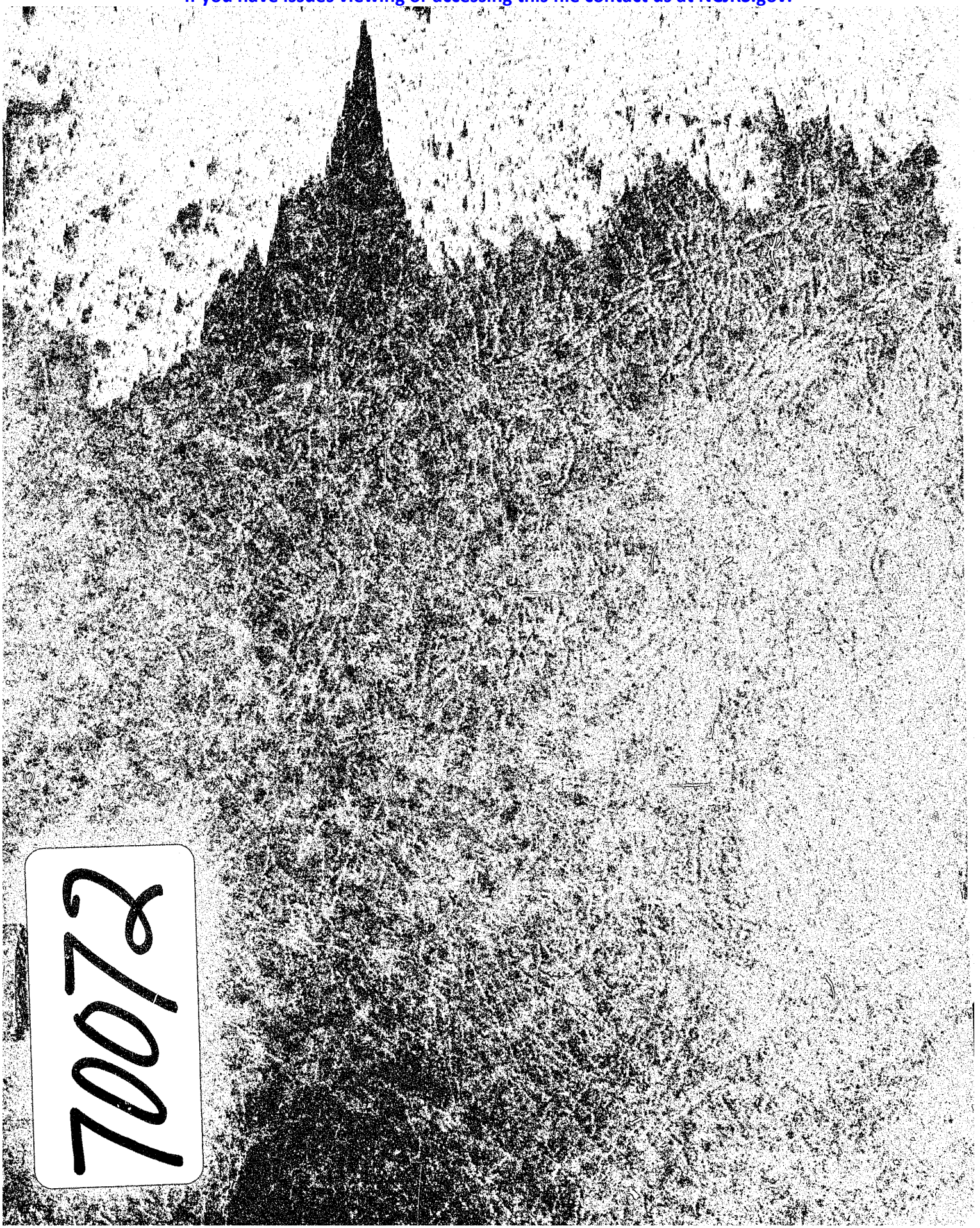

FURTHER STUDIES ON INTERFERING PEAKS IN GAS CHROMATOGRAPHIC EXCLUSION SCREENING OF DIRECT CHLOROFORM EXTRACTS OF BLOOD*

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The present report is a continuation of previous work on the interference by endogenous biochemicals in the gas chromatographic exclusion screening of directly extractable drugs (1).

In the previously reported study, interferences with drugs were examined which are screened for at 200°C and above on a moderately polar (OV-17) column. This included, among others, barbiturates, carbamates, glutethimide, propoxyphene, local anesthetics and meperidine.

Materials and Methods

Gas chromatographic retentions were determined using the following three percent solutions of biogenic compounds: Indole, 2-phenylethylamine, β-hydroxybutyric acid, glycine, cholesterol, cortisol, urea, tyramine and stearic acid.

Gas chromatograph with flame ionization detector and 4 ft x 1/8 inch stainless steel column packed with 3% OV 17 on 80/100 mesh chromosorb G HP (Supelco) were used. The gas chromatographic conditions were as follows.

Sample inlet (injection temperature)	: 190°C
Column oven temperature	: 160°C
Carrier gas - nitrogen	: 40 ml per min., 58 psi inlet pressure

H_2 and air flows were not measure, they were adjusted daily for maximum response to the test mixture components.

Sample injection: 10 μ ℓ Hamilton(R) liquid syringe and Hamilton(R) solid injector.

The method of extraction was utilized as before (1). For the exclusion of more volatile extractable agents, such as methyl

salicylate, phenyl salicylate, terpin hydrate, acetanilid, carbromal and amphetamine, the same column can be used at lower temperature; in the present case, this was 160°C.

Results

With glycine, cholestrol, cortisol, urea, tyramine and stearic acid, no response was obtained with 60 γ at an attenuation of about X 1000. The detection limits of indole, 2-phenylethylamine and β-hydroxybutric acid by gas chromatography is 300 nanogram, 300 nanogram, and 2.5 microgram respectively.

In figure 1 are shown gas chromatograms obtained with the above mentioned volatile agents. Figure 2 shows the gas chromatogram of the three endogenous compounds - indole, 2-phenylethylamine and β-hydroxybutyric acid, β-hydroxybutyric acid giving a barely discernible response when extracted from blood.

Table 1 shows the interferences of endogenous agents with drugs.

Table 2 shows detection limits for endogenous agents (Indole, 2-Phenylethylamine and β-Hydroxybutyric acid) in blood.

Discussion

Whether or not the three agents under study actually interfere, depends also on their extractability in this procedure. In the procedure, a portion of the chloroform extract is evaporated on a solid injector prior to introduction into the gas chromatograph. The results obtained when 1 ml blood containing 0.4 mg% indole, 50 mg% 2-phenylethylamine and 75 mg% β-hydroxybutyric acid was extracted with 0.2 ml chloroform, 2 microlitres of the extract were evaporated on a solid injector and introduced into the gas chromatograph at an attenuation of X 32. Theoretically corresponding

to 4 γ indole, 500 γ phenylethylamine and 750 γ β-hydroxybutyric acid, the actual amounts seen correspond to less than 1% of this.

By this procedure, individually determined limits for indole, 2-phenylethylamine and β-hydroxybutyric acid in blood were found in Table 2. These three endogenous agents, i.e. indole, 2-phenylethylamine and β-hydroxybutyric acid have same or almost coincidence retention distance with some drugs (table 1). So interference is evident. These and sometimes even higher concentrations may reasonably be expected at times in postmortem blood. Thus, these three agents can result in false positives and in falsely elevated values of drugs with the same or similar retention times.

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References

1. Niyogi, S. K. and Rieders, F.

Interfering Peaks in Gas chromatographic Exclusion Screening of Direct Chloroform Extracts of Blood. Acta pharmacol. et. toxicol, (in press).

Table 1

Interferences of endogenous agents with drugs

Endogenous Agent	RD	Interferes with	RD
2-Phenylethylamine	6	Methyl Salicylate	6.5
		Terpin Hydrate	6.5 (smaller of two peaks)
		Phenyl Salicylate	5 (smaller of two peaks)
Indole	13.5	Amphetamine	15.5
β-Hydroxybutyric acid	(13.5) 30	(Amphetamine)	15.5
		Acetanilid	28
		Carbromal (?)	26, 40
		(Amphetamine iso-Thiocyanate)	31

RD - retention distance in mm

Table 2

Detection limits for the endogenous agents in blood

Indole - 0.1 mg/100 ml
2-Phenylethylamine - 40 mg/100 ml
β-Hydroxybutyric acid -40 mg/100 ml

Legend of Figure 1

Drugs	Quantity	Retention Distance (RD) in mm
Methyl salicylate	400 nanogram	6.5
Terpin hydrate	1 microgram	6.5; 10
Acetanilid	400 nanogram	28
Carbromal	1 microgram	26; 40
Amphetamine* (from urine)	10 nanogram	15
Phenyl salicylate	1 microgram	5; 100

All injections were solid except amphetamine

* showed a peak, isothiocyanate, RD - 31 mm

Legend of Figure 2

Extract of the mixture of three endogenous agents from blood

Endogenous Agents	Retention Distance (RD) in mm
2-phenylethylamine	6
Indole	13.5
β-hydroxybutyric acid	13.5, 30

Blood containing 0.4 mg% Indole, 50%mg% phenylethylamine and 75% β-hydroxybutyric acid.

1 ml blood extracted with 0.2 ml $CHCl_3$.

2 µl $ChCl_3$ extract, solid injection.

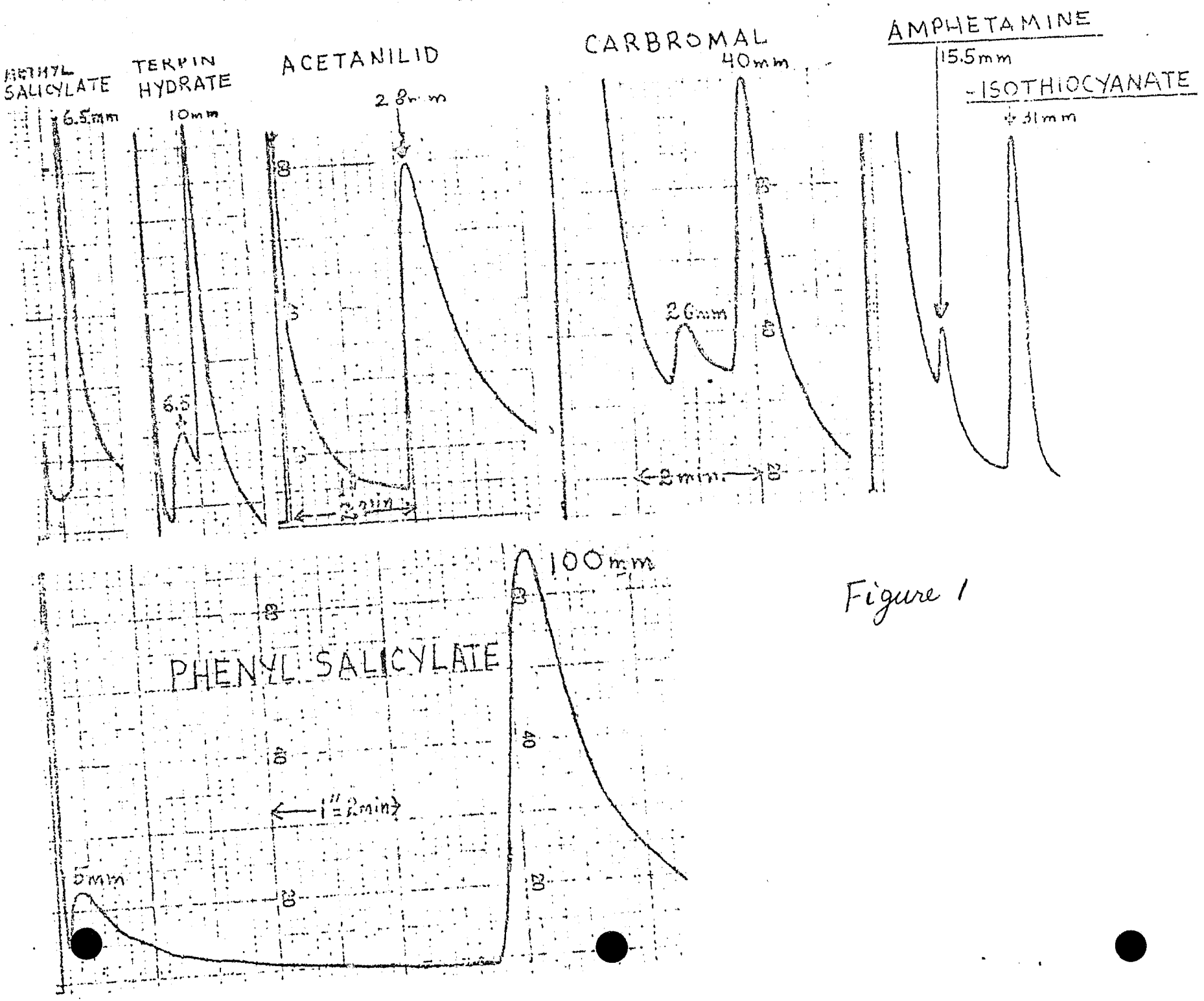
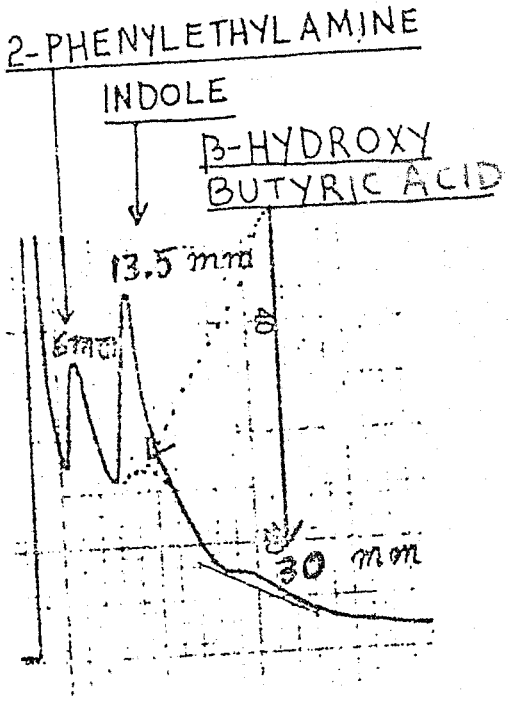

METHYL SALICYLATE
6.5mm
TERPIN HYDRATE
10mm
ACETANILID
28mm
CARBROMAL
40mm
26mm
2min
AMPHETAMINE
15.5mm
-ISOTHIOCYANATE
31mm
PHENYL SALICYLATE
100mm
1"=2min
2min

Figure 1

Figure 2


2-PHENYLETHYLAMINE
INDOLE
B-HYDROXY
BUTYRIC ACID
13.5 mm
6mm
30 mm

END