BIOCHEMISTRY DEPARTMENT

PAGE	METHOD No.	SUBJECT
1 of 25	AG-335	DETERMINATION OF METHIDATHION AND SOME OF ITS METABOLITES IN MILK BY GAS CHROMATOGRAPHY
EDITION 1/9/79	Replaces AG-98	
SUBMITTED BY: R. A. Kahrs		

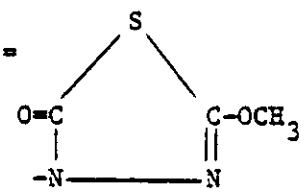
APPROVED BY: R.T. Murphy

1.0 SCOPE

This method describes procedures for the analysis of milk for the following compounds:

Methidathion	$R - CH_2-S-\overset{S}{\overset{\|\|}{P}}(O\ CH_3)_2$
GS-13007	$R - CH_2-S-\overset{O}{\overset{\|\|}{P}}(O\ CH_3)_2$
GS-28368	$R - CH_2-S-CH_3$ (sulfide)
GS-28369	$R - CH_2-SO_2-CH_3$ (sulfone)
GS-28370	$R - CH_2-SO-CH_3$ (sulfoxide)

R =



2.0 PRINCIPLE OF THE METHOD

Acetone is added to the milk to extract methidathion and its degradation products. The precipitated milk solids are filtered off and these are extracted again with toluene to complete the extraction.

The acetone-water and the toluene extracts are combined in a separatory funnel and shaken. Two phases are formed. The toluene phase (upper phase) contains the methidathion, GS-13007, sulfide, sulfone and most of the sulfoxide. Some of the sulfoxide remains in the aqueous phase. The toluene phase is evaporated to dryness and taken up in n-hexane. This solution is extracted with acetonitrile to recover the compounds sought while the milk fat and other interfering materials remain in the hexane.

BIOCHEMISTRY DEPARTMENT

PAGE	METHOD No.	SUBJECT
2 of 25	AG-335	DETERMINATION OF METHIDATHION AND SOME OF ITS METABOLITES IN MILK BY GAS CHROMATOGRAPHY
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The acetonitrile is evaporated to dryness and then the sample is taken up in toluene for gas chromatography to determine parent compound, oxygen analogue, sulfide, sulfone and part of the sulfoxide. The aqueous phase is extracted with methylene chloride to recover the remainder of the sulfoxide. This is evaporated to dryness and taken up in toluene for gas chromatography to determine the remainder of the sulfoxide.

3.0 REAGENTS

Acetone:	Nanograde
Toluene:	Nanograde
Hexane:	Nanograde
Acetonitrile:	Nanograde
Dichloromethane:	Spectroquality
Sodium Sulfate:	Reagent Grade
Methidathion:	Analytical Standard
GS-13007:	Analytical Standard
GS-28368:	Analytical Standard
GS-28369:	Analytical Standard
GS-28370:	Analytical Standard

4.0 EQUIPMENT

Jars:	16 oz flint, wide mouth
Buchner Funnel:	6 cm
Filter paper:	Whatman No. 1
Vacuum adapter:	For erlenmeyer flasks: similar to catalog No. K-20500 adapter, 24/40, Kontes Technical Glassware catalog TG-20, Kontes Glass Co. Vineland, NJ.
Filter vac:	Rubber seal between Buchner funnel and vacuum adapter
Separatory funnels:	125, 250 and 500 ml
Air manifold:	N-EVAP by Organomation or equivalent
Gas Chromatograph:	Microtek MT-220 equipped with a Flame Photometric Detector

BIOCHEMISTRY DEPARTMENT

GREENSBORO, N.C.

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3 of 25	AG-335	DETERMINATION OF METHIDATHION AND SOME OF ITS METABOLITES IN MILK BY GAS CHROMATOGRAPHY
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5.0 PROCEDURE

5.1 PREPARATION OF SAMPLE

Milk samples to be analyzed should be thawed slowly at a temperature between 20-40°C to prevent excessive coagulation and souring. The entire sample should be thoroughly mixed by a vigorous shaking to insure uniformity in sampling.

5.2 EXTRACTION

A 50 gram sample (50 ml) of milk is transferred to a 16 oz wide mouth jar equipped with a plastic cap. A polyethylene liner is placed under the cap to avoid dissolving extraneous material from the cap lid, and to avoid loss of solvent. Two hundred ml of acetone are added to the jar and the mixture is shaken for 1/2 hour. The acetone-aqueous extract is filtered through two pieces of filter paper in a Buchner funnel under suction. The acetone-aqueous extract is then transferred to a 500 ml separatory funnel. The resulting filter pad (not paper) is reshaken with 10 ml of distilled water and 50 ml of acetone for 1/2 hour.

The acetone-water extract is again filtered and combined in the separatory funnel. The resulting filter pad is shaken a final time with 100 ml of toluene for 1/2 hour. The toluene extract is filtered similarly to the acetone extract. The pad is washed with a 75 ml portion of toluene. The toluene and acetone-aqueous extracts are combined and vigorously shaken for three minutes, and the phases allowed to separate.

5.3 PARTITION CLEAN-UP

5.3.1 Toluene-Acetone Phase:

The toluene-acetone phase is evaporated to an oily residue which is then dissolved in 50 ml of hexane, and transferred to a 125 ml separatory funnel. A 25 ml portion of acetonitrile is used to rinse the flask and is added to the separatory funnel, and the entire mixture is shaken for one minute. The acetonitrile partitioning is repeated twice more with fresh 10 ml portions of acetonitrile. The acetonitrile fractions are combined in a 125 ml separatory funnel and washed with a fresh 50 ml portion of hexane by shaking for one minute. The hexane wash is discarded.

5.3.2 Aqueous Phase:

The aqueous phase is transferred to a 250 ml separatory funnel and partitioned with dichloromethane. Three successive two minute extractions using 50 ml portions of dichloromethane are dried through a 1 inch pad of sodium sulfate. The pad is then rinsed with a 25 ml portion of dichloromethane. This partitioning procedure cannot be too severe or an emulsion will form. The use of an end over end movement in rotating the funnel will minimize the chance of an emulsion.

5.4 DETERMINATION OF METHIDATHION, GS-13007, GS-28368, GS-28369, GS-28370

5.4.1 Designation of Sample Fractions:

In this part of the procedure, there are now two fractions of sample extract to analyze. The acetonitrile fraction will contain methidathion, GS-13007, GS-28368, GS-28369, and 50% of the GS-28370 present in the sample, and therefore, a multiple analysis will be performed. The dichloromethane fraction contains only the remaining 50% of the GS-28370 present in the sample, requiring only a single analysis.

5.4.2 Determination Procedures for Both Fractions:

The acetonitrile or dichloromethane is evaporated to 2 ml using a flash evaporator. The residue is transferred quantitatively with dichloromethane to a 2 dram vial, and taken to dryness. The residue is dissolved in an appropriate amount of Nanograde toluene. The final determination is done by means of a gas chromatograph equipped with a Flame Photometric Detector, with a sulfur specific filter (394 mμ). The conditions used for gas chromatography are given in Table I.

The gas chromatographic method is standardized by injecting known amounts of the various compounds. Stock solution of these are made by dissolving 100 mg of compound in 100 ml of acetone. Dilutions are made from this using toluene to obtain solutions ranging from 1 to 3 ng per μl in concentration. A constant volume of 5 μl equivalent to 5 to 15 ng of compound are used for standardization. Typical chromatograms are shown in Figure 1 and 1-A. The Flame Photometric Detection system, when employed in the sulfur specific mode, has been shown to be volume dependent. In order to obtain uniformity in the response of the detector, only equal volumes of standard and sample are injected (i.e., every injection in the sulfur specific mode is done with 5 μl injections).

A standard graph is prepared from the chromatograms of standards. Peak areas are calculated by multiplying the peak height by the base at half-height. These are plotted against the weight of compound injected. Peak areas of unknown samples are compared directly with such a graph to obtain the amount of compound present in the sample.

6.0 RECOVERY STUDIES

The recovery of methidathion and metabolites added before extraction to raw cow's milk are shown in Table II, and typical chromatograms shown in Figure 2, 3, 4, 5, 6. This table shows the sample code, the interval in days before and after the start of dosing, the individual compounds that were added, the amount of compound added (in terms of ppm), grams injected, the ppm of each compound detected in the acetonitrile and dichloromethane, the combined ppm found and the percent recovered.

The recoveries from cow's milk are shown at the level of 0.01 ppm of added compound. The pre-treatment samples represent an extraction by blending. No decrease in the percent recovery is obtained when using a shaking extraction. From this data, a lower limit of detectability for raw cow's milk was set at 0.005 ppm for three metabolites and at 0.01 ppm for methidathion and GS-13007 because of their longer elution time. The recoveries of individual compounds are (a) methidathion 88% to 120%, (b) GS-28368 60% to 70%, (c) GS-28369 135% to 160%, (d) GS-28370 130% to 190% of theoretical (refer to section 8.0 Discussion of Method).

7.0 ANALYSIS OF TREATED SAMPLES

For the complete description of sample coding, see the Dawson Report[1]. The TM refers to milk from the cow which was dosed, the CM to milk from the control cow. Minus (-) refers to pre-treatment, and plus (+) to post-treatment. A, refers to AM samples and P, to PM samples, the cows being milked at 6 am and 6 pm. There was one exception, the 18th day of post-treatment (+18). On this day, the treated cow was milked every 6 hours. These milk samples were designated as follows:

TM + 18 F (12 N)	12:00 PM
TM + 18 F (6 P)	6:00 PM
TM + 18 F (12 M)	12:00 AM
TM + 18 F (6 A)	6:00 AM

The "T" cow was dosed every morning at 8:00 AM.

Raw data obtained in the analysis of treated cow's milk are shown in Table III. This table provides data showing sample code, dosage rate of compound fed to cows in capsule form, the number of days after initial dosing, the number of hours after daily dosage when sample was taken, the weight of sample injected, the individual compounds being analyzed for, the amount (ng) of compound detected in acetonitrile and dichloromethane fraction, the ppm of each compound detected, and the combined ppm. Typical chromatograms are shown in Figure 3.

8.0 DISCUSSION OF METHOD

In setting the lower limit of detectability of 0.005 ppm for the three metabolites, various factors of compound stability were taken into account. When GS-13005 is fed to rats, three metabolites were found by Esser et al.[2] in urine. They were the sulfide (GS-28368), the sulfoxide (GS-28370), and the sulfone (GS-28369). The degradation of methidathion by transmethylation first produces the sulfide, which being unstable, readily oxidizes to the sulfoxide and subsequently, to the sulfone. Because of the ease of oxidation in milk, recoveries for the sulfoxide and sulfone should be somewhat higher when the milk is fortifed with all three standards. As shown in Table II, the recoveries for these compounds are more than 100%. Using the same extraction procedure, but not in the presence of milk, the expected recoveries of 90% to 100% are obtained.

9.0 REFERENCES

1. Murchison, T. E., Dawson Research Corp., "Metabolism Study of C^{14} Methidathion in a Cow", February 18, 1969.

2. Esser, H. O., Muche, W., and Alt, K. O., Helvetica Chemica Acta, 51, Fasc. 3, 513 (1968).

TABLE I GAS CHROMATOGRAPHIC CONDITIONS

Instrument:	Microtek MT 220 equipped with Flame Photometric Detector. Sulfur specific filter (394 mu).
Column:	3% SE 30 + 0.3% Epon 1001 on Gas Chrom "Q" (60/80 mesh) packed in Pyrex tubing (2 ft x 1/4 in).
Injector Temperature:	225°C
Column Temperature:	120°C & 170°C
Transfer Temperature:	250°C
Detector Temperature:	160°C
N_2 Carrier:	100 ml/min
O_2 Flow:	30 ml/min
Air Flow:	70 ml/min
H_2 Flow:	150 ml/min
Attenuation:	Input: 10^3, Output: 8
Minimum Detection Limit:	Methidathion------5 ng GS-28368----------5 ng GS-28369----------5 ng GS-28370----------5 ng
Volume Injected:	5 µl
Retention Times:	Methidathion @ 170°C-----2.5 min. ~~GS-28368 @ 120°C---------1.1 min.~~ GS-28369 @ 170°C---------1.1 min. GS-28370 @ 170°C---------1.7 min.
Chart Speed:	1/2 inch per min.
Response:	Peak areas in inches

NOTE: A 10% DC-200 on Gas Chrom "Q" gas chromatographic column may also be used for these determinations.

TABLE II RECOVERIES OF METHIDATHION AND ITS DEGRADATION PRODUCTS ADDED TO MILK

Sample Code	Experiment Day	Compound Determ.	PPM Added	Wt. Inj. (g) CH_3CN	Wt. Inj. (g) CH_2Cl_2	PPM Found CH_3CN	PPM Found CH_2Cl_2	Total PPM	% Recovery
CM-3	- 3	Methidathion	0.00	1.00	2.50	<0.01	<0.01	<0.01	--
Figs. 2,3,4		GS-28368	0.00	0.99	--	<0.005	--	<0.005	--
		GS-28369	0.00	1.00	2.50	<0.005	<0.005	<0.005	--
		GS-28370	0.00	1.00	2.50	<0.005	<0.005	<0.005	--
CM-3	- 3	Methidathion	0.01	1.00	2.50	0.009	<0.01	0.009	90
plus		CS-28368	0.01	0.78	--	0.007	--	0.007	70
0.01 ppm*		GS-28369	0.01	1.00	2.50	0.013	<0.005	0.013	130
Figs. 2,3,4		GS-28370	0.01	1.00	2.50	0.010	0.005	0.015	150
CM+17A	+17	Methidathion	0.00	1.20	1.20	<0.01	<0.01	<0.01	--
Figs. 4,5,6		GS-28368	0.00	1.15	1.20	<0.005	<0.005	<0.005	--
		GS-28369	0.00	0.78	1.20	0.006	<0.005	0.006	--
		GS-28370	0.00	1.20	1.20	<0.005	<0.005	<0.005	--
CM+17A	+17	Methidathion	0.01	0.60	1.20	0.012	<0.01	0.012	120
plus		GS-28368	0.01	1.14	1.20	0.006	<0.005	0.006	60
0.01 ppm*		GS-28369	0.01	0.60	1.20	0.016	<0.005	0.016**	160
Figs. 4,5,6		GS-28370	0.01	0.60	1.20	0.010	0.006	0.016	160

* 0.01 ppm of each of the 4 compounds was added

** When corrected for the check this value becomes 0.010 ppm (100% recovery).

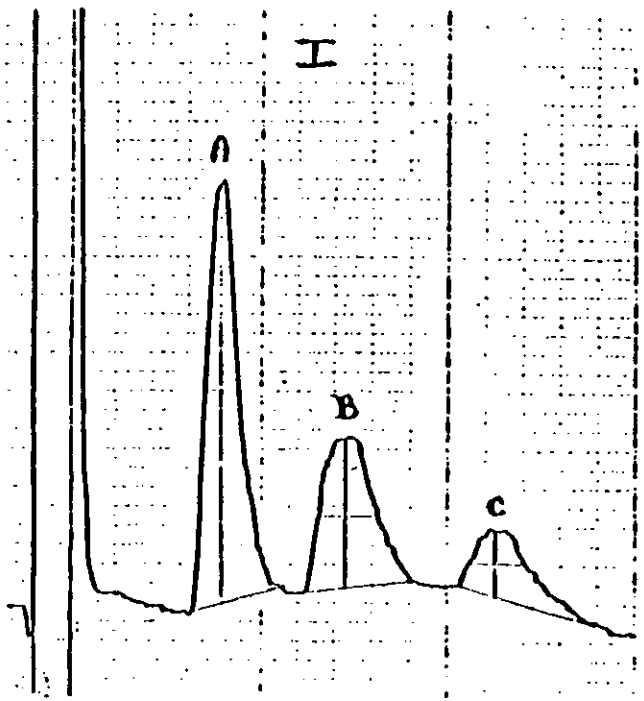
TABLE III RAW DATA FROM DETERMINATION OF METHIDATHION AND ITS DEGRADATION PRODUCTS IN MILK

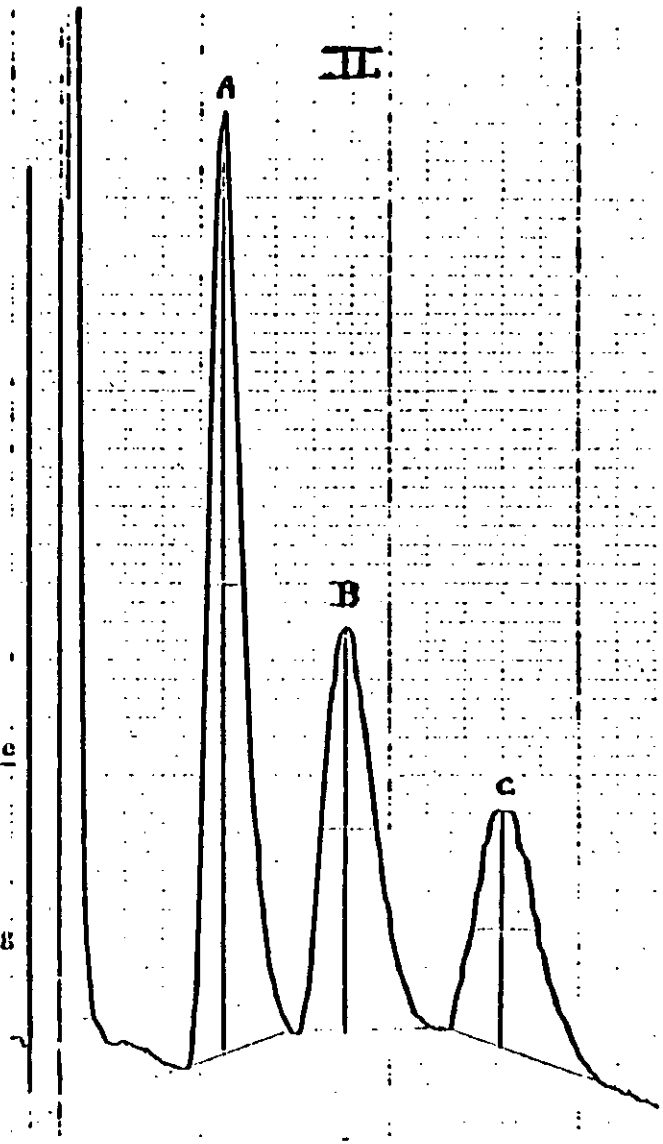
Sample* Code	Experiment Day	Dosage mg/kg/day	Hours After Dosage	Wt. Inj. (g) CH_3CN	Wt. Inj. (g) CH_2Cl_2	Compound Determ.	Ng Found CH_3CN	Ng Found CH_2Cl_2	PPM Found CH_3CN	PPM Found CH_2Cl_2	Total PPM
TM-3	- 3D	1	--	1.0	2.5	Methidathion	< 5	< 5	<0.01	<0.01	<0.01
				1.0	-	GS-28368	< 5	--	<0.005	--	<0.005
				1.0	2.5	GS-28369	< 5	< 5	<0.005	<0.005	<0.005
				1.0	2.5	GS-28370	< 5	< 5	<0.005	<0.005	<0.005
TM+17P	+17D	1	12	0.6	1.2	Methidathion	< 5	< 5	<0.01	<0.01	<0.01
Figs. 7,8				1.1	0.8	GS-28368	< 5	< 5	<0.005	<0.005	<0.005
				0.3	1.2	GS-28369	6.9	5	0.023	<0.005	0.023
				0.3	0.8	GS-28370	10.3	12.3	0.035	0.016	0.051
TM+17A	+17D	1	24	1.2	1.2	Methidathion	< 5	< 5	<0.01	<0.01	<0.01
Figs. 7,8				1.1	1.2	GS-28368	< 5	< 5	<0.005	<0.005	<0.005
				0.8	1.2	GS-28369	5.7	< 5	0.007	<0.005	0.007
				1.2	1.2	GS-28370	< 5	5.3	<0.005	<0.005(t)	<0.005(t)
TM+18F	+18D	1	18	0.35	-	Methidathion	< 5	--	<0.01	--	<0.01
(12M)				1.2	-	GS-28368	< 5	--	<0.005	--	<0.005
				0.35	-	GS-28369	3.9	--	0.011	--	0.011
				0.35	-	GS-28370	5.8	--	0.017	--	0.017
TM+18F	+18D	1	12	0.30	-	Methidathion	< 5	--	<0.01	--	<0.01
(6P)				1.2	-	GS-28368	< 5	--	<0.005	--	<0.005
				0.30	-	GS-28369	4.7	--	0.016	--	0.016
				0.30	-	GS-28370	4.9	--	0.016	--	0.016
TM+18F	+18	1	6	0.35	-	Methidathion	< 5	--	<0.01	--	<0.01
(12N)				1.2	-	GS-28368	< 5	--	<0.005	--	<0.005
				0.35	-	GS-28369	9.8	--	0.028	--	0.028
				0.35	-	GS-28370	9.8	--	0.028	--	0.028

TABLE III RAW DATA FROM DETERMINATION OF METHIDATHION AND ITS DEGRADATION PRODUCTS IN MILK (cont'd)

Sample* Code	Experiment Day	Dosage mg/kg/day	Hours After Dosage	Wt. Inj. (g) CH_3CN	Wt. Inj. (g) CH_2Cl_2	Compound Determ.	Ng Found CH_3CN	Ng Found CH_2Cl_2	PPM Found CH_3CN	PPM Found CH_2Cl_2	Total PPM
TM+18F	+18	1	24	0.53	--	Methidathion	< 5	--	<0.01	--	<0.01
(6A)				1.1	--	GS-28368	< 5	--	<0.005	--	<0.005
				0.53	--	GS-28369	4.9	--	0.009	--	0.009
				0.57	--	GS-28370	< 5	--	<0.005	--	<0.005
TM+25P	+25	1	12	0.96	1.5	Methidathion	< 5	<5	<0.01	<0.01	<0.01
Fig. 9				1.2	0.2	GS-28368	< 5	<5	<0.005	<0.005	<0.005
				0.32	1.5	GS-28369	6.7	<5	0.021	<0.005	0.021
				0.32	0.9	GS-28370	8.6	9.1	0.027	0.010	0.037
TM+28P	+28	2	12	1.2	--	Methidathion	< 5	--	<0.01	--	<0.01
Figs. 10,11				0.56	--	GS-28368	4.6	--	0.008	--	0.008
				0.19	--	GS-28369	11.4	--	0.061	--	0.061
				0.19	--	GS-28370	11.3	--	0.061	--	0.061
TM+28A	+28	2	24	1.1	--	Methidathion	< 5	--	<0.01	--	<0.01
Fig. 10				0.35	--	GS-28368	4.3	--	0.012	--	0.012
				0.22	--	GS-28369	4.7	--	0.022	--	0.022
				0.22	--	GS-28370	5.6	--	0.026	--	0.026
TM+32P	+32	2	96	0.60	1.25	Methidathion	< 5	<5	<0.01	<0.01	<0.005
Fig. 12				1.25	1.25	GS-28368	< 5	<5	<0.005	<0.005	<0.005
				0.60	1.25	GS-28369	2.7	<5	<0.005	<0.005	<0.005
				0.60	1.25	GS-28370	< 5	5.3	<0.005	<0.005	<0.005

* See Section 7.0 for Sample Code explanation.





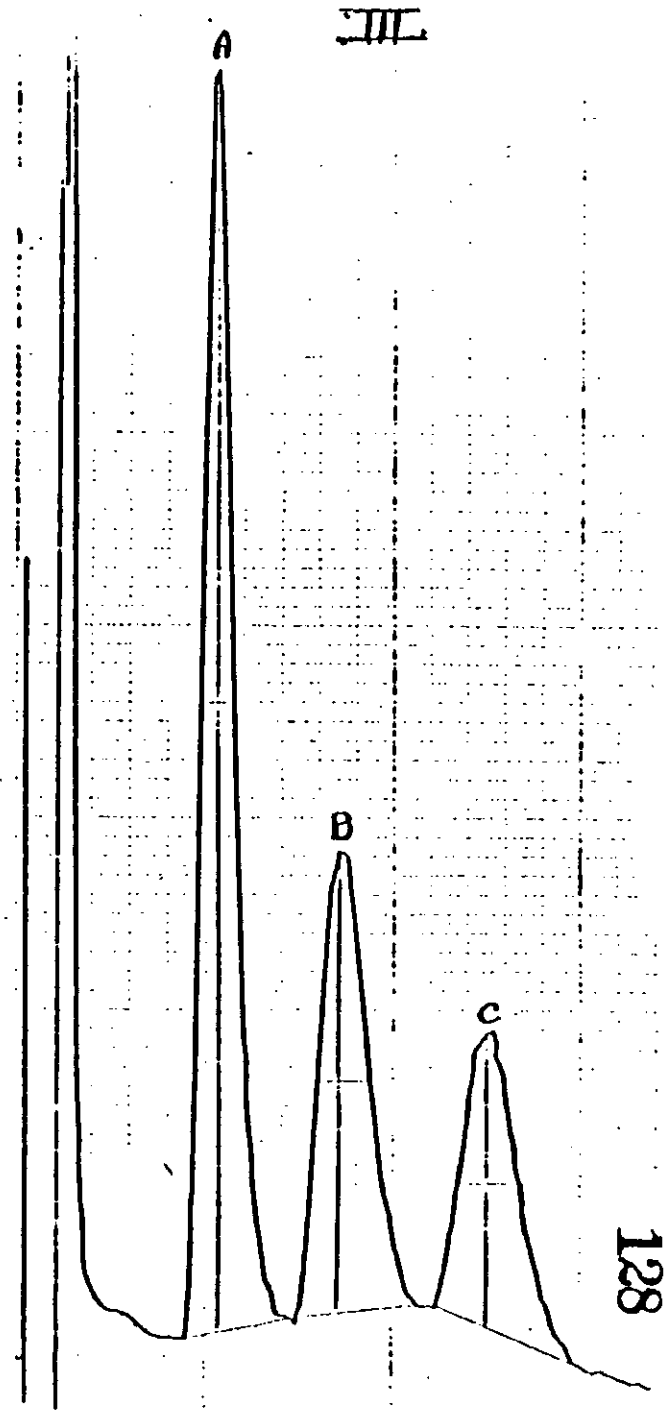


Fig. 1. Standardization of GLC Response

A. GS-28369
B. GS-28370
C. Methidathion
Column Temperature: 170°C
I 7.5 ng II 10 ng III 12.5ng

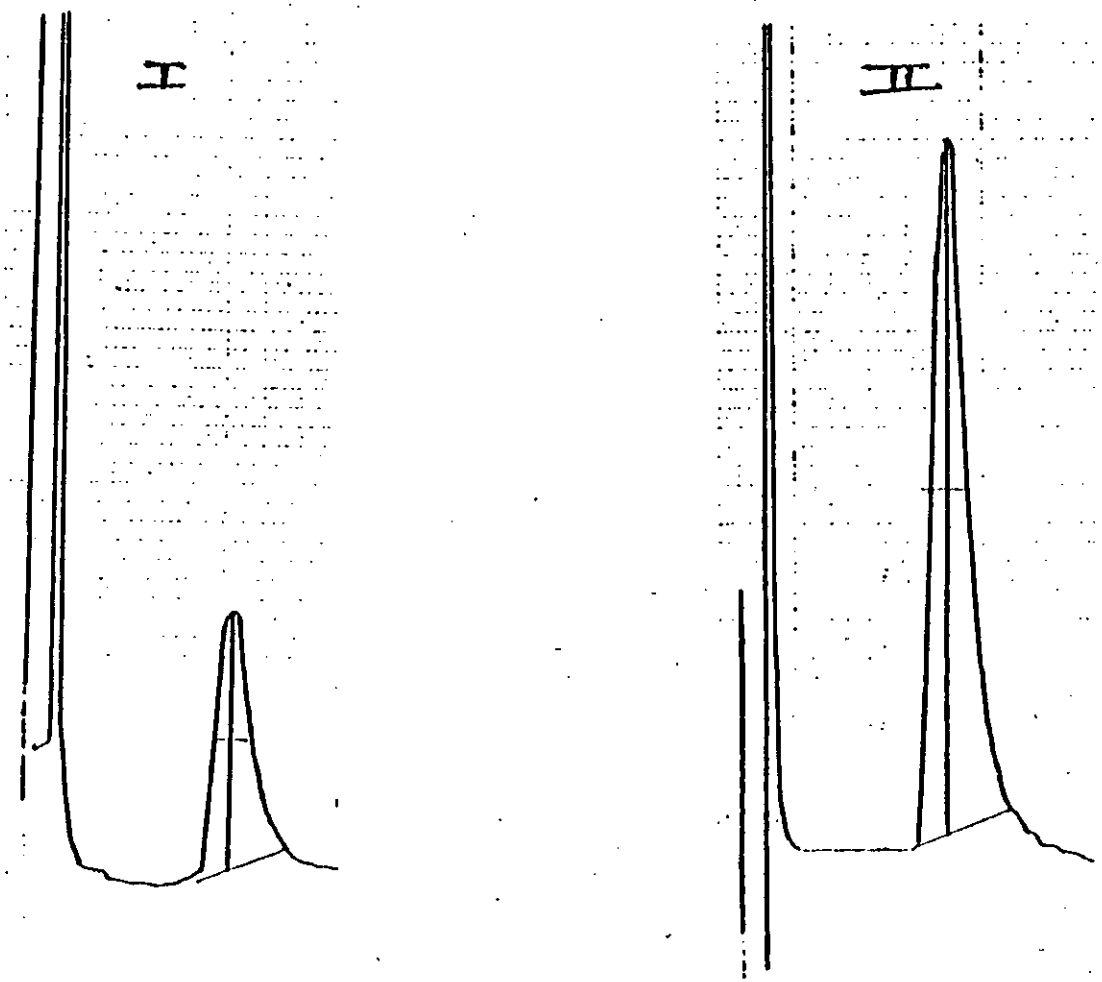


Fig. 1-A Standardization of GLC Response to GS-28368 (sulfide).

I 5 ng II 7.5 ng Column Temperature: 120°C

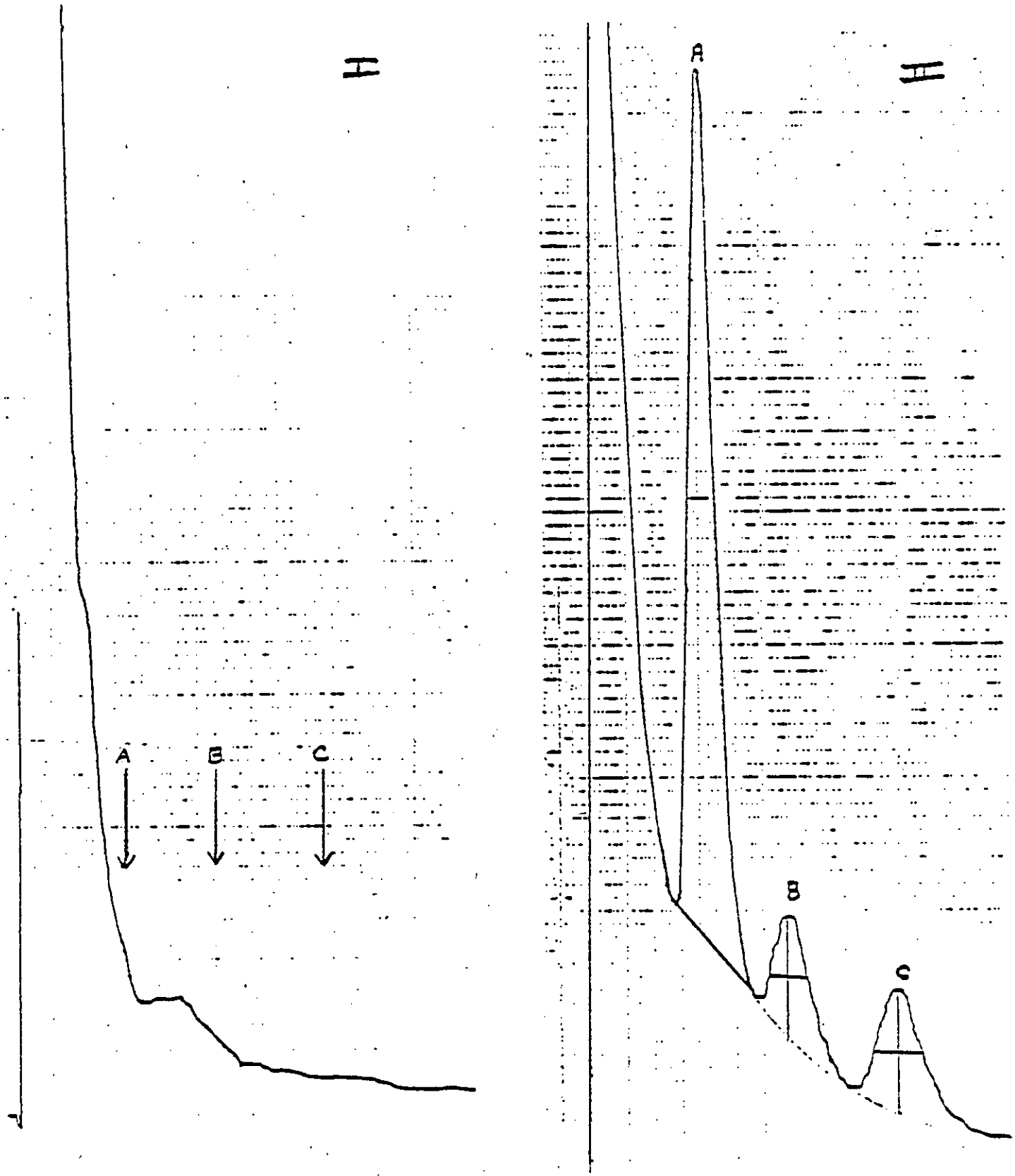


Fig. 2. Gas Chromatograms of Recoveries of Added Compounds (Acetonitrile Fraction).
A. GS-28369 B. GS-28370 C. Methidathion
I. Check Milk CM-3
II. Check Milk CM-3 plus 0.01 ppm of each compound.

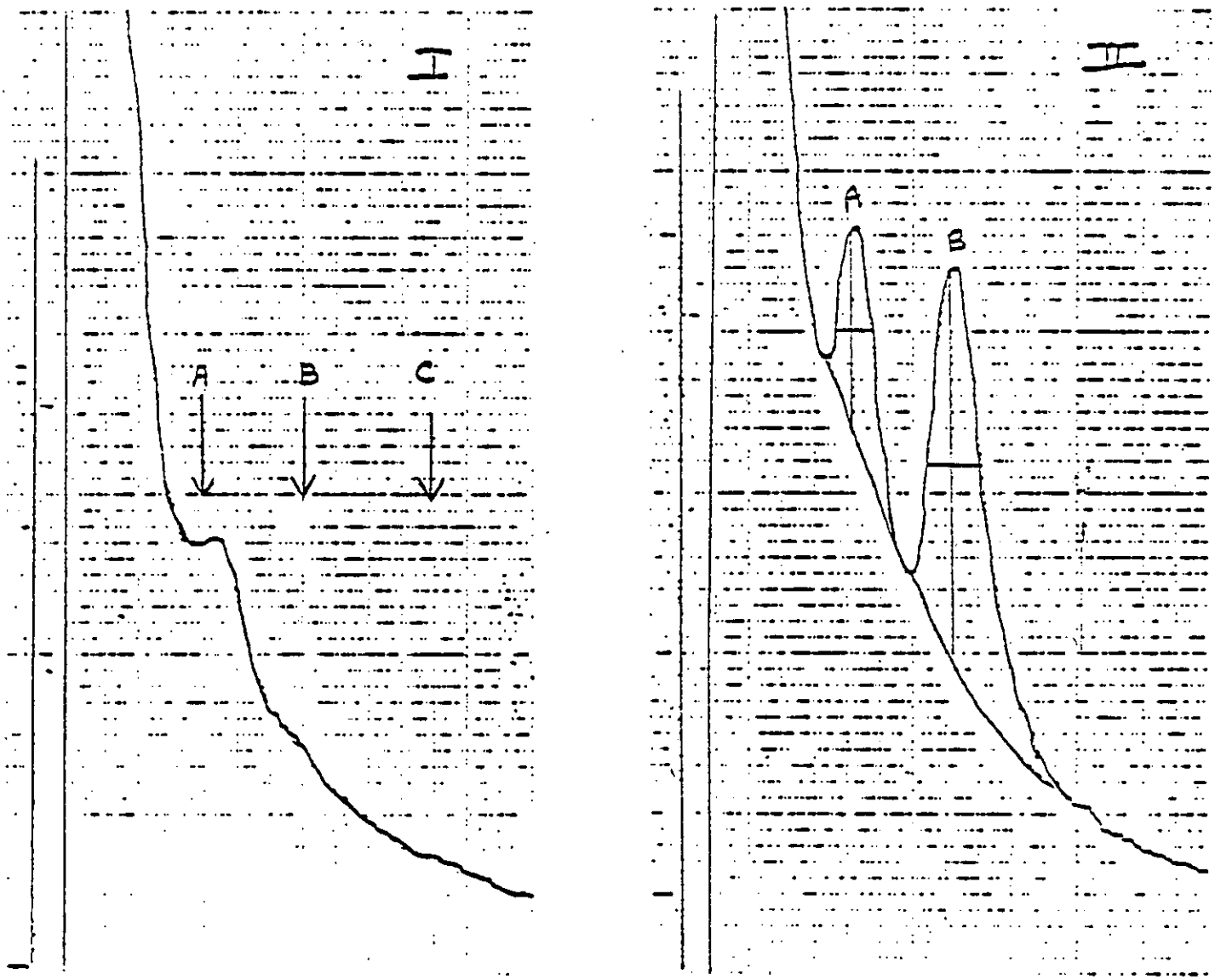


Fig. 3 Gas Chromatograms of Recoveries of Compounds Added to Milk (Methylene chloride fraction).

A. GS-28369 B. GS-28370 C. Methidathion

I. Check milk CM-3
II. Check milk CM-3 plus 0.01 ppm of each compound.

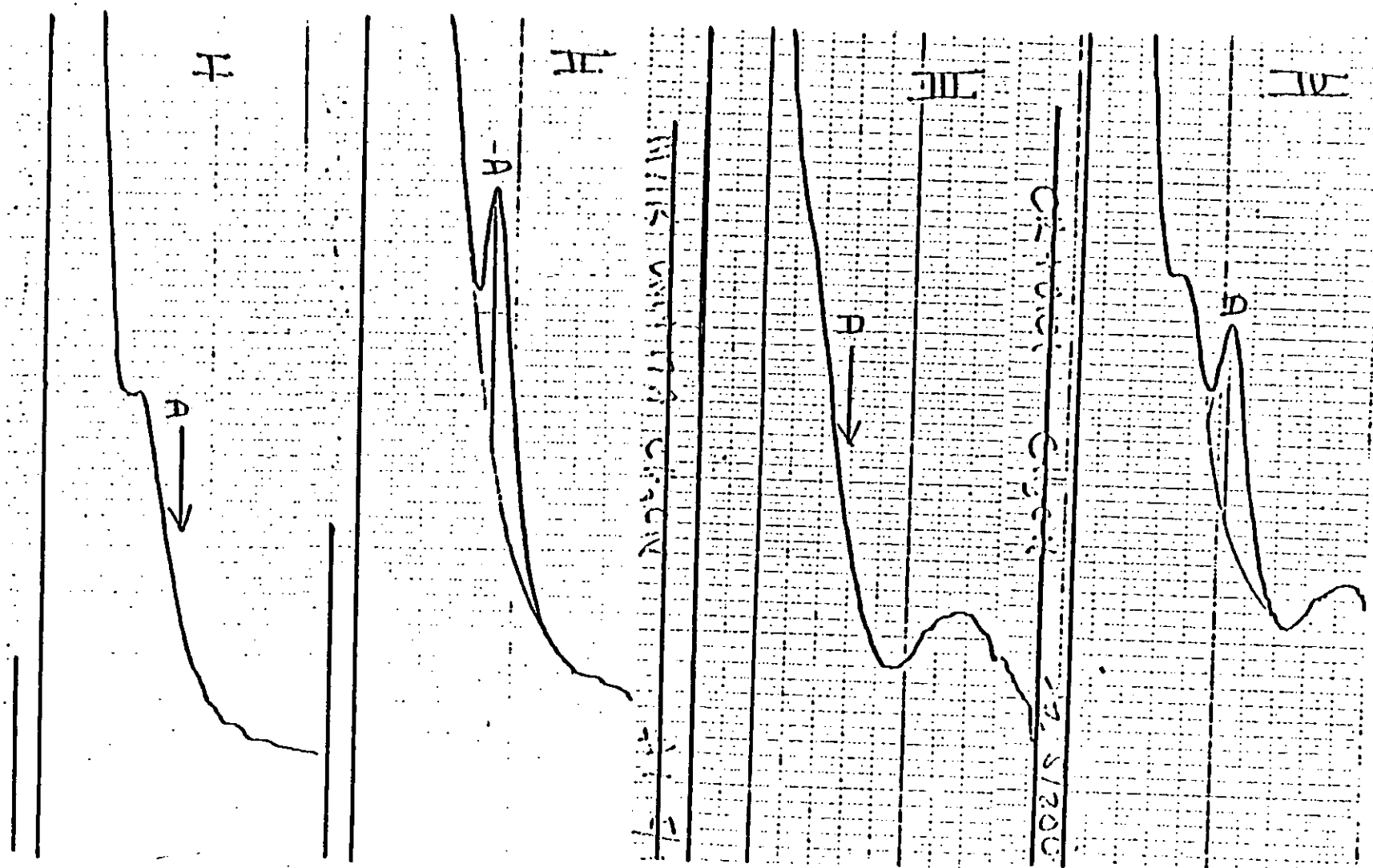


Fig. 4 Gas Chromatograms of Recoveries of Compounds Added to Milk. (Acetonitrile Fraction) (120°C)

I Check Milk CM-3
II Check Milk CM-3 plus 0.01 ppm of GS-28368 (D)
III Check milk CM+17A
IV Check milk CM+17 plus 0.01 ppm of GS-28368 (D)

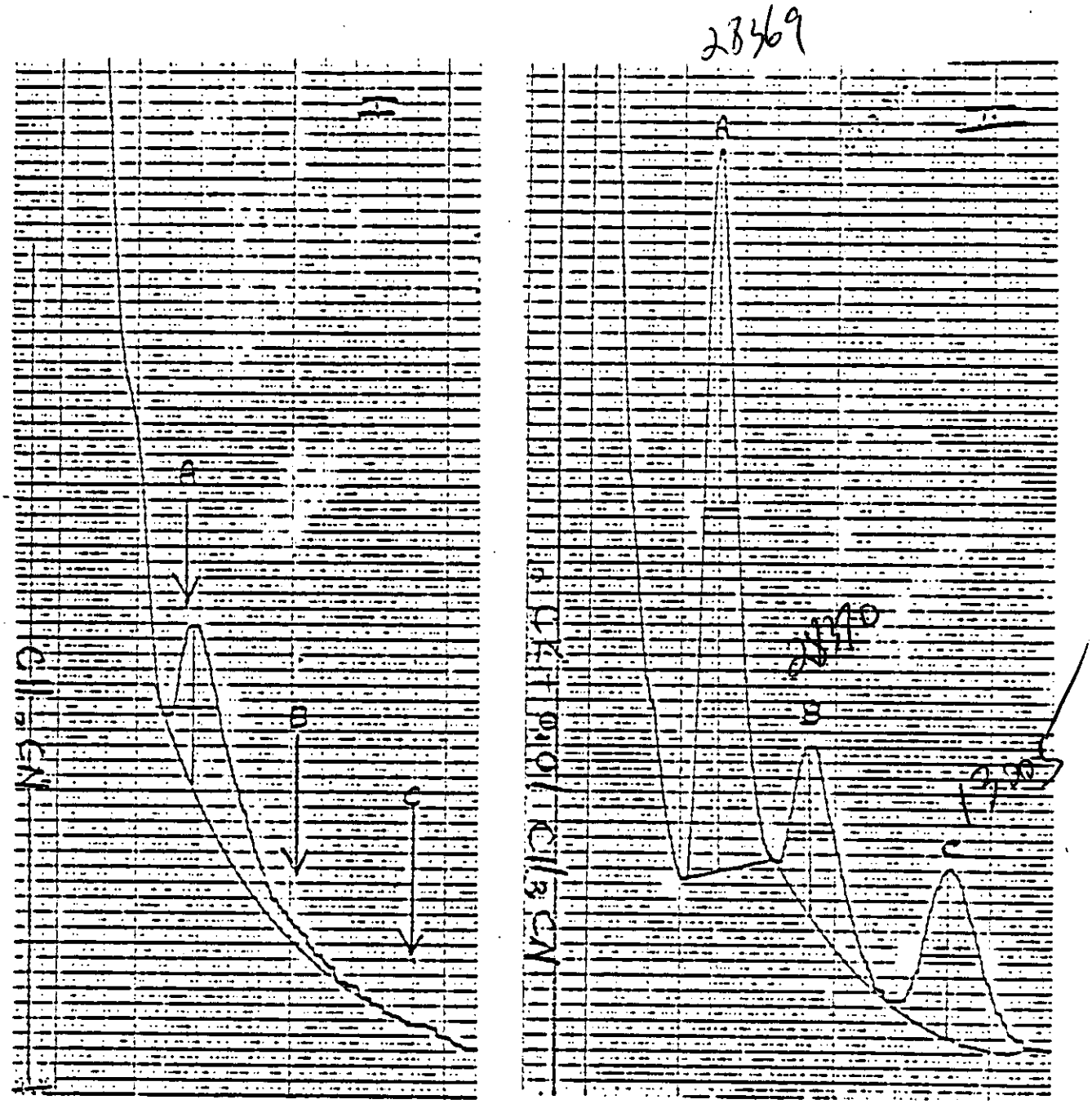


Fig. 5 Gas Chromatograms of Recoveries of Compounds Added to Milk (Acetonitrile Fraction).

A. GS-28369 B. GS-28370 C. Methidathion

I Check milk CM + 17A
II Check milk plus 0.01 ppm each of the above compounds.

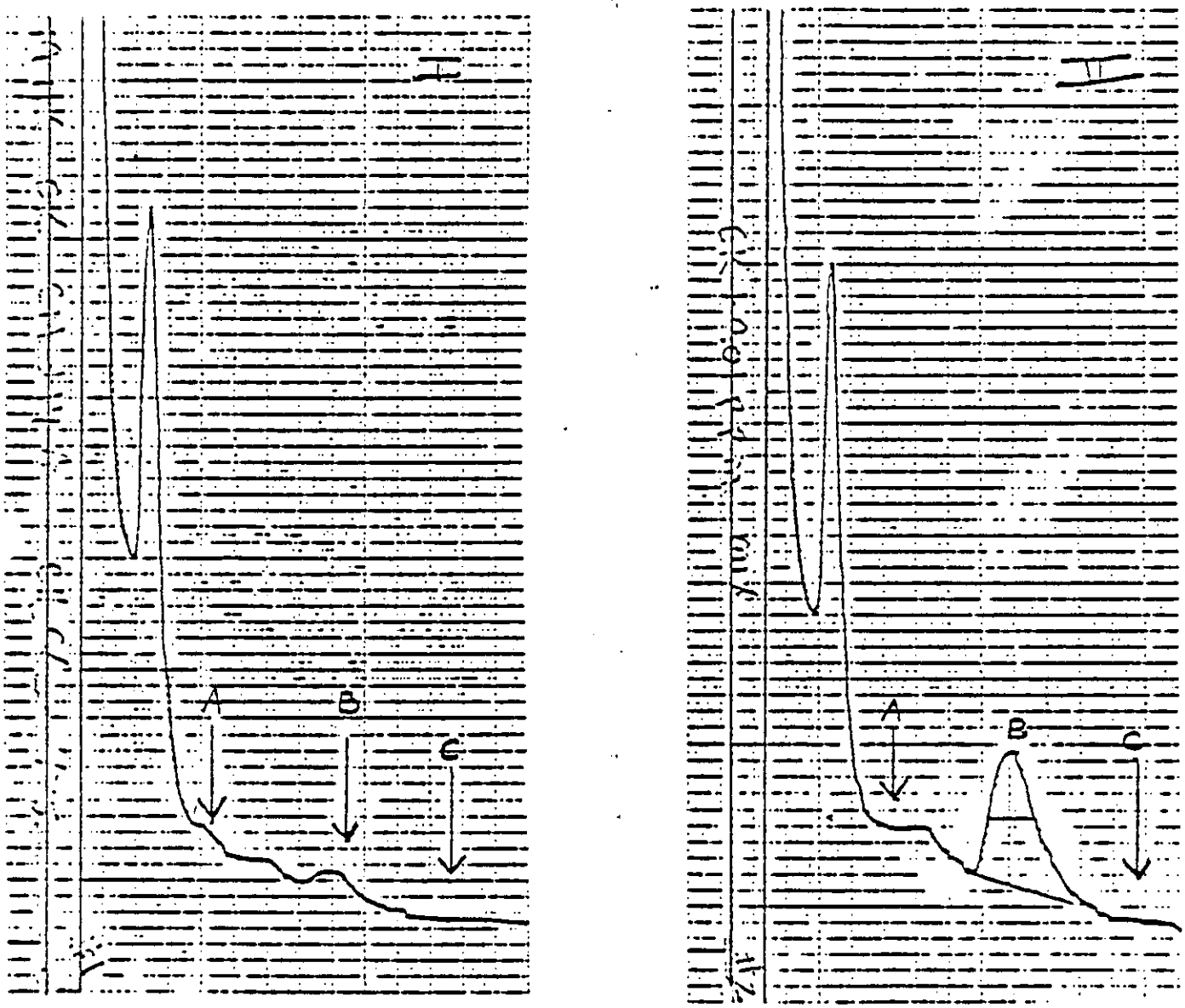


Fig. 6 Gas Chromatograms of Compounds Added to Milk (Methylene Chloride fraction).

I. Check Milk CM + 17A
II. Check Milk plus 0.01 ppm of GS-28370.

A. GS-28369 B. GS-28370 C. Methidathion

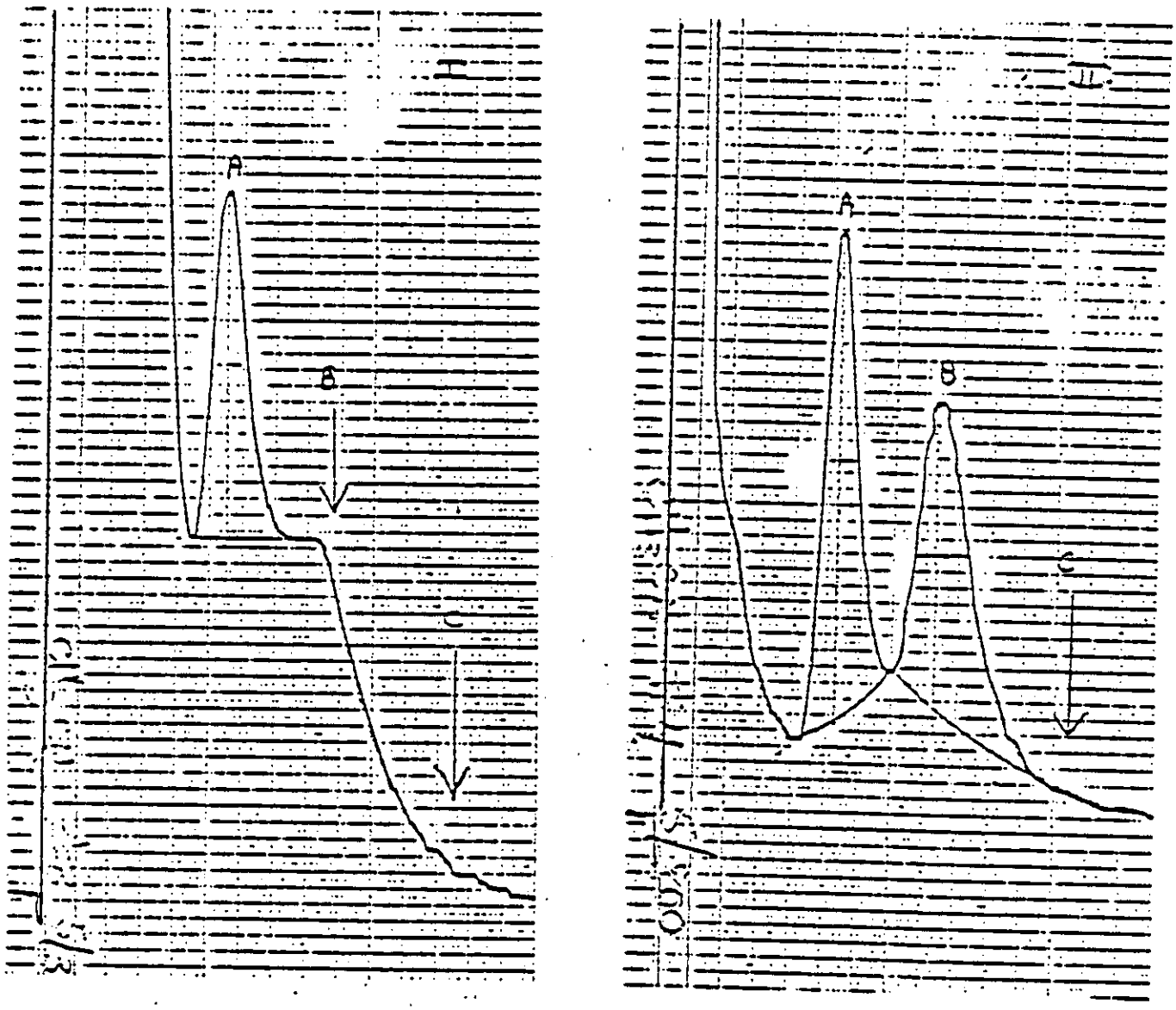


Fig. 7 Gas Chromatograms from Analysis of Treated Milk Samples. (Acetonitrile Fraction).

I. Sample Code TM + 17 A
II. Sample Code TM + 17 P

A. GS-28369 B. GS-28370 C. Methidathion

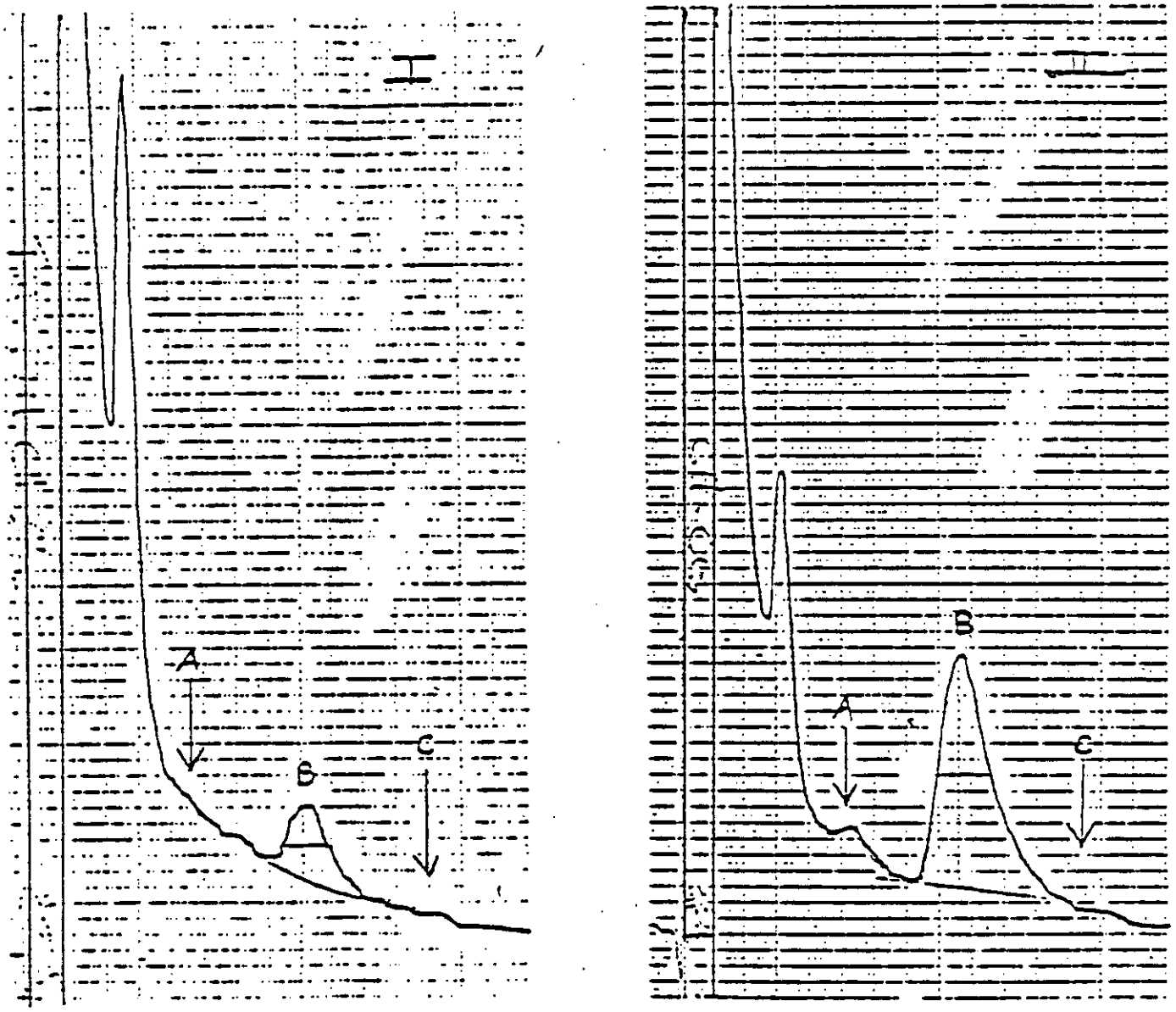


Fig. 8 Gas Chromatograms from Analyses of Treated Milk Samples (Methylene chloride fraction).

I. Sample code TM + 17A
II. Sample code TM + 17P

A. GS-28369 B. GS-28370 C. Methidathion

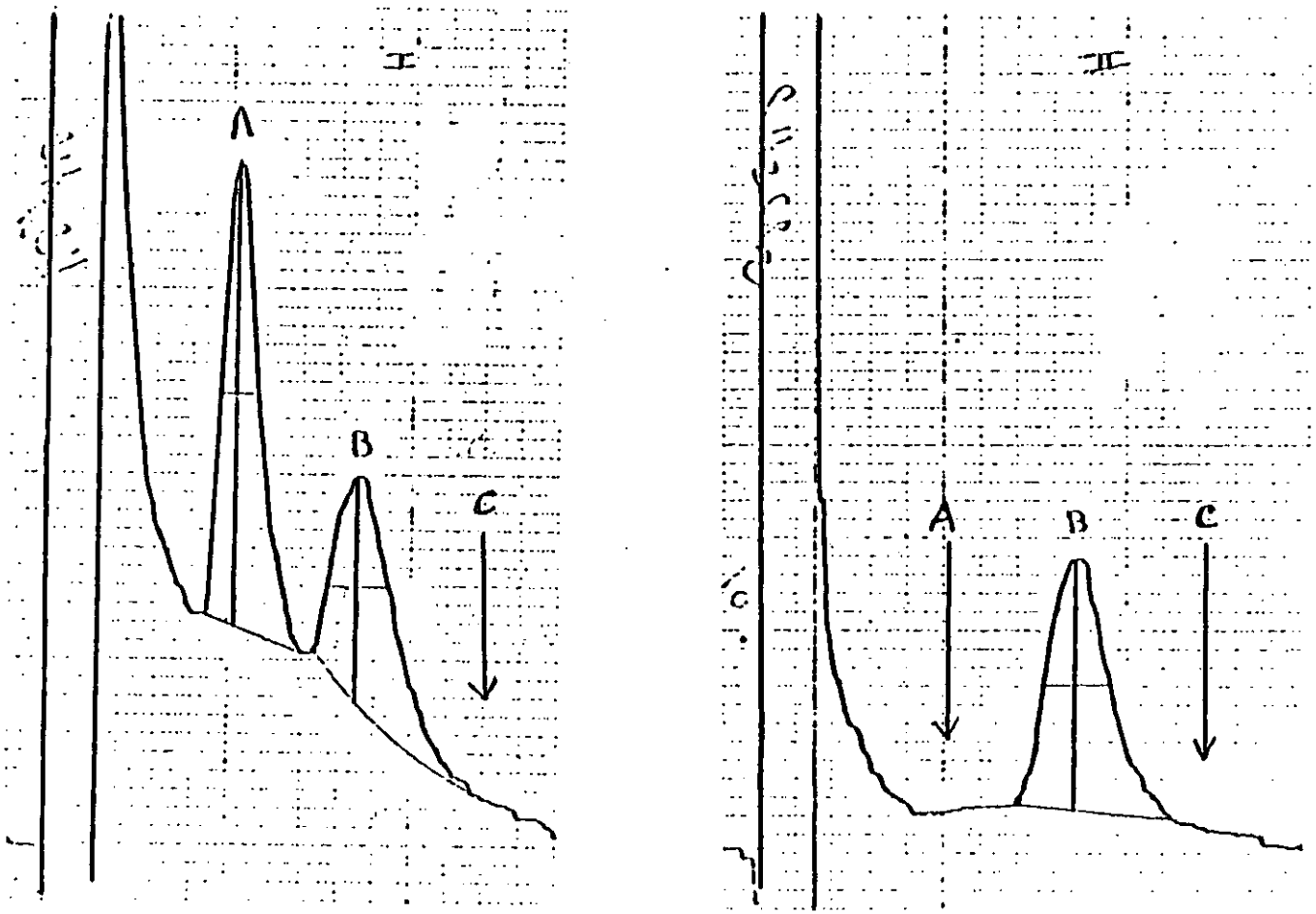
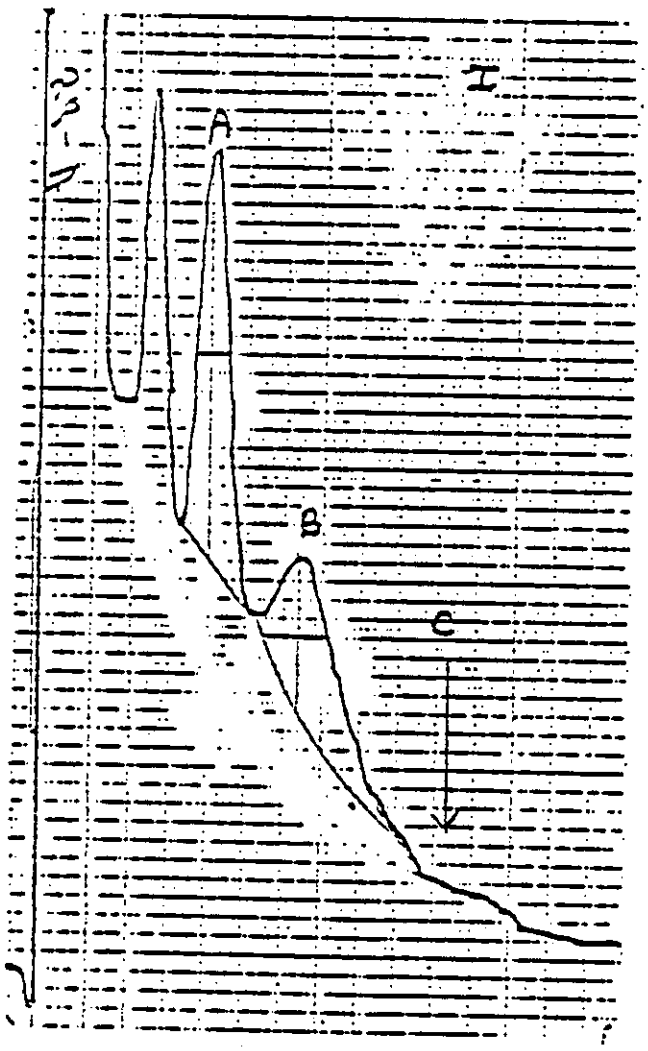


Fig. 9 Gas Chromatograms from Analyses of Treated Milk Samples

Sample Code TM + 25 P

I. Acetonitrile fraction.
II. Methylene chloride fraction

A. GS-28369 B. GS-28370 C. Methidathion



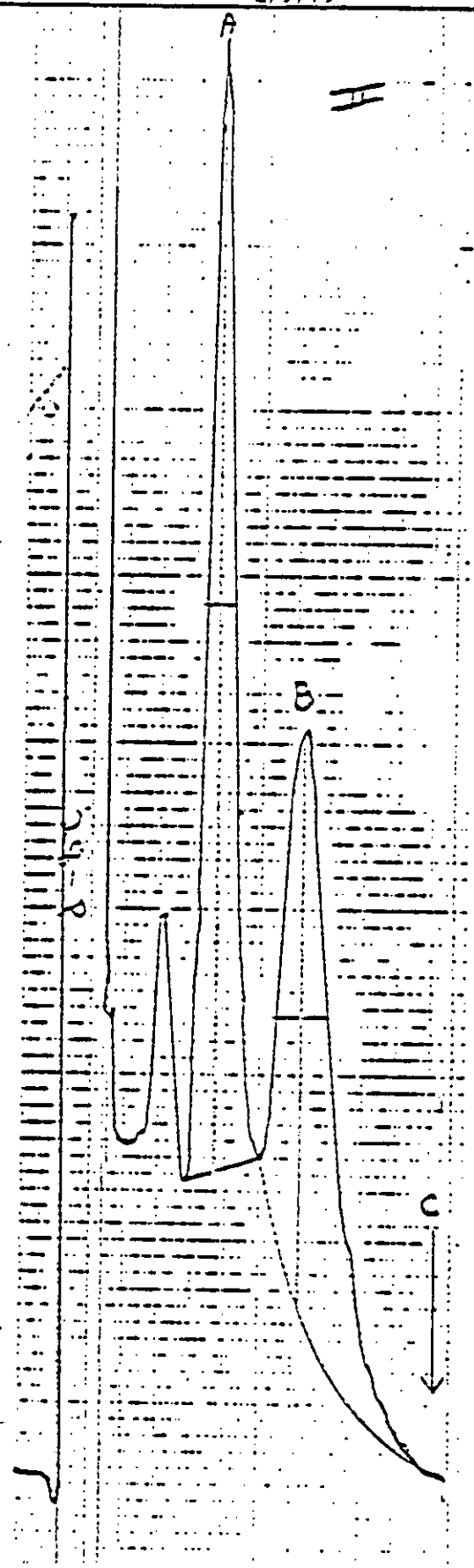


Fig. 10

Gas Chromatograms from Analysis of Treated Milk Samples (Acetonitrile fraction).

I. Sample code TM + 28 A
II. Sample code TM + 28 P
A. GS-23869 B. GS-28370
C. Methidathion

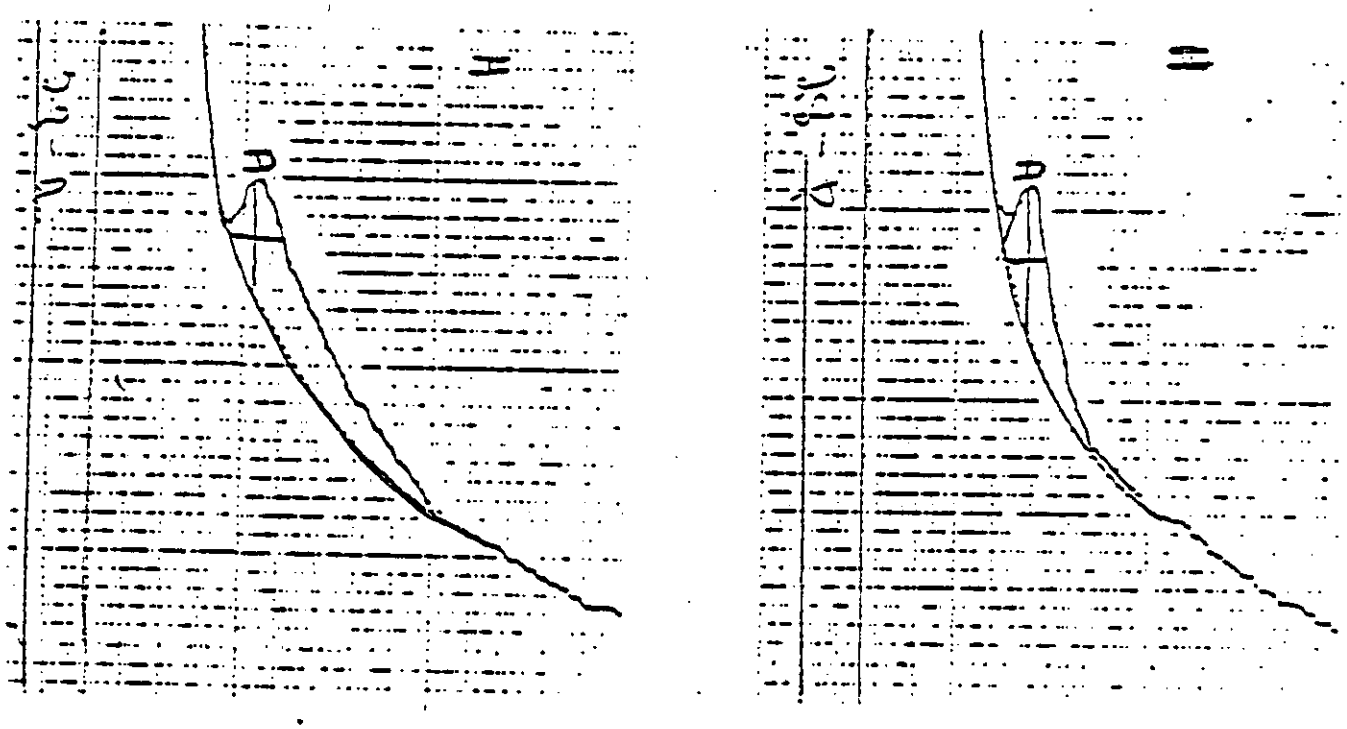


Fig. 11 Gas Chromatograms from Analysis of Treated Milk. (Acetonitrile fraction, 120°C)

I Sample code TM + 28A
II Sample code TM + 28P

D. GS-28368

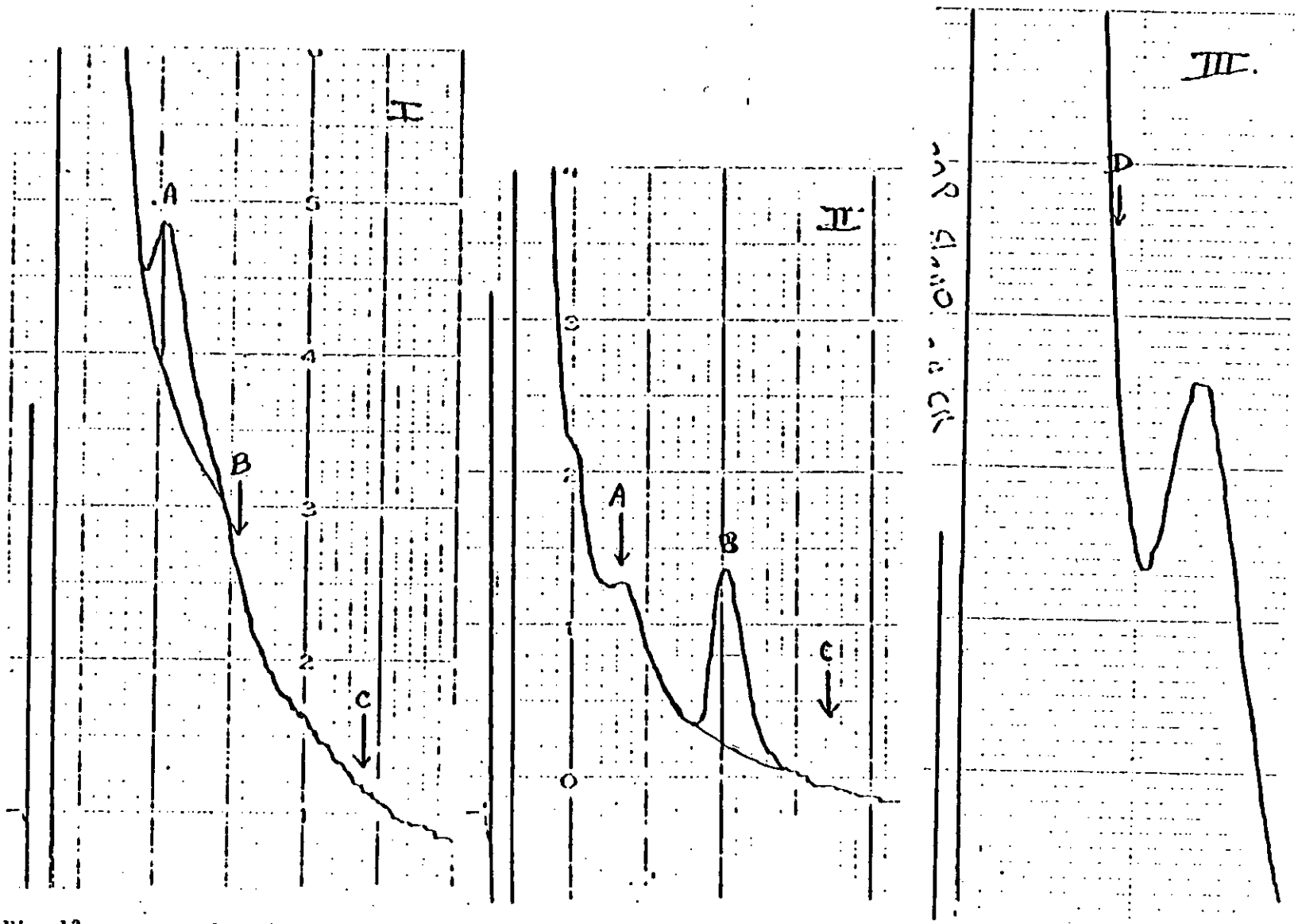
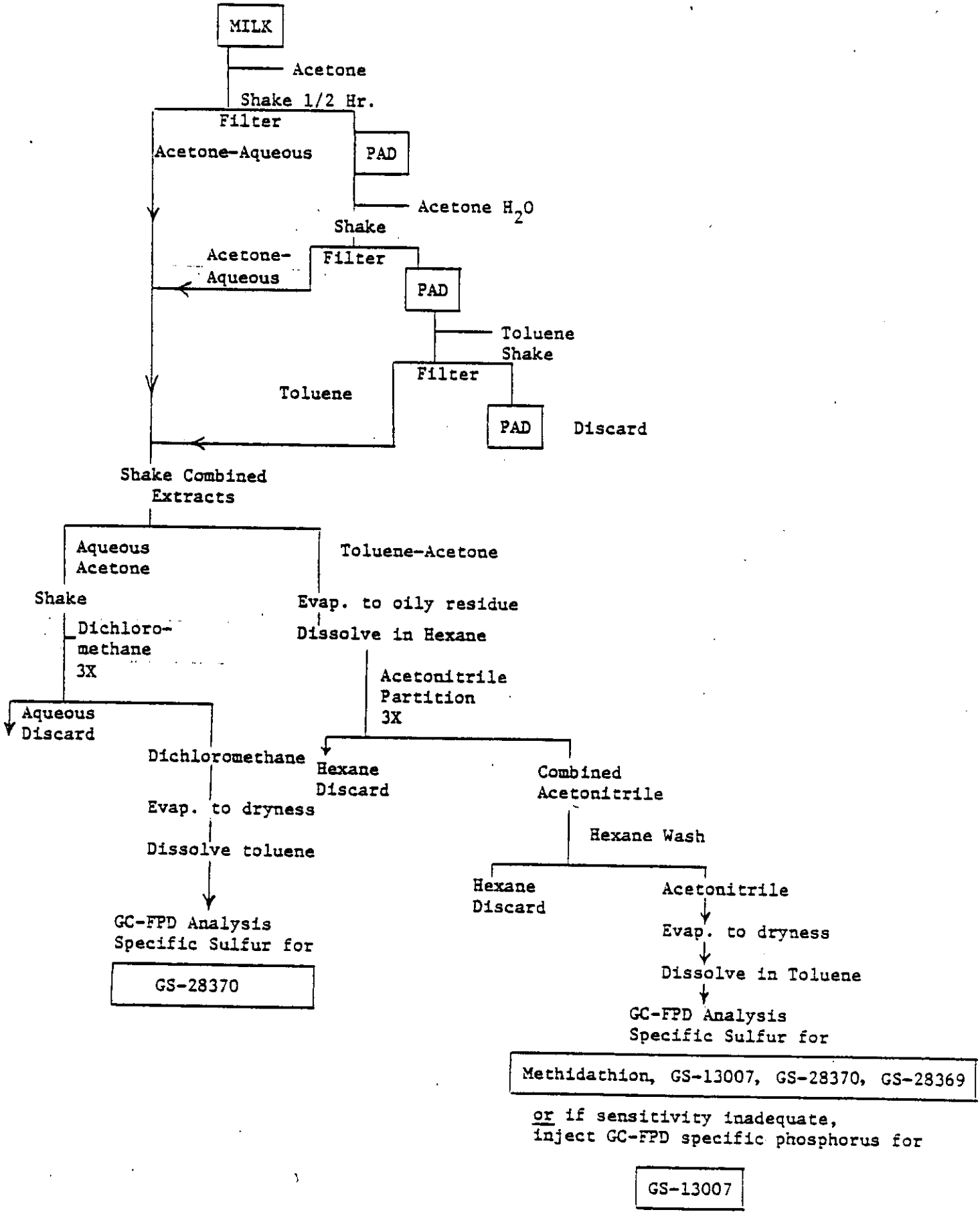


Fig. 12 **Gas Chromatograms From Analyses of Treated Milk Samples.**

Sample TM + 32 P

I. Acetonitrile Fraction
II. Methylene Chloride Fraction
III Acetonitrile Fraction at 120°column temperature.

A. GS-28369 B. GS-28370 C. Methidathion D. GS-28368


MILK
Acetone
Shake 1/2 Hr.
Filter
Acetone-Aqueous
PAD
Acetone H_2O
Shake
Filter
Acetone-Aqueous
PAD
Toluene
Shake
Filter
Toluene
PAD
Discard
Shake Combined Extracts
Aqueous Acetone
Toluene-Acetone
Shake
Dichloro-methane 3X
Aqueous Discard
Dichloromethane
Evap. to dryness
Dissolve toluene
GC-FPD Analysis Specific Sulfur for
GS-28370
Evap. to oily residue
Dissolve in Hexane
Acetonitrile Partition 3X
Hexane Discard
Combined Acetonitrile
Hexane Wash
Hexane Discard
Acetonitrile
Evap. to dryness
Dissolve in Toluene
GC-FPD Analysis Specific Sulfur for
Methidathion, GS-13007, GS-28370, GS-28369
or if sensitivity inadequate, inject GC-FPD specific phosphorus for
GS-13007

ADDENDUM TO AG-335

GC CONDITIONS USED BY ADC LABS

7/15/82

RAKahn

All samples were analyzed according to methodology supplied by Ciba-Geigy Corporation. Milk samples were analyzed as described in Method No. AG-335 "Determination of Methidathion and Some of Its Metabolites in Milk by Gas Chromatography". Tissue samples were analyzed according to Method No. AG-334 "Determination of Residues of Methidathion and Its Metabolites, GS-13007, GS-38269, and GS-28370 in Animal Tissue by Gas Chromatography Employing Flame Photometric Detection". These methods were used without modification of any steps, except for filtration of liver samples. Liver extracts were filtered through two pieces of filter paper instead of one.

All gas chromatography was done on a Tracor Model 222 gas chromatograph operated as follows:

Column: 4' x 1/4" i.d. glass packed with 10% DC-200 on Gas Chrom Q, 80/100 mesh

Injection Port Temperature:	239°C
Column Temperature:	200°C
Detector Temperature:	219°C
N_2 Carrier Flow (Rotameter):	10
O_2 Flow (Rotameter):	8
Air Flow (Rotameter):	100+
H_2 Flow (Rotameter):	100

Detector Filter: Sulfur for milk; Phosphorous for tissues

Attenuation: 10^3 x 32 for milk; 10^3 x 128 & 10^3 x 64 for tissues

Injection Volume: 5 µl

Chart Speed: 0.25 in/min

Retention Time: GS-13007 - 4.25 min
GS-13005 - 5.83 min

ADDENDUM TO AG-335

GC CONDITIONS USED BY ADC LABS

7/15/82

RAKuhn

All samples were analyzed according to methodology supplied by Ciba-Geigy Corporation. Milk samples were analyzed as described in Method No. AG-335 "Determination of Methidathion and Some of Its Metabolites in Milk by Gas Chromatography". Tissue samples were analyzed according to Method No. AG-334 "Determination of Residues of Methidathion and Its Metabolites, GS-13007, GS-28369, and GS-28370 in Animal Tissue by Gas Chromatography Employing Flame Photometric Detection". These methods were used without modification of any steps.

All gas chromatography was done on a Tracor Model 222 gas chromatograph operated as follows:

Column: 4' x 1/4" i.d. glass packed with 10% DC-200 on Gas Chrom Q, 80/100 mesh

Injection Port Temperature:	239°C
Column Temperature:	163°C
Detector Temperature:	219°C
N_2 Carrier Flow (Rotameter):	10
O_2 Flow (Rotameter):	8
Air Flow (Rotameter):	100+
H_2 Flow (Rotameter):	100
Detector Filter:	Sulfur
Attenuation:	10^3 x 32 and 10^3 x 64
Injection Volume:	5 µl
Chart Speed:	0.25 in/min
Retention Time:	GS-28369 - 5.24 min GS-28370 - 6.03 min