Synthesis and Characterization of FC-95-^{14}C

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Specific Activity and
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Summary

The synthesis of a 20.0 g lot of FC-95-^{14}C (carbon-14 label α to sulfur atom) is described. The specific activity is 0.459 ± 0.008 μCi/mg. Thin-layer and column chromatography showed the FC-95-^{14}C to be at least 99% radiochemically pure. The FC-95-^{14}C was found suitable for metabolism studies.

Introduction

A series of experiments is planned to investigate the metabolism of FC-95 (a sulfonic acid salt) and the possibility of FC-807 being biotransformed to FC-95 and/or the alcohol (FM-3422). To facilitate these experiments, carbon-14 labeled FC-95 was synthesized and characterized. The synthetic pathway illustrated in Figure 1 is described.

A. Synthesis of FC-95-^{14}C

I. Fractional Distillation of $C_7F_{15}{}^*CF_2SO_2F$ (Step 1)

The crude cell drainings from cell run R-3256-B were filtered through glass wool to remove the cell tars. The fluorochemical product was washed twice with cold, dilute saturated $KHCO_3$ and twice with water; the product was dried over silica gel. A yield of 293.7g of base-washed cell drainings was obtained from cell run R-3256-B. GLC analysis showed 71.1% $C_8F_{17}SO_2F$. One hundred sixty grams of the base-washed cell drainings was purified by fractional distillation (see Table 1). Analysis for percent $C_8F_{17}SO_2F$ was done by gas chromatography (area percent). Fractions 11-16 were combined to give 98.3 g of product which was found to contain 98.15% $C_8F_{17}SO_2F$.

II. Preparation of FC-95-^{14}C ($KO_3S{}^*CF_2C_7F_{15}$)

Water (31.1g) and potassium hydroxide pellets, (85% assay, 15.53g) were added to a 500 ml three-necked round-bottomed flask which had been equipped with a reflux condenser, thermometer, an air-driven mechanical stirrer, Tru-Bore® stirrer assembly and a small, pressure-equalized addition funnel. The flask contents were heated to 78-80°C. Fractional $C_7F_{15}{}^*CF_2SO_2F$ (27.0g, 98.15% assay) was added dropwise through the addition funnel at a rate sufficient to maintain the temperature between 80-83°C. Gummy, white solids were formed during the addition. Upon completion of the addition of the radioactive perfluoro-octane-sulfonyl fluoride, the mixture was heated at 85°C for 3 hours. At the end of the three hour reaction time, the gummy mass had broken into smaller solid pieces. Water (10.8 ml) was added. The pH of the solution was ∿ 13.5. The upper aqueous phase was removed (aspirator) and the solids were washed successively with water (54.0 ml) at 45-50°C. The solids were washed once more with water (17.8 ml), stirred at a high agitation rate, and the upper aqueous phase was discarded. Isopropanol (19.15g) and water (17.0g) were added to the solid product, and the mixture was heated at reflux temperature for one hour. All of the solids had dissolved after heating the mixture for one hour. The flask contents were poured into a glass evaporating dish. The flask was washed with isopropanol (24.7 ml)-water (17.0 ml). Concentration of the product was done on a steam bath. A final drying of the potassium perfluorooctane sulfonate was done at 60°C for 3 hours in a vacuum desiccator. The dry powder was triturated once with Freon 113 to remove small traces of silicon oil impurity used as stirrer lubricant. The dried product (27.8g) was assigned the following L number: FC-95-^{14}C, 20.0g, L-4544.

FC-95 normally is produced from one-plate distilled perfluorooctane-sulfonyl fluoride (assay 66-67%). The high assay $C_7F_{15}{}^*CF_2SO_2F$ (98.15%) will also be used to prepare FC-807-^{14}C.

B. Specific Activity Determination of FC-95-^{14}C

Three standard solutions of FC-95-^{14}C[a] (L-4544, see Synthesis Section) were prepared by weighing[b] I, 25.00 mg; II, 26.84 mg; and, III, 26.79 mg into three 25 ml class A volumetric flasks. Solution I was prepared with methanol and solutions II and III were prepared with a 1:1 mixture (volume:volume) of water and methanol. The volumes were adjusted to 25 ml with the appropriate solvent and mixed by inverting by hand. Calibrated micropipettors[c] were used to aliquot six 10 µl and six 50 µl aliquots of each solution directly into scintillation counting vials. To three of the vials containing 10 µl aliquots and to three containing 50 µl aliquots from each primary solution, 1 ml of water and 15 ml of Aquasol®[d] were added. The remaining vials were prepared with 2.5 ml of methanol and 7.5 ml of MTSS[e]. The samples were cooled to refrigerator temperature and allowed to equilibrate in the dark before they were counted two times at 5 minutes each with a Packard Model 3385 Liquid Scintillation Spectrometer. The counting efficiency was determined for each sample by the internal standard method. The averaged data were reduced to dpm and the µCi/mg was calculated for each vial. The data are shown in Table 2. The mean µCi/mg found for each of the weighings was within 2% of each of the other means. The means between Aquasol®and MTSS were within 1.2% and the means between 10 µl and 50µl were within 1.3%. The overall average specific activity based on the 36 replicates (12 from each primary weighing) was 0.459 ± 0.0080 µCi/mg.

C. Radiochemical Analysis of FC-95-^{14}C

I. Thin-Layer Chromatography Systems and Carbon-14 Analysis

The analysis of radiochemical purity was carried out with a variety of thin-layer chromatography systems using either SGF 250 micron pre-scored Uniplates[f] or pre-adsorbent SGF 250 micron pre-scored Uniplates[f]. Plates were routinely developed in 10"x12"x4" thin-layer tanks[g] fitted with glass lids and lined with saturation pads[g]. Plates were allowed to develop 15 cm and were scraped laterally in 0.5 cm wide segments. The scraping was accomplished with a custom-made template and sharpened stainless steel spatula ground to exactly 0.5 cm width.

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[a] Riker Isotope Number 442.

[b] Weighings were accomplished on a 5-place Mettler H64 electronic balance which had been recently calibrated by 3M Metrology.

[c] L/I Micropipettor, Lab Industries, Berkeley, California.

[d] New England Nuclear, Boston, Massachusetts.

[e] Modified TSS:25.2 g PPO, 1.01g Dimethyl POPOP and 3.8 liters toluene.

[f] Analtech, 75 Blue Hen Drive, Newark, Delaware.

[g] Supelco Inc., Bellefonte, Pennsylvania.

The scraped silica gel segments were collected in scintillation vials containing 2.5 ml of methanol. To the methanol, 7.5 ml of MTSS was added. The samples were counted and the counts per minute (cpm) were corrected for background using a suitable blank (usually two 0.5 cm segments scraped from below the origin on the plate being assayed). The cpm were not corrected for efficiency. The carbon-14 content of each segment was expressed as percent of total carbon-14 on the plate:

$$\frac{\text{cpm on segment}}{\text{sum of cpm on plate}} \times 100 = \% \text{ carbon-14 content in segment.}$$

The percent carbon-14 content of each segment was plotted versus segment number. This provided a thin-layer radiochromatogram showing the radioactivity peaks corresponding to separated components in the material applied to the plate.

II. Solutions Used for Radiochemical Purity Analysis

Solution 1: The FC-95-^{14}C made from 25.00 mg of FC-95-^{14}C in 25.0 ml methanol described in the section on Specific Activity Determination, this report, was designated as Solution 1.

Solution 2: 28.12 mg of FC-95-^{14}C, L-4544, (Riker Isotope Inventory Number 442) was placed into a 10 ml volumetric flask and the volume adjusted to 10 ml with methanol. The solution was inverted several times to ensure mixing.

III. Column Chromatography of FC-95-^{14}C

A silicic acid (Unisil[a]) column was prepared by pouring a chloroform slurry of silicic acid into a 14mm I.D. glass column[b] to a height of 21 cm. A 1.0 ml aliquot of Solution 2 was applied to the top of the column. Three successive 200 ml fractions were collected after applying 200 ml of $CHCl_3$, Fraction 1; 200 ml of chloroform-methanol 1:1 (volume: volume), Fraction 2; and 200 ml of methanol, Fraction 3. Each of the 200 ml fractions was evaporated with a rotating evaporator, transferred to a 12 ml centrifuge tube, evaporated to near dryness with a stream of nitrogen, and reconstituted with 0.20 ml of chloroform-methanol 1:1 (volume:volume). Fifty microliters was applied to thin-layer plates and analyzed as described previously.

IV. Results and Discussion

Since the chemical identity of the FC-95-^{14}C is well established by synthesis (see synthesis of FC-95-^{14}C, this report), unlabeled FC-95 was not co-chromatographed with the labeled FC-95-^{14}C. The results of the radiochemical purity analysis of FC-95-^{14}C by thin-layer chromatography are shown in Table 3 and in Figures 2-5. These analyses did not discern any impurities in FC-95-^{14}C. Thin-layer analysis of three column fractions from a silicic acid column showed 99.1% of the radioactivity recovered in Fraction 2 (1:1 chloroform-methanol) (see Figure 6). Fraction 3 (methanol) contained 0.8 per cent of the

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[a] Unisil, Activated Silicic Acid, 100-200 mesh. Clarkson Chemical Company, Inc., Williamsport, Pennsylvania.

[b] Column was fitted with a sintered-glass base and stopcock.

recovered radioactivity (see Figure 7). The impurity contained in Fraction 3 has a different R_f (0.40) from that of FC-95 (0.27) in the same solvent systems. The impurity was not identified.

Overall, the radiochemical purity of FC-95-^{14}C is at least 99%. The FC-95-^{14}C is suitable for metabolism studies.

Acknowledgement

The authors gratefully acknowledge the gas chromatographic analysis of the carbon-14 labeled $C_7F_{15}CF_2SO_2F$ fractions done by Mr. Todd Mathisen of Commercial Chemicals Division and the assistance of John C. Hansen and Larry Headrick during the electrochemical fluorination of labeled $C_7F_{15}CF_2SO_2F$.

List of Tables and Figures

Table 1

Data from Fractional Distillation of Base-Washed $C_7F_{15}\overset{*}{C}F_2SO_2F$

Fraction	bP °C	Yield (g)	Area % [a]				
			LB'S [b]	C_8F_{18}	$C_6F_{13}SO_2F$ $C_7F_{15}SO_2F$	$C_8F_{17}SO_2F$	HB'S [c]
1	< 40	4.3	99.3				
2	< 80	10.8	95.3	2.30	0.12	2.20	---
3	80-83	8.7	80.99	8.82	0.95	7.86	---
4	83-92	4.1	73.55	12.46	1.55	13.36	---
5	92-94	5.7	63.63	15.39	2.17	18.94	---
6	94-97	4.3	50.23	18.54	3.09	27.52	---
7	97-104	1.8	41.92	21.18	3.80	32.42	---
8	104-108	2.5	22.02	18.90	5.27	53.51	---
9	108-118	1.8	12.16	15.03	5.82	66.66	---
10	118-123	2.7	6.17	11.12	5.84	76.78	---
11	126-128	9.4	7.23	5.44	5.33	87.53	---
12	128-130	7.1	0.49	1.93	4.39	95.66	---
13	131- 33	9.4	---	---	0.43	96.64	0.46
14	138- 40	31.2	---	---	1.52	97.92	0.49
15	> 140	16.0	---	---	0.22	99.55	0.09
16	---	25.2	---	---	0.058	93.22	---
Bottoms	---	---	---	---	---	5.13	64.0
Blend FR 11-16						98.15	

[a] Area percent was determined by gas chromatographic analysis.

[b] Low boiling non-functional fluorocarbons below C_8F_{18}.

[c] High-boiling, unidentified fluorocarbons boiling above $C_8F_{17}SO_2F$.

Table 2

Specific Activity Determination of FC-95-^{14}C

Comparison of Primary Solutions

Solution I (25.00 mg/25.0 ml) µCi/mg	Solution II (26.84 mg/25.0 ml) µCi/mg	Solution III (26.79 mg/25.0 ml) µCi/mg
0.4535	0.4610	0.4660
0.4558	0.4530	0.4647
0.4582	0.4535	0.4598
0.4658	0.4652	0.4532
0.4816	0.4607	0.4554
0.4736	0.4611	0.4644
0.4521	0.4565	0.4425
0.4637	0.4422	0.4576
0.4589	0.4486	0.4567
0.4637	0.4540	0.4625
0.4653	0.4576	0.4490
0.4685	0.4518	0.4513
$\bar{X} \pm$ S.D. 0.4634 ± 0.0085	0.4554 ± 0.0063	0.4569 ± 0.0071

Comparison of Scintillation Solvents

MTSS µCi/mg	Aquasol® µCi/mg
0.4658	0.4535
0.4816	0.4558
0.4736	0.4582
0.4637	0.4521
0.4653	0.4637
0.4685	0.4589
0.4652	0.4610
0.4607	0.4530
0.4611	0.4535
0.4540	0.4565
0.4576	0.4422
0.4518	0.4486
0.4532	0.4660
0.4554	0.4647
0.4644	0.4598
0.4625	0.4425
0.4490	0.4576
0.4513	0.4567
$\bar{X} \pm$ S.D. 0.4614 ± 0.0084	0.4558 ± 0.0067

Comparison of 10 µl and 50 µl Aliquots

10 µl µCi/mg	50 µl µCi/mg
0.4535	0.4521
0.4558	0.4637
0.4582	0.4589
0.4658	0.4637
0.4816	0.4653
0.4736	0.4653
0.4610	0.4685
0.4530	0.4565
0.4535	0.4422
0.4652	0.4486
0.4607	0.4576
0.4611	0.4518
0.4660	0.4425
0.4647	0.4576
0.4598	0.4567
0.4532	0.4625
0.4554	0.4490
0.4644	0.4513
0.4615 ± 0.0076	0.4557 ± 0.0075

Table 3

Thin-Layer Chromatography Systems for FC-95-^{14}C

Plate No.	Type of Plate	Solvent System[a]	R_f[b] of FC-95-^{14}C
1	Uniplate	50 chloroform 50 acetone	0.17
2	Uniplate	100 chloroform 100 methanol 2 acetic acid[c]	0.70
3	Uniplate	100 butanol 10 water 10 acetic acid[c]	0.67
4	Uniplate	150 chloroform 50 methanol 5 ammonium hydroxide[c]	0.30
5	Pre-adsorbent Uniplate	100 butanol 10 water 10 acetic acid[c]	0.67

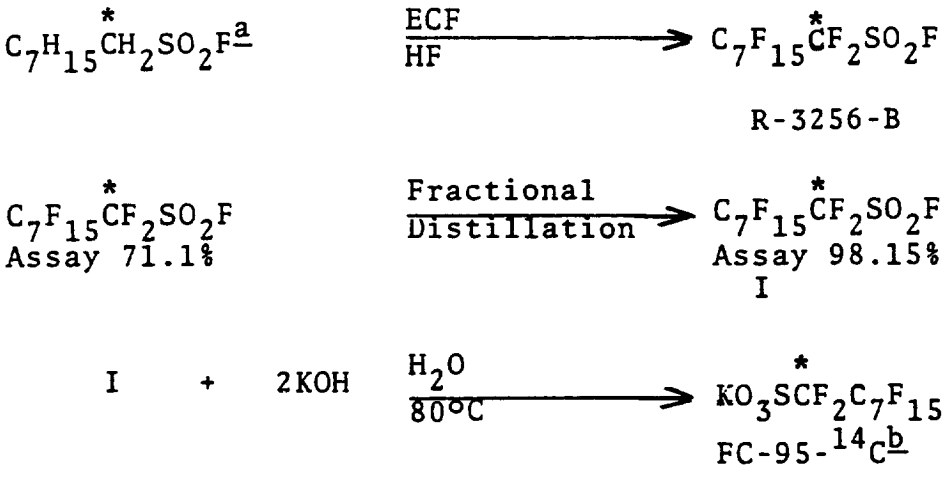
[a] Solvents were prepared volume:volume; a 100 ml aliquot of solvent mixture was added to chromatography tank.

[b] R_f is of only radioactive peak on plate.

[c] Acetic acid and ammonium hydroxide were concentrated.

Figure 1

Pathway for Synthesis of FC-95-^{14}C



* Denotes position of Carbon-14.

[a] $C_7H_{15}\overset{*}{C}H_2SO_2F$ was prepared by Pathfinder Laboratories.

[b] FC-95-^{14}C, potassium perfluorooctanesulfonate.

Figure 2

Thin-layer Radiochromatogram of
FC-95-^{14}C, Plate No. 1

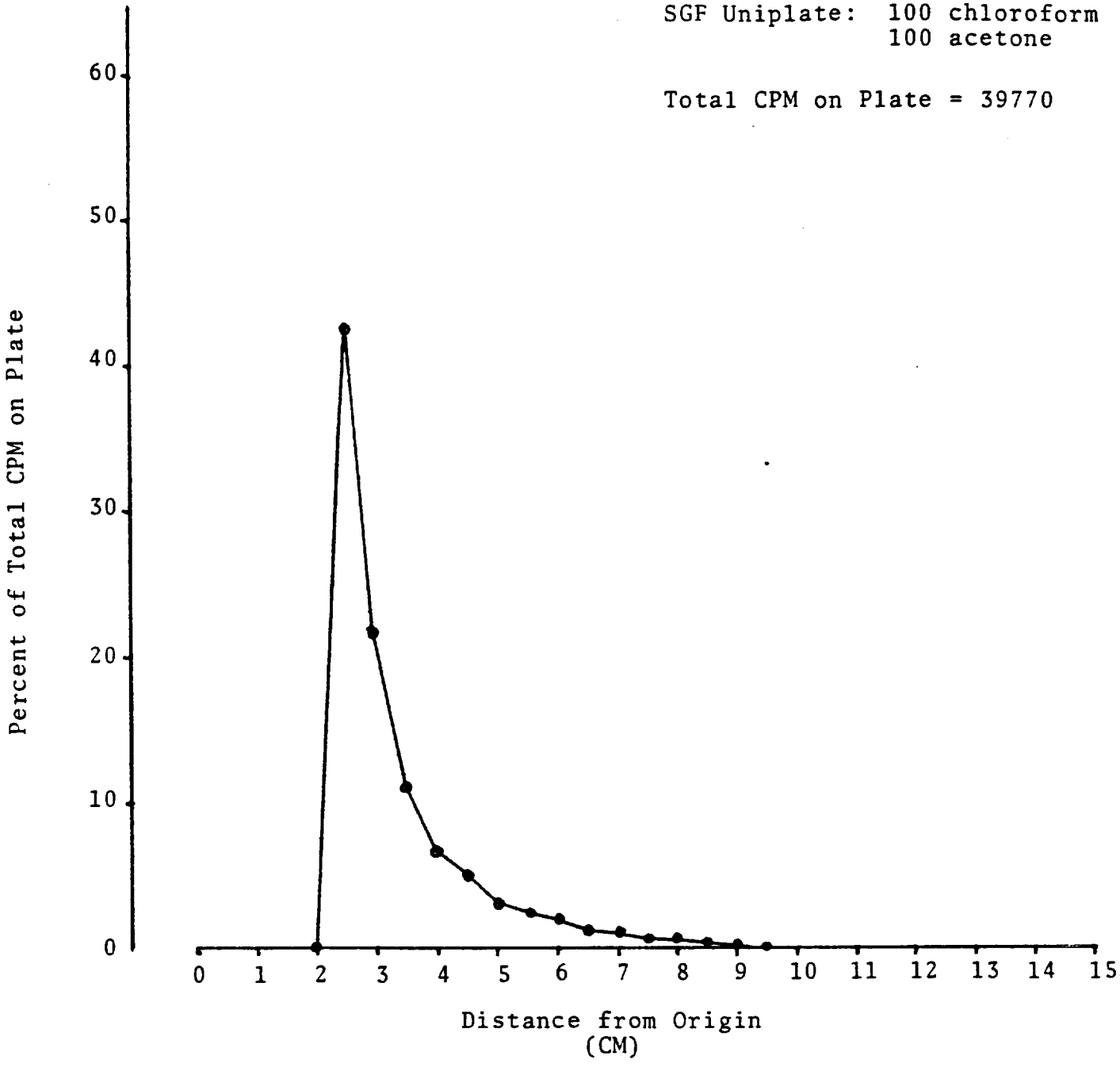


Figure 3

Thin-layer Radiochromatogram of
FC-95-^{14}C, Plate No. 2

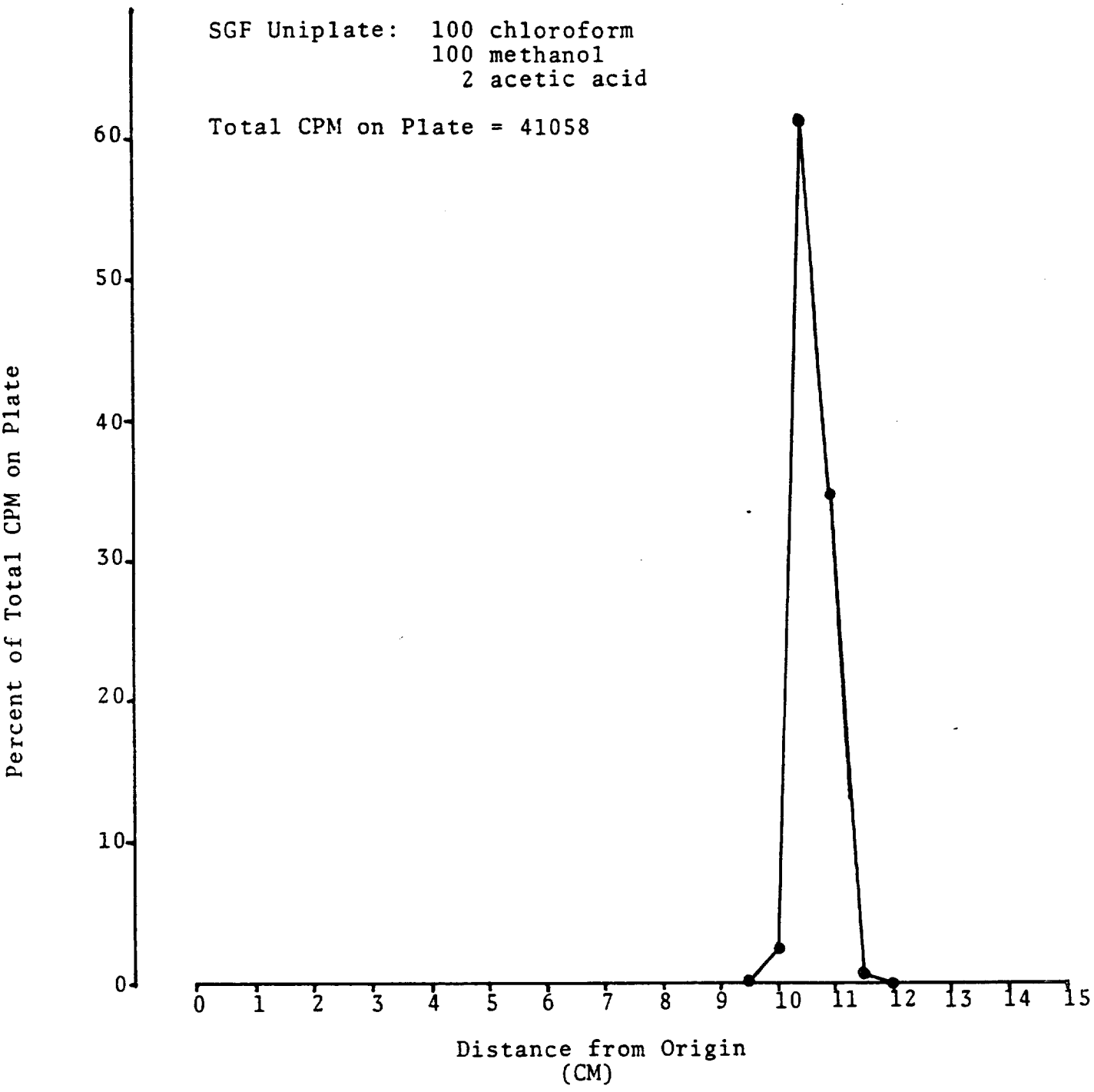


Figure 4

Thin-layer Radiochromatogram of
FC-95-^{14}C, Plate No. 3

SGF Uniplate: 100 butanol
10 water
10 acetic acid

Total CPM on Plate = 38549

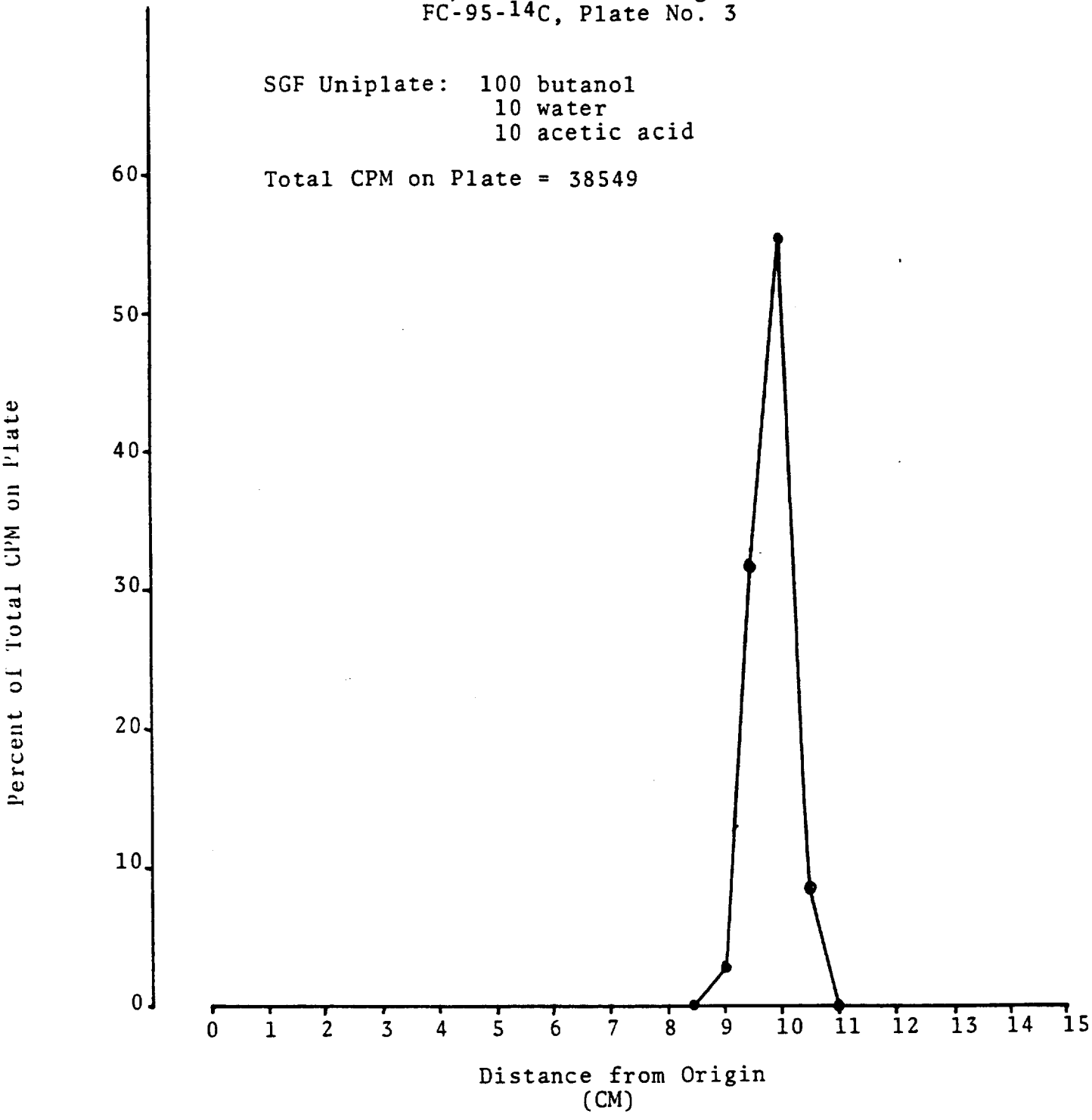


Figure 5

Thin-layer Radiochromatogram of
FC-95-^{14}C, Plate No. 4

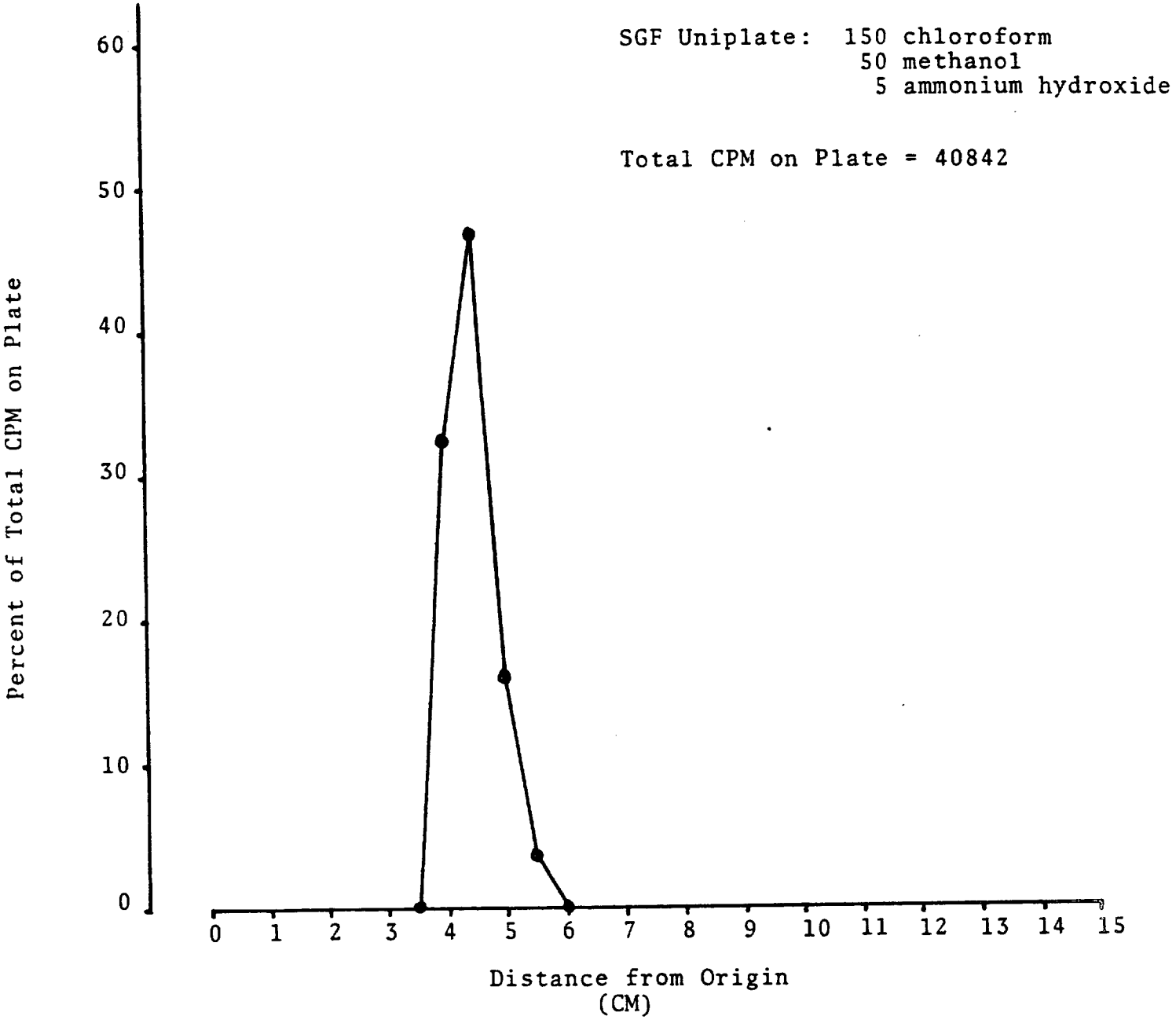


Figure 6

Thin-layer Radiochromatogram of
FC-95-^{14}C Silicic Acid Column Eluent, Fraction 2
(Chloroform-Methanol)

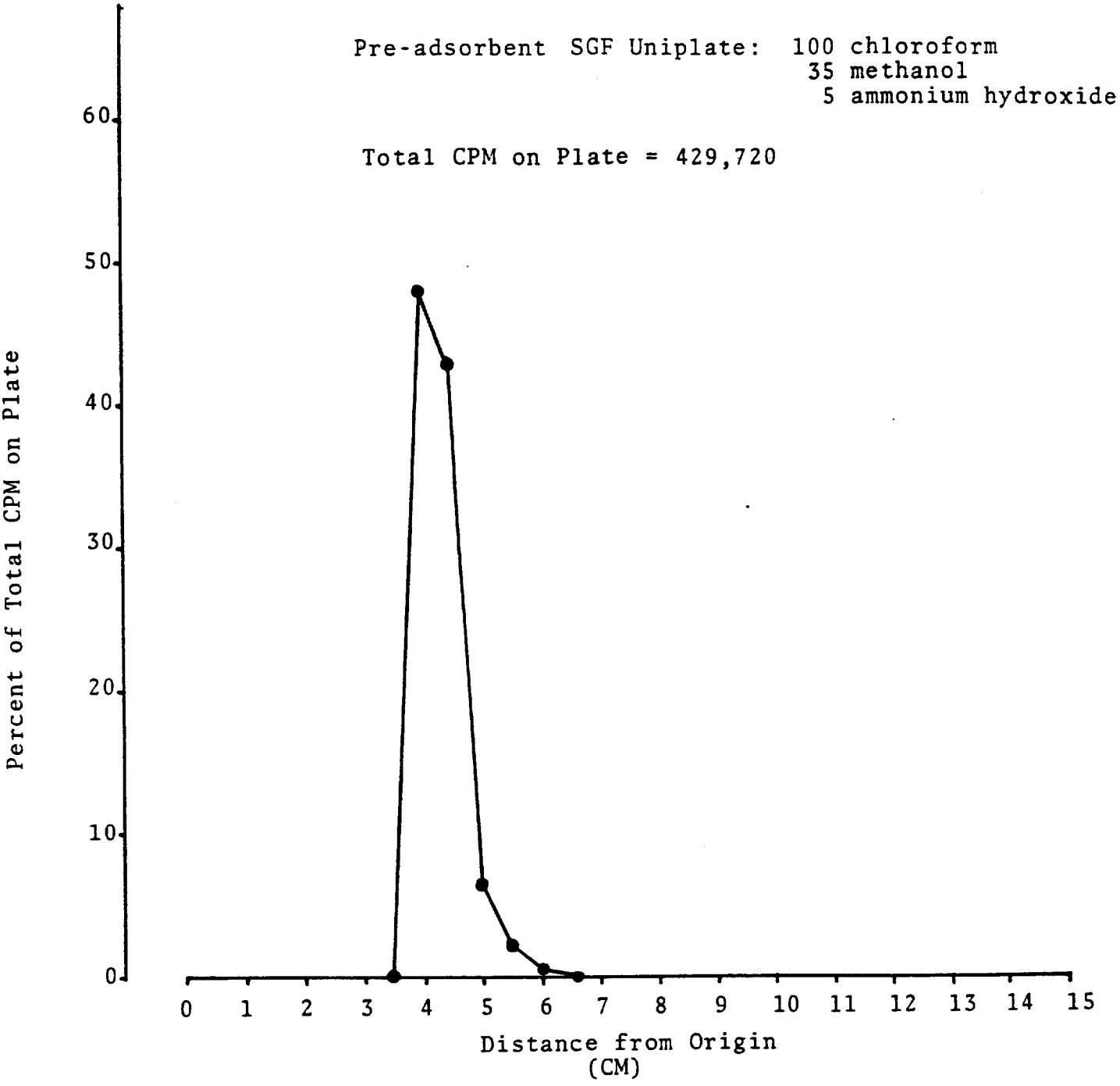


Figure 7

Thin-layer Radiochromatogram of
FC-95-^{14}C Silicic Acid Column Eluent, Fraction 3
(Methanol)