasil 'A' on a Whatman No. 54 filter paper. Rinse the flask with distilled water (20 ml) and add this to the funnel.
 - 4.7.3 Add concentrated hydrochloric acid (150 ml). Heat on a gently boiling water bath for 90 minutes. Allow to cool completely.
 - 4.7.4 Transfer the extract to a 500 ml separating funnel and extract with diethyl ether (200, 150, 100 ml). Discard the aqueous layer.
 - 4.7.5 Wash the combined ether extracts with a saturated solution of sodium hydrogen carbonate (2 x 50 ml) and discard the aqueous phase. Dry the ether over anhydrous sodium sulphate (50 g) for 10 minutes.
 - 4.7.6 Filter the extract through a No. 4 filter paper into a K-D flask, rinse the conical flask and sodium sulphate with diethyl ether (50 ml) and filter this into the K-D.
 - 4.7.7 Reduce the contents of the K-D to less than 5 ml on a boiling water bath.
 - 4.7.8 Transfer the extract from the K-D tube to a flat bottomed glass vial and take to dryness under a stream of nitrogen on a dri-block at 50°C.

4.8 Acetylation of NC 8493

- 4.8.1 Dissolve the residue in toluene (0.5 ml); add acetic anhydride (0.2 ml) and pyridine (50 μ l). Warm on a hot-plate at 95°C for 15 minutes (avoid distilling off the solvents by placing a vial cap loosely on top of the vial).
- 4.8.2 Take the extract to dryness under a stream of nitrogen on a dri-block at 50°C.

4.9 Sep-pak clean-up of metabolites

- 4.9.1 Dissolve residue in dichloromethane (3 ml) and transfer onto a Sep-pak florisil cartridge.
- 4.9.2 Rinse the vial with dichloromethane (2 ml) and transfer this on to the cartridge.
- 4.9.3 Elute with 96 + 4 v/v dichloromethane + ethyl acetate (10 ml). Collect this fraction.
- 4.9.4 Take cleaned extract just to dryness on a dri-block at 50°C under a stream of nitrogen or air.
- 4.9.5 Dissolve the residue in appropriate volume (usually 0.5 ml) of marker solution (10 μ g/ml MPDMS in ethyl acetate) prior to injection into GLC.

4.10 Calibration

Prepare 2 sets of 3 calibration standards containing 1, 3, and 10 μ g NC 8438 or NC 9607 and NC 8493 as appropriate. Acetylate the latter (NC 9607 and NC 8493) under the same conditions used for the samples (section 4.8.1) after removal of the solvent. Dissolve in 1 ml of 10 μ g/ml MPDMS in ethyl acetate.

4.11 Gas chromatographic analysis

- 4.11.1 Inject an aliquot of each solution into the gas chromatograph (section 4.2) by manual injection.
- 4.11.2 If necessary, further dilute the extracts with marker solution before re-injection.

4.12 Calculation of results

- 4.12.1 In each chromatogram measure (either manually or using a reporting integrator system) the peak heights of NC 8438, NC 9607 and NC 8906 (acetylated NC 8493) and express these as a percentage of the height of the MPDMS peak.
- 4.12.2 For each analyte, perform a power regression analysis to define calibration curves with equations of the form $y = ax^n$ where y = analyte peak height ratio and x = $\mu\text{g/ml}$ analyte. The value of n is typically between 1.5 and 2.0.
- 4.12.3 Use these calibrations to determine the concentrations of NC 8438, total (free and conjugated) NC 9607 and total NC 8493 in each extracted sample.
- 4.12.4 Calculate the residue level as mg/kg (ppm) by reference to the weight of sample analysed (25 g) and any dilutions made in section 4.10.2.

With fortified recoveries, calculate the recovery efficiency as follows.

$$\% \text{ recovery} = \frac{\text{residue (mg/kg)} \times 100}{\text{fortification level (mg/kg)}}$$

5 RESULTS

5.1 Limit of determination

The peak obtained from a 1 μg standard of any of the analytes gives the smallest peak which can be measured with reasonable accuracy, such a peak representing a residue of 0.03 mg/kg. A level of 0.05 mg/kg is therefore considered to be an appropriate limit of determination for all compounds determined.

5.2 Recovery efficiency

The individual recovery efficiency results for ethofumesate, NC 9607 and NC 8493 are detailed in Table 1. Similar results were obtained from both grass and sugarbeet (immature plants, roots and leaves) samples, with an overall mean of 98.2% for ethofumesate and means of 76% and 71% for NC 9607 and NC 8493 respectively.

When testing conjugate recoveries, fortifications are made using the corresponding 'free' metabolites, as the specific conjugated materials are not available for this purpose. These tests therefore assume that the acid hydrolysis of conjugates is quantitative, based on radiolabelled studies.

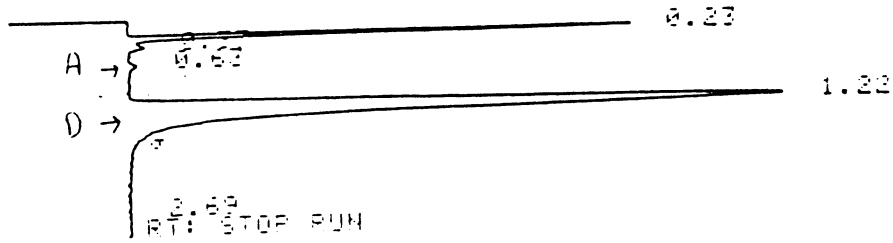
5.4 Typical chromatograms

Examples of typical chromatograms from this study have been included in the following order.

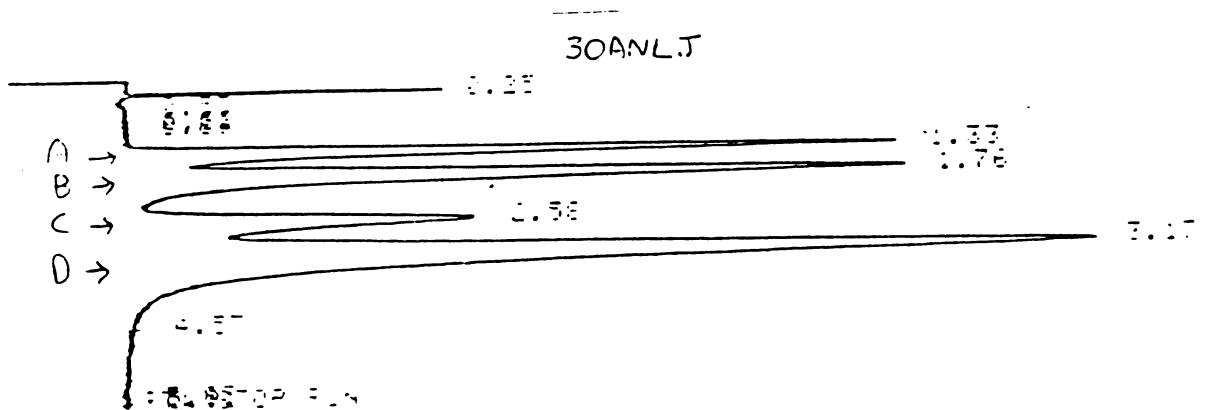
Chromatogram	Type	Analysis reference	Description
5.4.1	Calibration standard	02 CBNQ	1 µg/ml ethofumesate
5.4.2	Calibration standard	30 ANLJ	10 µg/ml NC 9607 and NC 8493 (NC 8906)
5.4.3	Control extract	30 ANAJ	Untreated grass sample containing an apparent ethofumesate residue of 0.019 mg/kg
5.4.4	Control extract	06 ATAJ	Untreated sugarbeet root sample containing a non-detectable residue for total NC 9607
5.4.5	Recovery test (0.03 mg/kg)	02 CBBQ	Sugarbeet leaves sample 108% ethofumesate (corrected for 02 CBAQ)
5.4.6	Recovery test (4.0 mg/kg)	25 ANBJ	Grass sample 73% NC 9607 and 90% NC 8493 (corrected for 25 ANAJ)
5.4.7	Sample extract	30 ANEJ	Grass sample containing 0.23 mg/kg ethofumesate
5.4.8	Sample extract	30 ANFJ	Grass sample containing 1.28 mg/kg total NC 9607 and 0.52 mg/kg total NC 8493
5.4.9	Sample extract	02 CBHQ	Sugarbeet leaf sample containing <0.05 mg/kg ethofumesate
5.4.10	Sample extract	11 CAVQ	Immature sugarbeet sample containing 8.1 mg/kg total NC 9607 and <0.05 mg/kg total NC 8493

In each chromatogram, peak A represents ethofumesate, peak B NC 9607, peak C NC 8906 and peak D the marker compound (MPDMS).

5.4.1



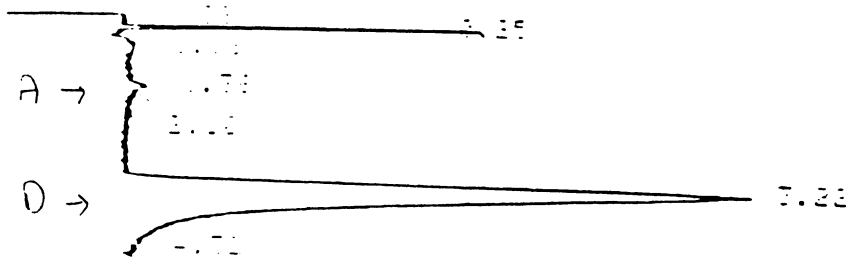
5.4.2 *



* At this time of analysis, this standard was prepared from a solution containing ethofumesate (peak A) as well as the two metabolites (Peaks B and C), although calculations of ethofumesate residues were based upon a separate standard containing ethofumesate only.

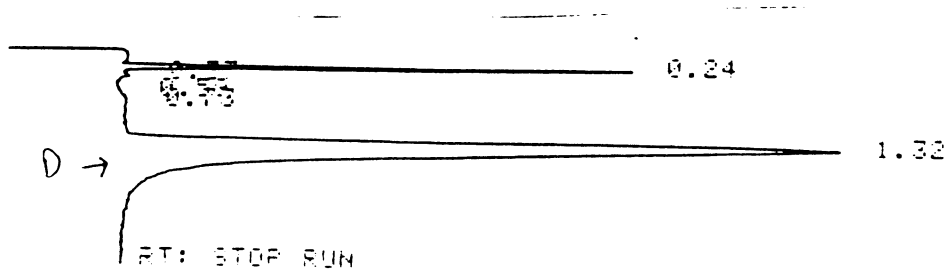
5.4.3

30ANAJ → 1/2 ml



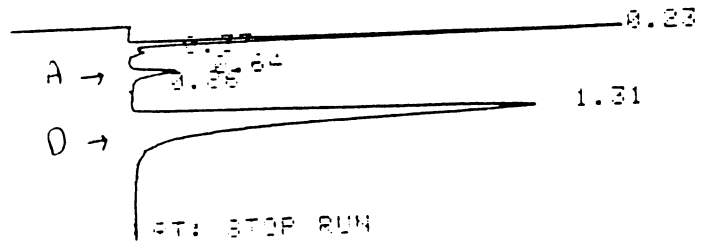
5.4.4

06ATAJ → 1/2 ml



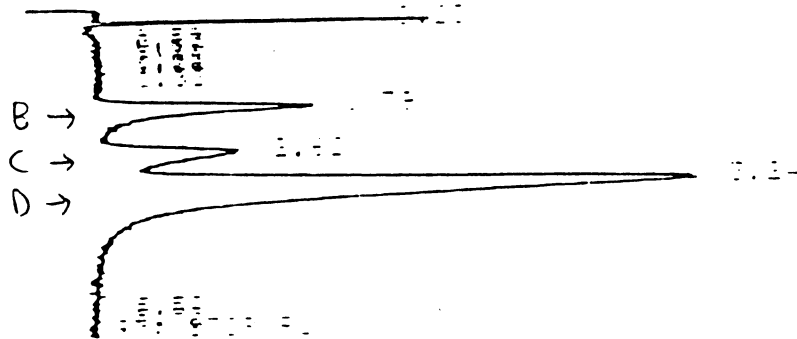
5.4.5

UACBBQ → 1/2 ml

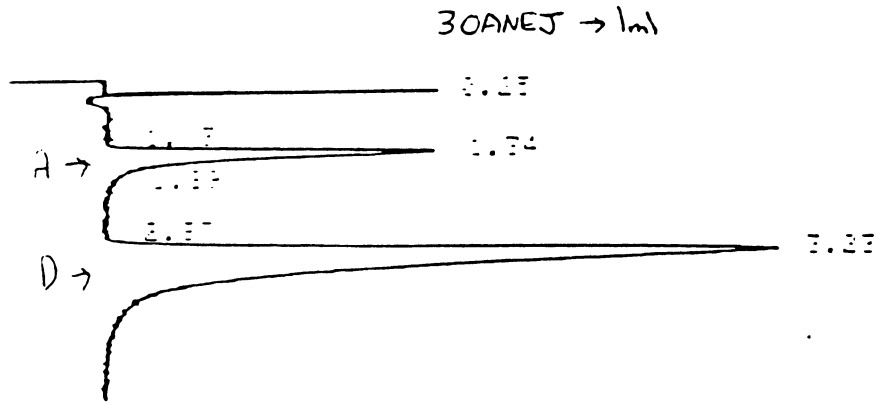


5.4.6

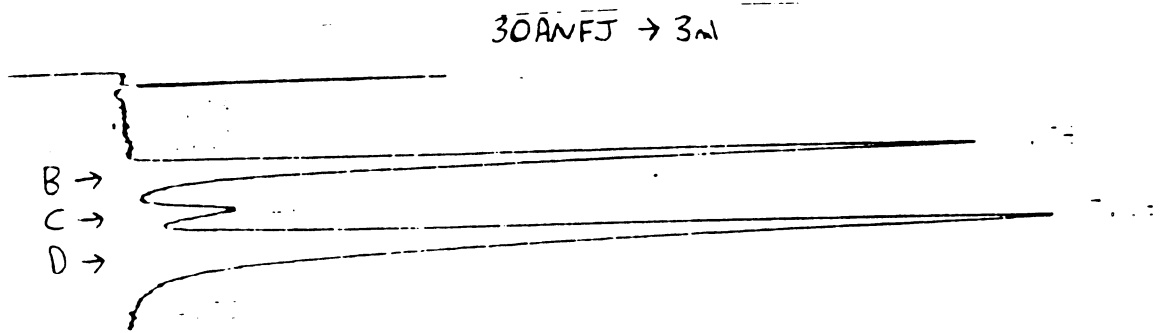
Q5ANBJ → 12 ml



5.4.7

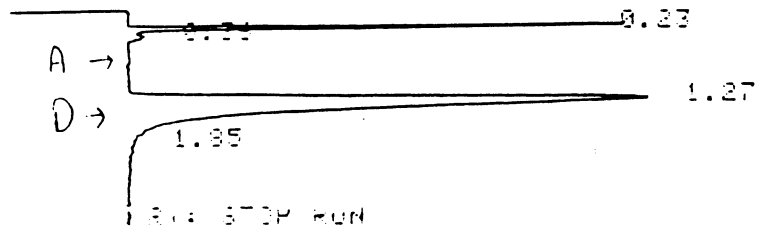


5.4.8



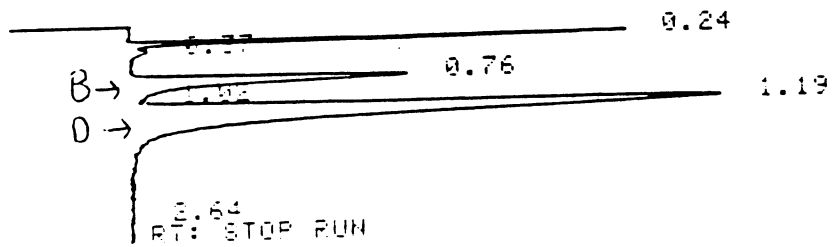
5.4.9

O2 CBHQ → 1/2 ml



5.4.10

11CAVQ → 5 ml



6 REFERENCES

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Registration reference NC 8438/M3 and M4

EPSR/JDM/WM
4th February, 1986
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