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**GLC METHOD OF ANALYSIS FOR MAJOR  
FORMETANATE-DERIVED RESIDUES IN NECTARINES**

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### Formetanat method

The "G.L.C Method of Analysis for Major Formetanate-Derived Residues in Nectarines", dated 11.9.87, is our best method for formetanate in fruit crops. However, there are two minor modifications:

- 1.) Page 13, 4.5.5 Clean up  
 II Wait for 20 min... which already contains 25 ml of 5N HBr.
- 2.) Page 14, 4.5.6 Bromination  
 I Transfer the concentrate....., fill up with water to a minimum of 25 ml.

The bromination of formetanate <sup>is a</sup> problematical part of the method. It is very important to add 1ml of a saturated sodium sulfite solution immediately (after 5 sec.).

If you have further questions, please ask me.  
 Viel Glück! (good luck!)  
 A. (Name - Rüdiger)

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## 1. SUMMARY

- The method of analysis presented here allows the quantification of residues of the pesticide formetanate and metabolites containing the 3-aminophenol moiety in nectarines.
- Formetanate-related compounds are extracted from the nectarine matrix with acidified organic solvents and transformed to 3-aminophenol by basic hydrolysis. After liquid-liquid extraction on solid phase 3-aminophenol is brominated to 3-amino-2,4,6-tribromophenol. For final quantitation of this target GLC/ECD is used.
- The method has been tested from 0.05 to 10 mg/kg and found to produce a linear response of the analytical target over the whole range.
- The overall recovery efficiency in the range of 0.05 - 10 mg/kg was  $74 \pm 11$  %.
- The limit of determination was established at 0.05 mg/kg.
- Analysis of a batch of six samples takes up to 1.5 days from weighing of the samples to preparation of the final solutions for GLC determination.

## 2. INTRODUCTION

Formetanate hydrochloride (3-dimethylamino-methyleneaminophenyl methylcarbamate x hydrochloride) is an acaricide/insecticide. It acts by inhibition of acetylcholinesterase and is effective for the control of spider mites, rust mites, certain aphids, thrips, lygus bugs, leaf hoppers, slugs and snails on horticultural, agronomic and ornamental plants.

Different residue methods have been developed for the determination of formetanate hydrochloride in crops and other commodities. A colorimetric method<sup>3)</sup> is based on the production of azo dyes of 3-aminophenol, a GLC-method<sup>3)</sup> quantifies the brominated 3-aminophenol, an HPLC-method<sup>4)</sup> determines formetanate as analytical target using RP/UV.

As all of these methods suffer either from lacking quality, insufficient sensitivity and/or problems with blank values an improved method has been established to overcome these problems.

Metabolism studies on different plants<sup>1),2)</sup> showed that formetanate was partly degraded to different metabolites which virtually all contain the 3-aminophenol moiety. Therefore this compound, 3-aminophenol, was chosen as analytical target for the evaluation of a total residue method in nectarines.

### 3. PRINCIPLE OF METHOD

Formetanate-related compounds are extracted from the matrix with acidified organic solvents and are unified to 3-aminophenol by basic hydrolysis. After liquid-liquid extraction on solid phase 3-aminophenol is brominated to 3-amino-2,4,6-tribromophenol. For final quantitation of this target GLC/ECD is used.

### 4. ANALYTICAL METHOD

#### 4.1 STANDARD SOLUTIONS

Formetanate x HCl - Prepare a stock solution by dissolving about 25 mg of analytical standard in 50 ml of 1 N hydrochloric acid, prepare working solutions from the stock by further dilution. Reprepare all solutions in a three month interval.

3-Amino-2,4,6-tribromophenol Prepare a stock solution by dissolving about 10 mg of analytical standard in 20 ml of toluene, prepare working solutions from the stock by further dilution. Reprepare all solutions monthly.

#### 4.2 APPARATUS

Standard laboratory glassware  
Rotary vacuum evaporator with water bath  
Macerator or homogenizer  
Centrifuge  
Electrically heated mantles (250 ml), mirror burner  
Gas chromatograph with electron capture detector

4.3 GAS CHROMATOGRAPH, OPERATING CONDITIONS

INSTRUMENT	COLUMN (fused silica capillary)	CARRIER	SPLIT (ml/min)	MAKE UP (ml/min)	OVEN (°C)	INJECTOR (°C)	DETECTOR (°C)	RETENTION TIME (min)
HP 5890 A	HP 17	He	45	Ar/CH <sub>4</sub>				5.4
	10 m,	0.2 bar		80	220	250	300	or
	0.53 mm i.d. 2 µm film	or 0.15 bar						7.3
HP 5730 A	CP Wax 57 CB	He	25	Ar/CH <sub>4</sub>				
	8 m,	0.8 bar		60	205	200	300	2.8
	0.32 mm i.d.							
HP 5890 A	HP 1	He	on					
	5 m,	5 ml	column	Ar/CH <sub>4</sub>	200	on	300	2.5
	0.53 mm i.d. 2.65 µm film	< 10 k Pa		75		column		

#### 4.4 REAGENTS

Organic solvents - methanol, dichloromethane, n-hexane, ethyl acetate, toluene are distilled in glass or purchased as p.a. commodity

Celite (filter aid) (Ferak, Berlin, Germany)

Hydrochloric acid, conc. p.a. (Ferak, Berlin, Germany)

Sodium hydroxide solution, 32 % p.a. (Riedel de Haen, Hannover, Germany)

Sodium hydroxide, solid p.a. (Merck, Darmstadt, Germany)

Extrelut 20, prepacked columns (Merck, Darmstadt, Germany)

Extrelut 20, refill pack (Merck, Darmstadt, Germany)

equivalent: fill an empty Extrelut column with 15 g of Chem-elut material; place filters on top and bottom of the column (see below).

- Chem-elut, Chem tube hydromatix (Analytchem Int., Frampton, USA)
- Filter, 10 mm and 25 mm (Schleicher & Schüll, Dassel, Germany)

Hydrobromic acid, 48 % (Riedel de Haen, Hannover, Germany)

Bromine, p.a. (Riedel de Haen, Hannover, Germany)

Sodium sulfite-7-hydrate, p.a. (Riedel de Haen, Hannover, Germany)

Formetanate x HCl, lot no. ST 016, quality certificate 301/85 (Schering AG, Wolfenbüttel, Germany)

3-Amino-2,4,6-tribromophenol, Charge no. 86 02499, quality certificate 359/86 (Schering AG, Wolfenbüttel, Germany)

## 4.5 EXPERIMENTAL PROCEDURE

### 4.5.1 Sample preparation

- immature fruits - finely chop and mix thoroughly
- mature fruits - remove stones and mix thoroughly the remaining pulp (weigh fruits before and after removal of stones)

### 4.5.2 Extraction of sample

- I Weigh 25 g of prepared sample into a suitable vessel for the macerator employed. Fortify at this stage with formetanate to check recovery efficiency.
- II Add 100 ml methanol and 1 ml hydrochloric acid, conc. (pH <3), macerate for 5 minutes and filter the macerate with suction over Celite.
- III Return the solid on the filter aid to the macerating flask, repeat the maceration for 5 minutes with methanol/dichloromethane (100 ml, 1/1, v/v), filter again with suction and wash the solid on the filter aid with 50 ml of methanol.
- IV Combine the filtrates and reduce to dryness using a vacuum rotary evaporator (bath temperature 50 °C).

### 4.5.3 Partition of extract

- I Dissolve the residue from 4.5.2 IV in 50 ml of water, transfer the solution to a 250 ml separating funnel (pH should be <3, if necessary add a drop of hydrochloric acid, conc.) and extract the water phase two times with 50 ml dichloromethane each.

- II Extract the water phase once with 50 ml of n-hexane and discharge the organic layers.

#### 4.5.4 Hydrolysis

- I Transfer the water layer from 4.5.3 II to a 250 ml round bottom flask and adjust the pH to approximately 7 using a 10 N sodium hydroxide solution.
- II Add 4 g of sodium hydroxide pellets and reflux for 20 minutes. Cool.
- III Centrifuge the mixture for 10 minutes at 4000 rpm and transfer the supernatant to a graduated cylindrical flask. Note the total volume of the supernatant! Discharge the pellet.

#### 4.5.5 Clean-up

- I Add 20 ml each of the solution from 4.5.4 III to the top of two Extrelut-columns (see p. 7; equivalently use 15 g chem-elut material).
- II Wait for 20 minutes and elute each column with 120 ml of ethyl acetate into the same 500 ml round-bottom flask which already contains 25 ml of  $X$ -N hydrobromic acid.  
*B. 2.2 4.2/9.2*
- III Reduce the solution to about 20 ml using a vacuum rotary evaporator (water bath 40 °C).

#### 4.5.6 Bromination

- I Transfer the concentrate from 4.5.5 III into a 50 ml separating funnel and, if necessary, fill up with water to ~~about~~ 25 ml.  
 Minimum of 25 ml
- II Add 1 ml of freshly prepared, saturated bromine water, swirl the solution and immediately (after 5 seconds) add 1 ml of a saturated solution of sodium sulfite. Swirl again (the solution will change its colour).
- III Extract the solution two times with 2 ml of toluene each, combine the toluene extracts in a 5 ml volumetric flask and make up to 5 ml with toluene.

Quantify this solution on a GC (see p. 6) at the same day.

#### 4.6 CALCULATION OF RESULTS

For each standard and sample extract the peak heights of preferably peak areas have to be measured.

For establishment of a calibration curve peak heights or areas from standards are plotted vs. the amounts of formetanate yielding a straight line corresponding to the general equation  $y = ax + b$  (term b normally can be neglected).

The amounts of formetanate in samples may be taken directly from the calibration curve.

Residue concentration of formetanate are calculated as follows

$$\text{residue (mg/kg)} = \frac{C \times V \times FI}{W \times FII}$$

- C = Amount of formetanate in a sample obtained from the calibration curve ( $\mu\text{g/ml}$ )
- V = Volume of the final solution (ml)
- W = Weight of the analysis sample (g)
- FI = Conversion factor from 3-amino-2,4,6-tribromophenol (ATP) to formetanate x HCl (FMT)
- $$\frac{\text{MW FMT}}{\text{MW ATP}} = \frac{257.8}{345.8} = 0.746$$
- FII = Factor resulting from the aliquot (40 ml) which is taken from the hydrolysis solution. This factor has to be calculated separately for each sample (see p. 9)!
- $$\text{FII} = \frac{V_A}{V_G}$$
- $V_A$  = Aliquot (ml), normally 40 ml
- $V_G$  = Volume of the total hydrolysis solution, see p. 9, step 4.5.4.III.

#### 4.7 DETERMINATION OF RECOVERY EFFICIENCY

$$\text{Recovery (\%)} = \frac{C \times V \times \text{FI}}{W_R \times \text{FII}}$$

$W_R$  = Amount of formetanate x HCl added ( $\mu\text{g}$ )

Other abbreviations see 4.6 .

## 5. RESULTS

When subjected to the different clean-up steps, hydrolysis and bromination, formetanate is converted in good yield to 3-aminophenol and finally to 3-amino-2,4,6-tribromophenol. The results are presented below. The pulp of nectarines was chosen as matrix.

The overall mean recovery rate was  $74 \pm 11 \%$ ,  $n = 21$ .

### 5.1 RECOVERY EFFICIENCY OF FORMETANATE x HCl IN NECTARINES

Fortification level (mg/kg)	Recovery rate (%)	Mean (%)	S.D. (%)
0.05	78	79	5
	84		
	74		
0.1	77	86	10
	96		
	85		
0.2	71	68	5
	63		
	73		
1	77	68	14
	84		
	78		
	51		
	56		
	54		
10	76	73	8
	70		
	72		
	69		
	78		
	75		

## 5.2 LIMIT OF DETERMINATION AND DETECTION

The limit of determination was established at the lowest fortification level, i.e., 0.05 mg/kg. Apparent residues in the batch of untreated nectarines used for establishment of this method were negligible. Concentrations of apparent residues ranged from <0.02 ng 3-amino-2,4,6-tribromo-phenol/ $\mu$ l to 0.03 ng/ $\mu$ l.

A lower limit of detection of 20 pg was attainable.

## 6. REFERENCES

1. Fate of formetanate hydrochloride in alfalfa by J. Celorio, 24.09.1986, Schering Report UPSR 30/86
2. Metabolism of formetanate acaricide in orange seedlings by C.O. Knowles, A.K. Sen Gupta  
J. Econ. Entomol. 63 615-620 (1970)
3. Anal. Methods Pestic. Plant Growth Reg. 12, 279-296 (1973)
4. Determination of the insecticide/acaricide formetanate in fresh fruit by RP/LC by J. Lawrence  
J. Agric. Food Chem. 29, 722-724 (1981)

**7. REPRESENTATIVE CHROMATOGRAMMS****1. Standards**

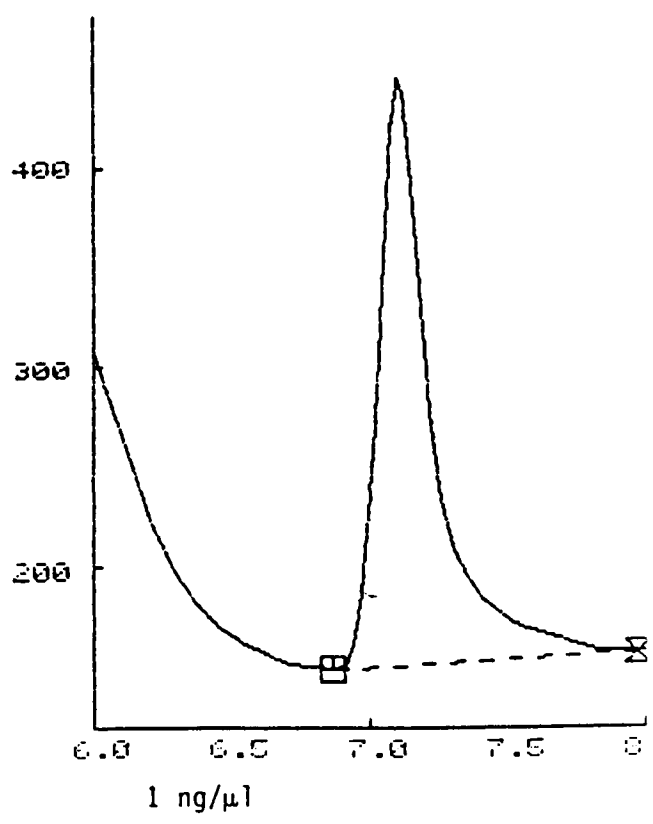
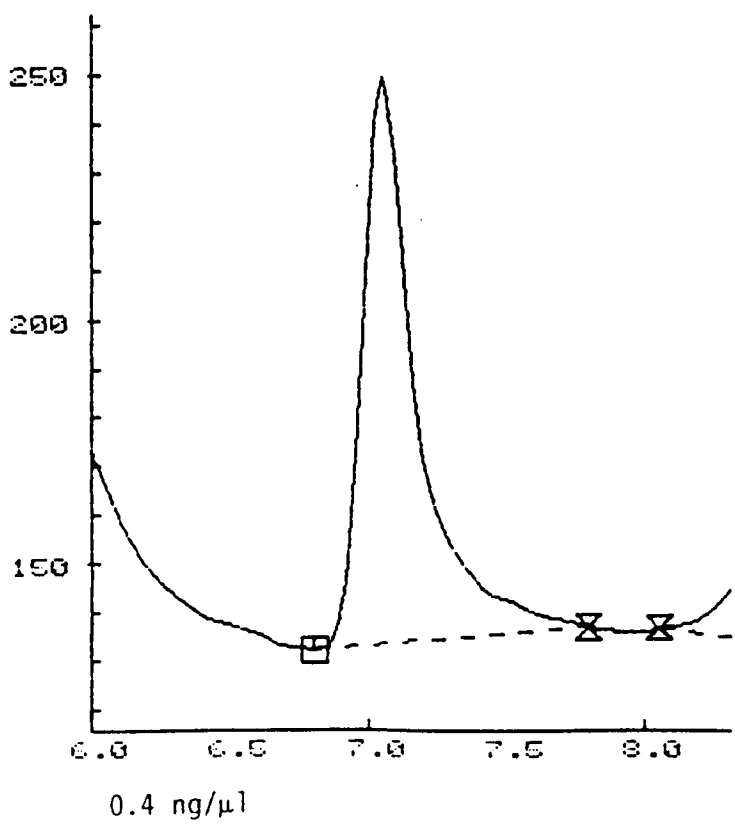
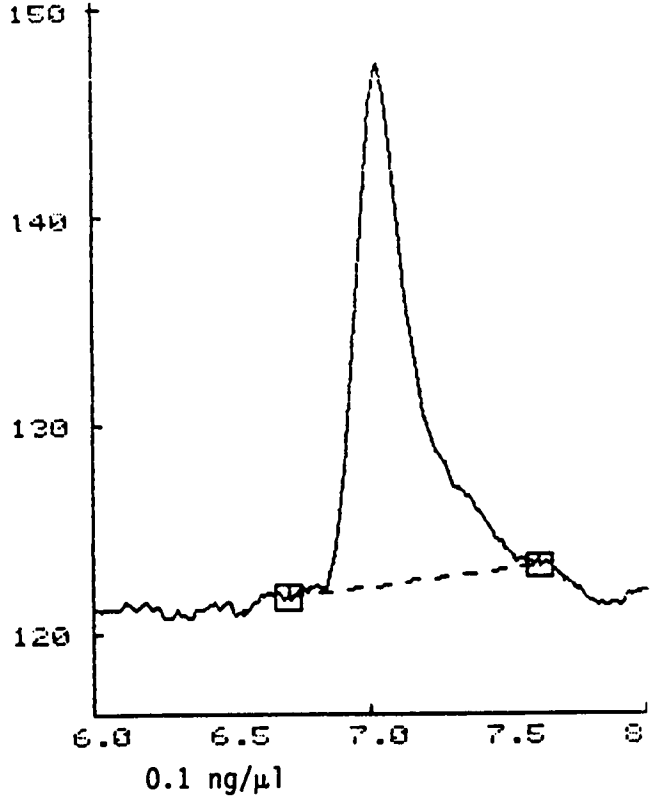
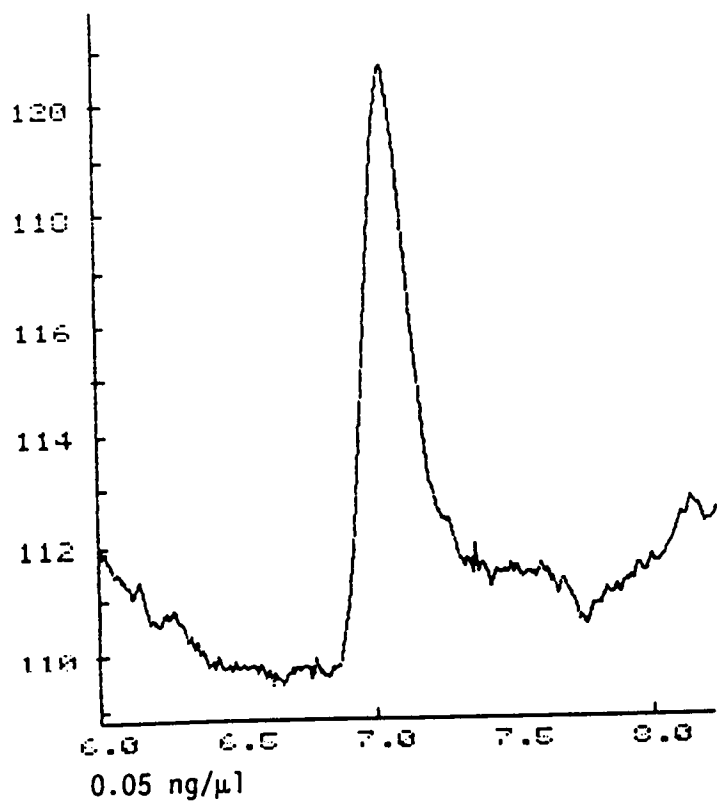
0.05, 0.1, 0.4, 1, 5, 10 ng/ $\mu$ l

**2. Recoveries**

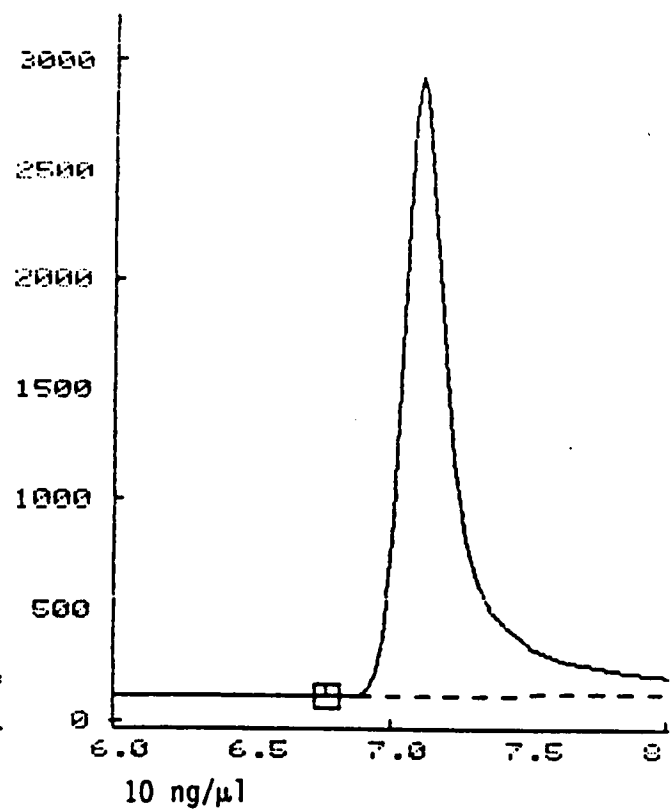
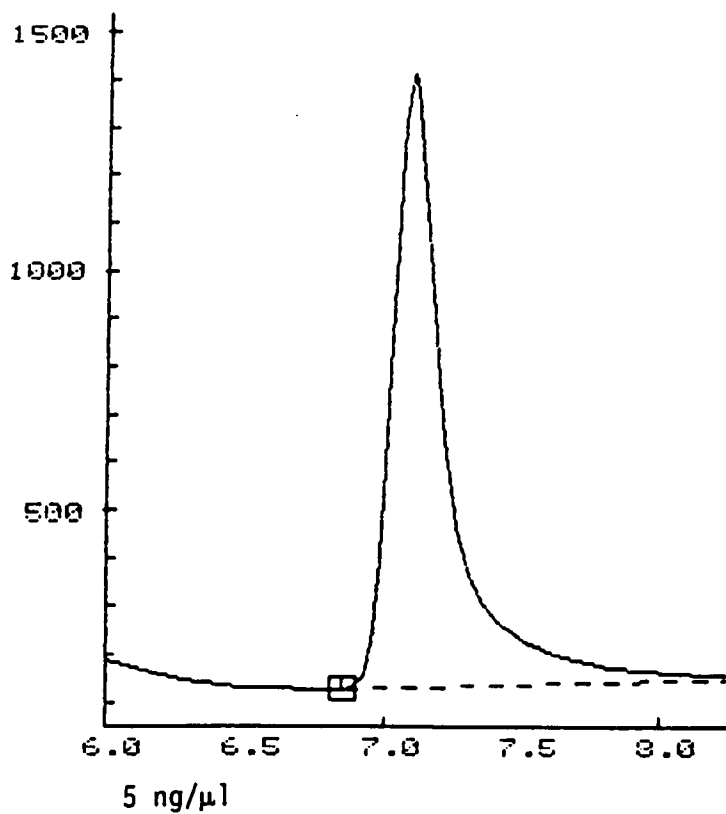
Fortification level	10	mg/kg
	1	mg/kg
	0.2	mg/kg
	0.05	mg/kg

**3. Control samples**

STANDARDS



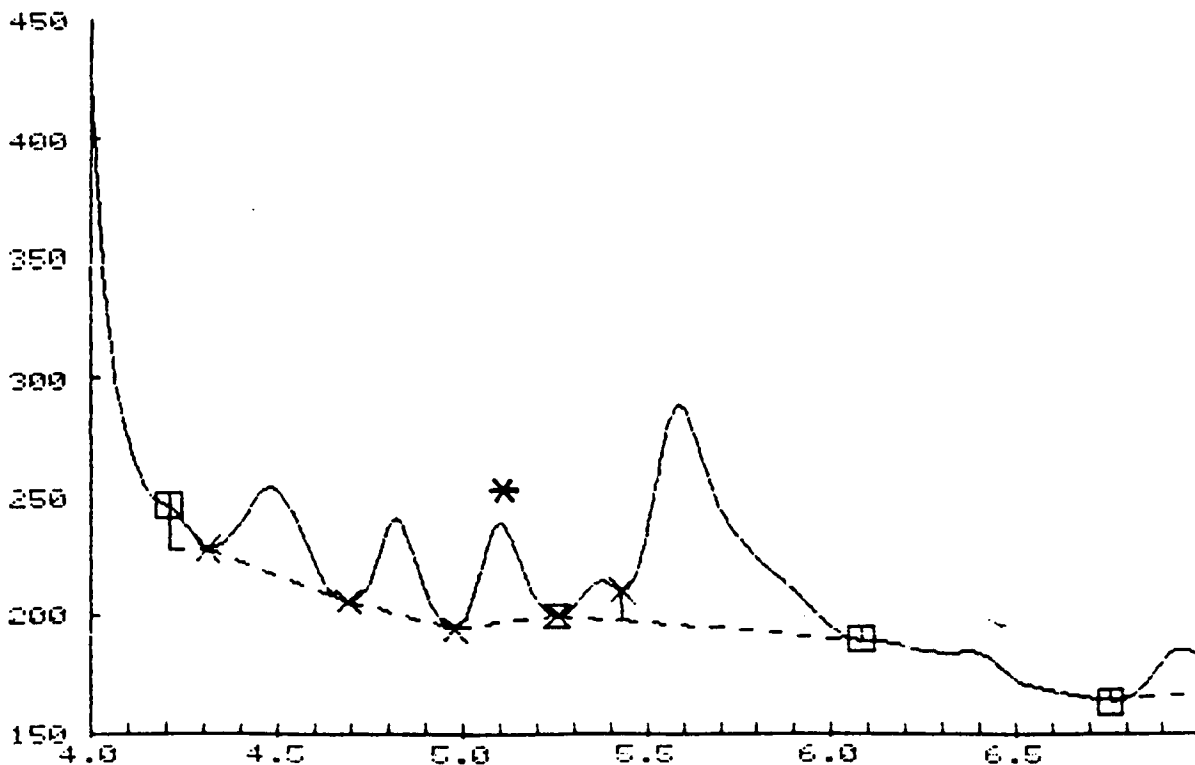
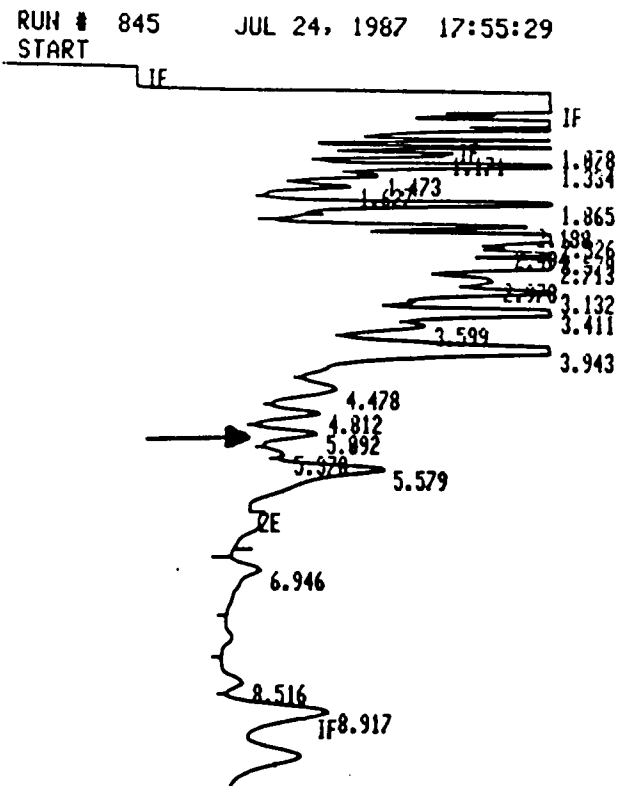
STANDARDS



RECOVERIES

Fortification level: 0.05 mg/kg

Sample contains 0.2 ng 2,4,6-tribromo-aminophenol/ $\mu$ l

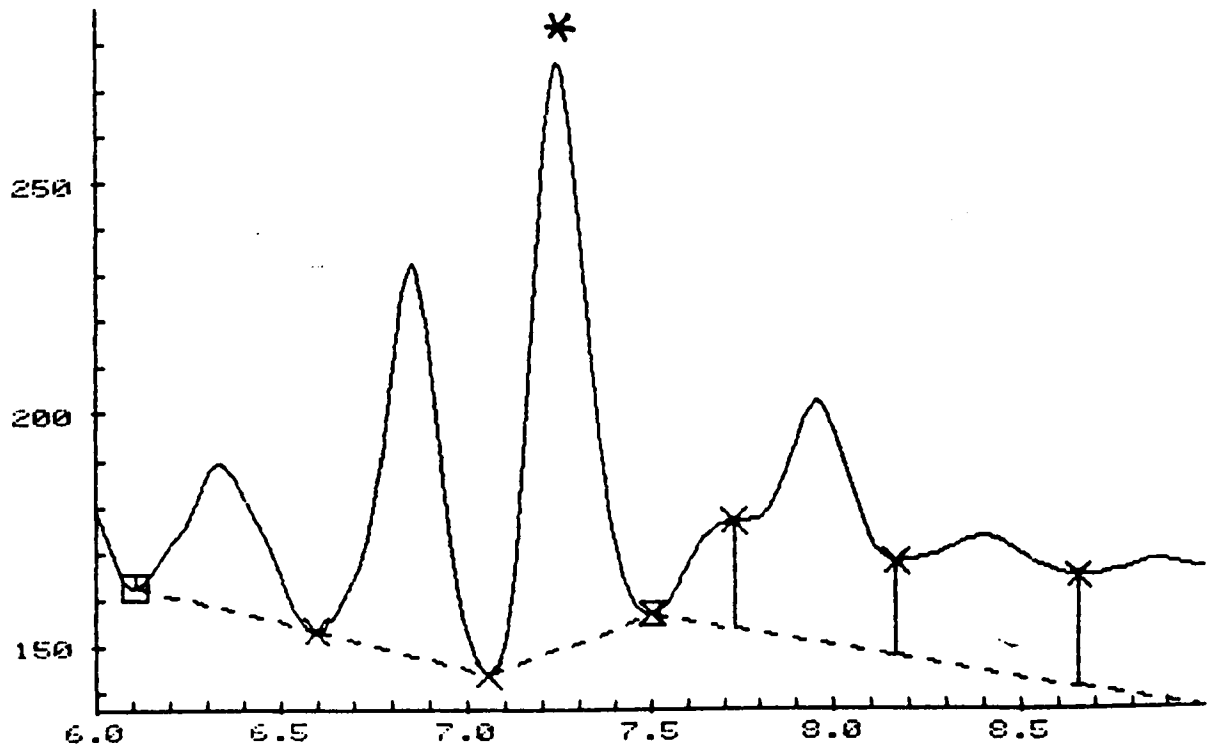
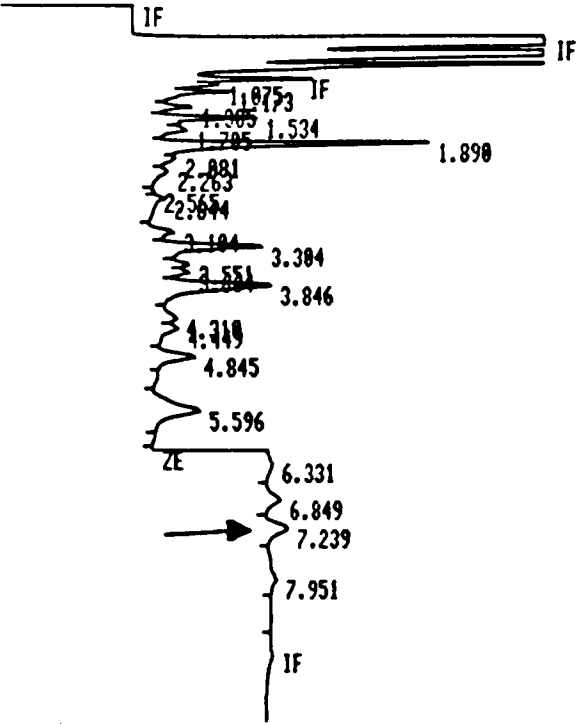


RECOVERIES

Fortification level: 0.2 mg/kg

Sample contains 0.47 ng 2,4,6-tribromo-aminophenol/ $\mu$ l

RUN # 942 AUG 4, 1987 21:20:37  
START

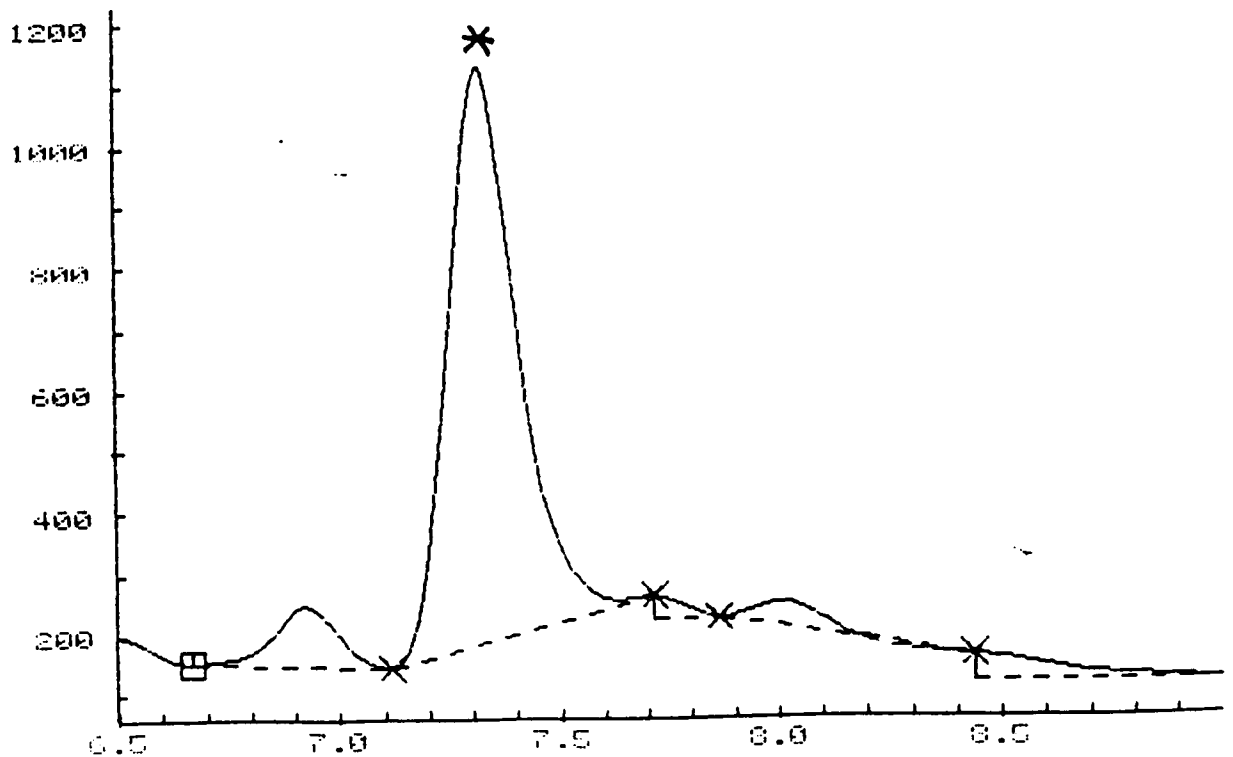
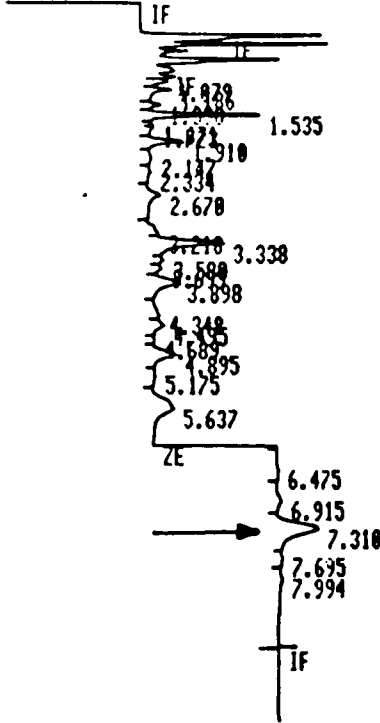


RECOVERIES

Fortification level: 1 mg/kg

Sample contains 2.4 ng 2,4,6-tribromo-aminophenol/ $\mu$ l

RUN # 1085 AUG 17, 1987 21:48:38  
START

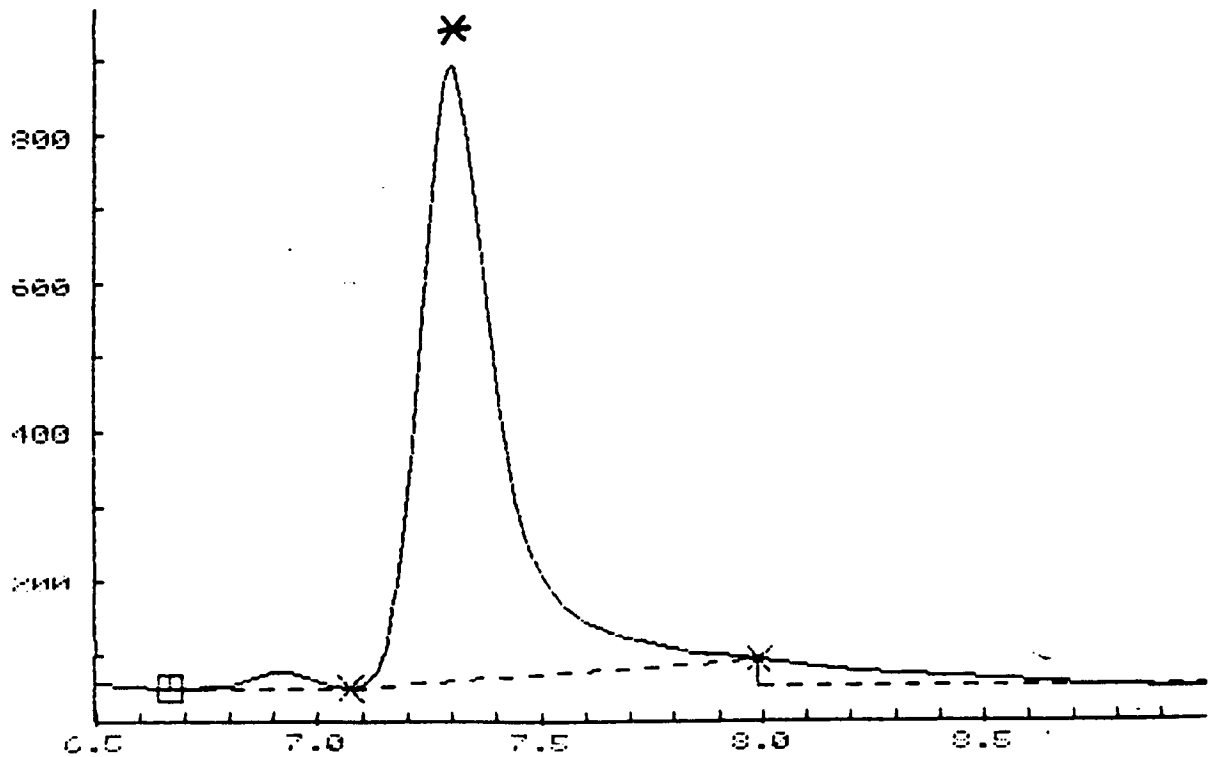
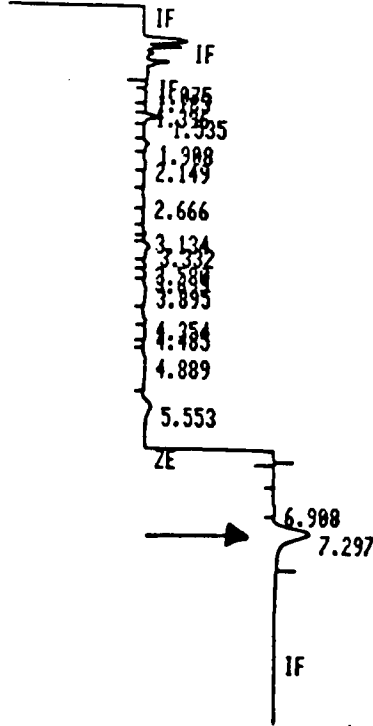


RECOVERIES

Fortification level: 10 mg/kg

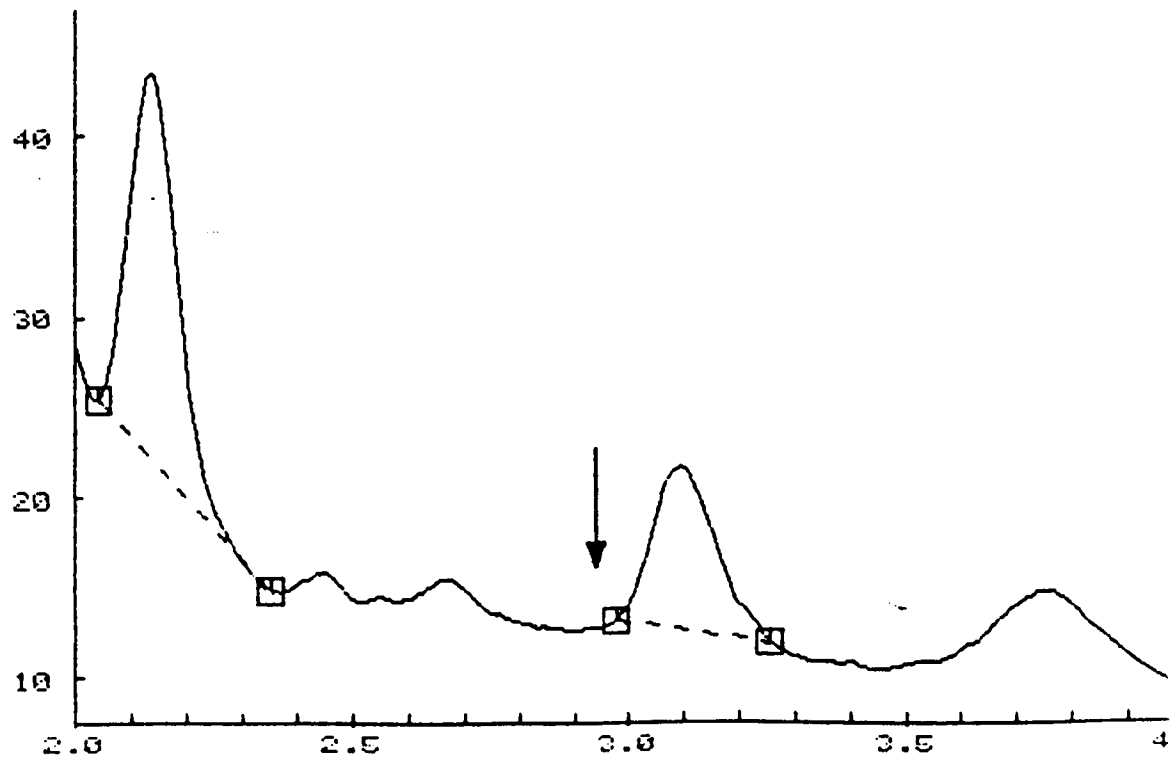
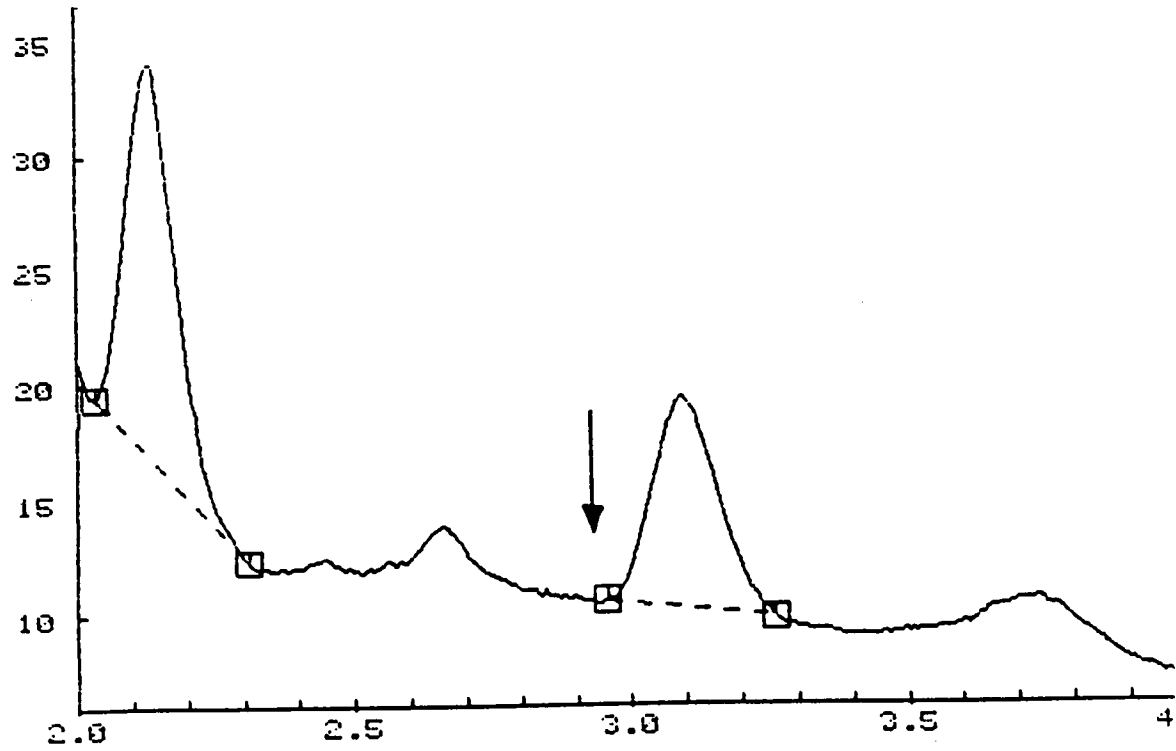
Sample contains 2.5 ng 2,4,6-tribromo-aminophenol/ $\mu$ l

RUN # 1101      AUG 18, 1987    03:26:58  
START



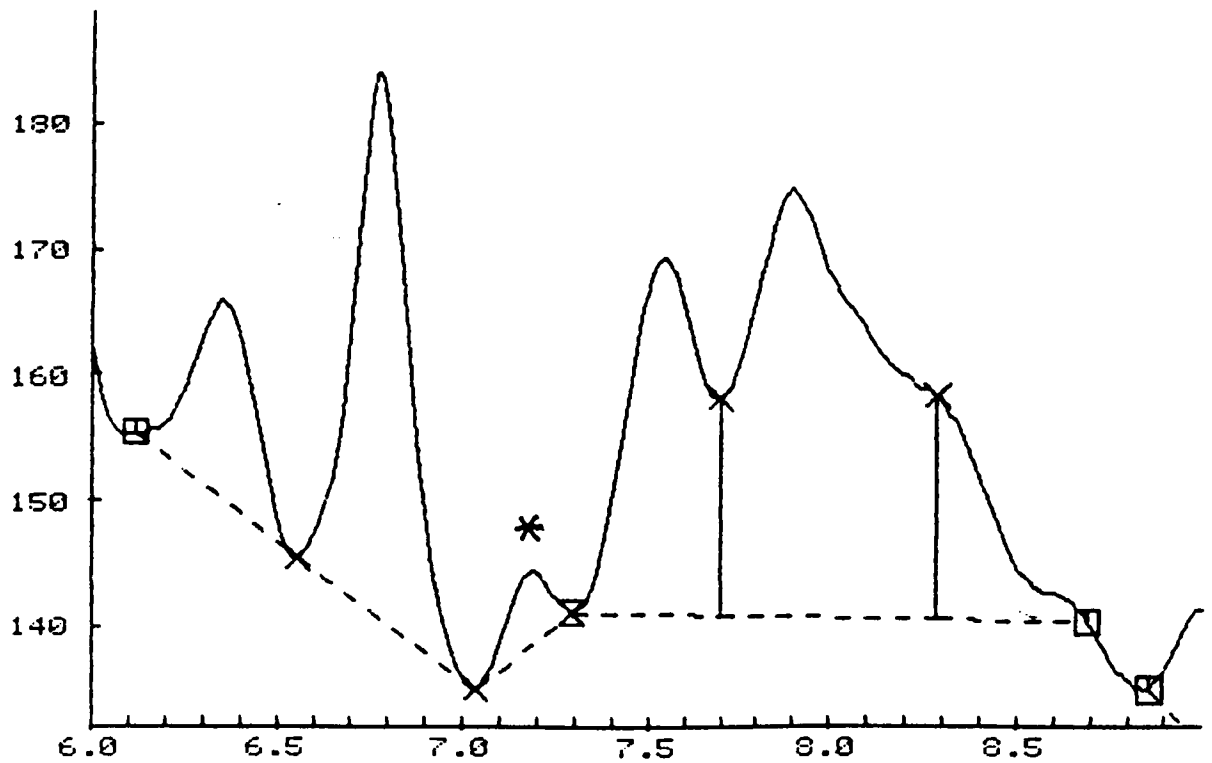
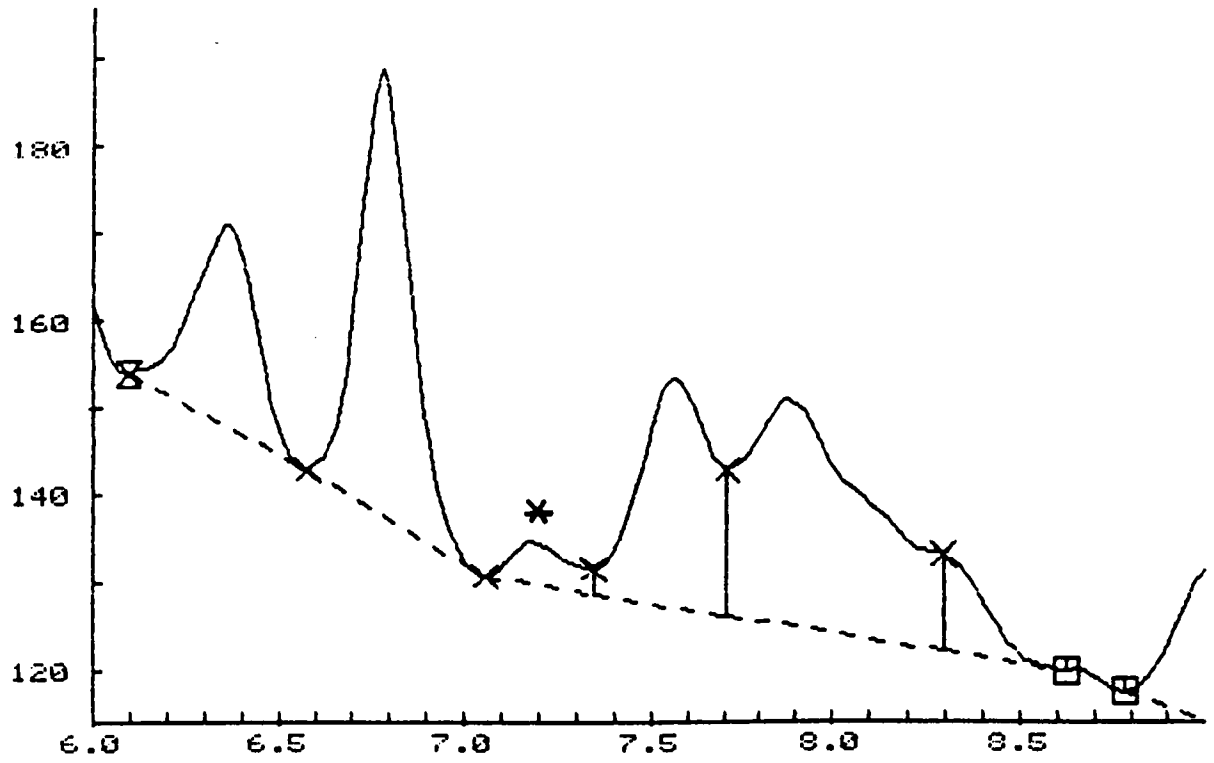
CONTROL SAMPLES

No apparent residues (<0.02 ng/μl) at the retention time of 2,4,6-tribromo-3-aminophenol

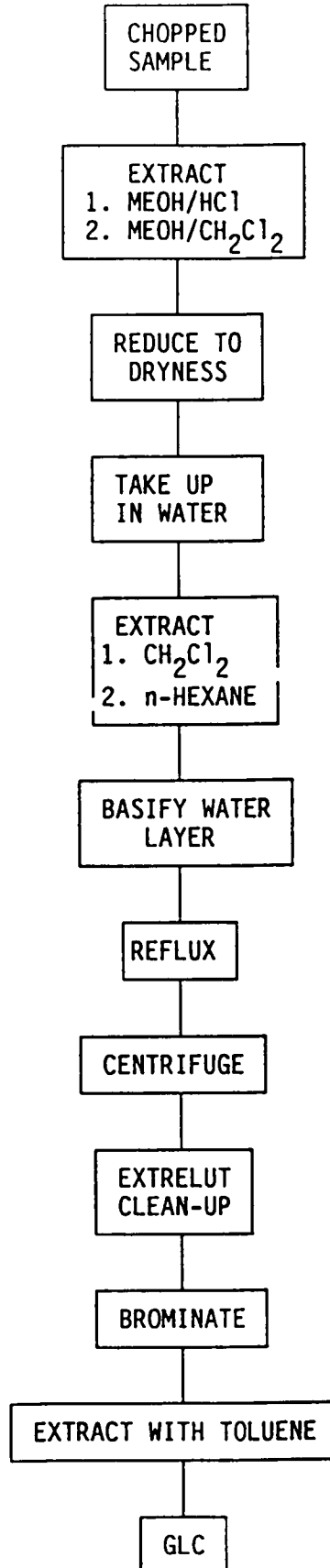


CONTROL SAMPLES

Apparent residues of 0.01 and 0.02 ng/ $\mu$ l



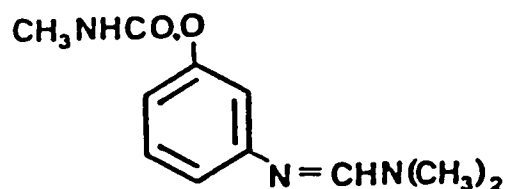
## 8. FLOW DIAGRAM



## 9. DESCRIPTION OF FORMETANATE

Name: 3-dimethylaminomethyleneaminophenyl  
methylcarbamate

Structural formula:



Empirical formula:  $C_{11}H_{15}N_3O_2$

Molecular weight: 221,3

Melting point: 102 - 103 °C (yellowish crystalline solid)

Vapor pressure:  $3 \times 10^{-5}$  Pa (25 °C) as base  
 $1.6 \times 10^{-6}$  Pa (25 °C) as HCl

Thermodynamic

acidity constant:  $pK_a = 8.0$  (25 °C, formetanate hydrochloride)

Solubility:	formetanate		formetanate x HCl	
dichloromethane	350.9 g/l	(20 °C)	0.29 g/l	(20 °C)
ethyl acetate	44.9	(20 °C)	0.015	(20 °C)
water	6.3	(25 °C)	822	(25 °C)
acetone, chloroform	100		< 1	
methanol	> 200		> 200	

Hydrolysis (22 °C):	k		t 0.5 (h)
	pH	(h <sup>-1</sup> )	
	5	$4.63 \times 10^{-4}$	1500
	7	$3.05 \times 10^{-2}$	23
	9	$3.47 \times 10^{-1}$	2

n-octanol/water partition coefficient (25 °C): 6 (base)  
< 0.002 (HCl)