work file

Synthesis and Characterization of FC-143-^{14}C

December 28, 1979

Conducted at: Commercial Chemicals Division
and Riker Laboratories, Inc.
3M
St. Paul, Minnesota 55101

During: April 1979 to July, 1979

Synthesis Conducted by: F. E. Behr

Specific Activity and Radiochemical
Purity Determination by: J. D. Johnson and S. J. Gibson

Report by:

Fred E Behr 1/17/80
F. E. Behr, Ph.D. Date
Research Specialist
Commercial Chemicals Division

James D. Johnson 1/17/80
J. D. Johnson Date
Senior Biochemical Pharmacologist
Riker Laboratories, Inc.

Specific Activity and Radiochemical
Characterization Section
Reviewed by:

R. E. Ober 1/23/80
R. E. Ober, Ph.D. Date
Manager, Drug Metabolism
Riker Laboratories, Inc.

Summary

The synthesis of a 20.0 g lot of FC-143-^{14}C is described (carbonyl carbon is labeled). The specific activity was determined to be 0.507 ± 0.012 μCi/mg. Radiochemical purity determination showed the FC-143-^{14}C to be at least 98% pure; the FC-143-^{14}C was found suitable for metabolism studies.

Introduction

A series of experiments is being planned to investigate the metabolism of FC-143 and the possibility of affecting the rate of elimination of FC-143-^{14}C from the body. To facilitate these experiments, carbon-14 labeled FC-143 was synthesized and characterized. The synthetic pathway illustrated in Figure 1 is described.

A. Synthesis of FC-143-^{14}C

I. Fractional Distillation of Cell Run, R-3265-B

Analysis by gas chromatography showed the cell drainings contained about 12.1% high boiling materials. The crude cell drainings were dried over anhydrous KF to remove HF. The solids were removed by filtration to yield 434.0 gms for distillation. Three fractions were collected (Table 1). Fractions 1 and 2 (389.3 g) were combined and will be used for the preparation of $C_7F_{15}\overset{*}{C}O_2NH_4$.

II. Hydrolysis and Stabilization of Distilled Cell Drainings from R-3265-B

Into a 500 ml three-necked round-bottomed flask which had been equipped with a mechanical stirrer, Tru-Bore® stirrer assembly, addition funnel, condenser, and thermometer was placed the combined cell drainings, Fractions 1 and 2, 389.3 g. A pre-mixed solution of NaOH (68.4g) and water (68.4 g) was added to the flask through a pressure-equalized addition funnel over 45 minutes at a rate sufficient to keep the temperature between 52-55°C. The mixture was heated to reflux (85°C) for 9 hours. After the 9 hour reaction time, the pH was found to be > 12. The mixture was cooled to 65°C and a mixture of water (257.0 ml) and 96% H_2SO_4 (94.3 ml) was added slowly to the flask contents. The final pH was < 1 (Congo red paper). The acidified mixture was heated at 80°C for 30 minutes, cooled to 65°C, and the bottom fluorochemical phase was separated. The yield was 356.7 g (91.6% mass yield).

III. Fractional Distillation of $C_7F_{15}\overset{*}{C}O_2H$

The apparatus consisted of a 500 ml three-necked round-bottomed flask, a small packed heated distillation column, pot thermometer, take-off head, a collection assembly, -78°C cold trap, magnetic stirrer assembly, and a mercury manometer. The base-stabilized radioactive fluorochemical (356.7 g), 96% sulfuric acid (12.0 ml), and Super-Cel (0.7 g) was added to the distillation flask. The

water aspirator vacuum was adjusted to 200 mm and the low boiling materials were removed. As the head temperature dropped below 40°C at 200 mm vacuum, the distillation was continued with lower vacuum (10-20 mm). Fractions were collected, weighed, and analyzed by gas chromatography.

The analysis was done by area percent and the assay reported was for percent $C_7F_{15}CO_2H$ (See Table II). Fraction 6, 44.0 g, was refractionated to give 95.0% $C_7F_{15}CO_2H$ (see Table III). Fraction 3, (24.3 g, 97.5% $C_7F_{15}CO_2H$), and Fraction 4, (13.4 g, 96.08% $C_7F_{15}CO_2H$) were combined for use in preparation of FC-143-^{14}C.

IV. Fractions 3 and 4 (30.0 g) were dissolved in FC-80 (90 ml). Anhydrous NH_3 was added until the pH was greater than 8. The pH was rechecked after 2 hours. The slurry was filtered, and the product was air dried in the hood. The final product was prepared by pulverizing the air dried solid and drying the solid at 65°C for 1 hour. Analysis by gas chromatography showed an assay of 98.8% $C_7F_{15}CO_2CH_3$. The yield was 31.1 g. The following L numbers were assigned: L-4585, FC-143-^{14}C, 100% solids, 20.0 g; L-4586, fractionated $C_7F_{15}CO_2H$, NB 52343:8-30, Fraction 4, 0.4 g.

B. Specific Activity Determination of FC-143-^{14}C

Three standard solutions of FC-143-^{14}C[a] (L-4585, see Synthesis Section) were prepared by weighing[b] I, 10.56 mg; II, 25.28 mg; and III, 20.06 mg into three 10 ml class A volumetric flasks. The volumes were adjusted to 10 ml with methanol and the flasks were 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 4. The overall average specific activity based on the 36 replicates (12 from each weighing) was 0.507 ± 0.012 µCi/mg. The mean µCi/mg found for each of the primary weighings was within 1.5% of the overall mean. The means between Aquasol® and MTSS were within 0.12%, and the means between 10 µl and 50 µl aliquots were within 2.0%.

---

[a] Riker Isotope Number 459.

[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, CA.

[d] New England Nuclear, Boston, Mass.

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

C. Radiochemical Purity Analysis of FC-143-^{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 systems using SGF 250 micron pre-scored Uniplates[a]. Plates were routinely developed in 10" x 12" x 4" thin-layer tanks[b] fitted with glass lids and lined with saturation pads[b]. 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. 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. Solution Used for Radiochemical Purity Analysis

The solution used to streak the thin-layer plates was prepared by placing 30.39 mg of FC-143-^{14}C (Riker Isotope Inventory Number 459) into a 10.0 ml class A volumetric flask, adjusting the volume to 10.0 ml with methanol, and mixing by hand by inverting. Fifty µl of this solution was applied in a narrow four cm long streak to each plate.

III. Results and Discussion

Since the chemical identity of the FC-143-^{14}C is well established by synthesis and gas chromatography (see section A, this report), unlabeled FC-143 was not co-chromatographed with the FC-143-^{14}C. The results of the radiochemical purity analysis are shown in Table 5 and in Figures 2-6. A small amount of impurity (< 2%) was apparent on Plate Number 2 (Figure 3). The other four solvent systems did not resolve this impurity. No attempt was made to identify the impurity found on Plate Number 2. Overall, on the basis of gas chromatography (section A, this report) and thin-layer chromatography, the radiochemical purity of FC-143-^{14}C is at least 98%. The FC-143-^{14}C is suitable for metabolism studies.

---

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

[b] Supelco Inc., Bellefonte, Pennsylvania.

Acknowledgement

The authors gratefully acknowledge the gas chromatographic analysis of the carbon-14 labeled cell drainings, fractionated acid, and FC-143-^{14}C done by Mr. Todd Mathisen of Commercial Chemicals Division. The authors gratefully acknowledge the assistance of John C. Hansen and Larry G. Headrick during the electrochemical fluorination of labeled $C_7H_{15}COCl$.

List of Tables and Figures

Table 1

Data from One Plate Distillation of Cell Run, R-3265-B

			Assay GLC (Area Percent)				
Fr	bp °C	WT (g)	LB's [a]	C_7F_{16}	C_8CE [b]	$C_7F_{15}COF$	HB's [c]
1	82-89	215.6	5.51	31.97	46.92	10.24	0
2	90-92	173.7	--	4.32	66.93	25.90	0
3	92-110	12.2	--	--	30.37	50.87	17.88
Bottoms	--	22.4	Mostly High Boilers				

[a] LB's : low boilers -- unidentified but with retention times < C_7F_{16}.

[b] C_8CE: C_8 cylic ether - various perfluorobutyltetrahydrofurans and isomers.

[c] HB's : materials boiling higher than $C_7F_{15}COF$.

Table 2

Data from Fractional Distillation of $C_7F_{15}^*CO_2H$

Fr	bp°C	WT(g)	Assay		
			Inerts	$C_5F_{11}CO_2H$ $C_6F_{13}CO_2H$	$C_7F_{15}CO_2H$
1	<35 (200mm)	102.3	97.98	---	---
2	35-40	103.6	99.98	---	---
3	26-47 (10mm)	2.4	2.18	9.41	87.9
4	98-101	2.1	5.97	13.19	79.8
5	101-103	1.2	---	15.85	78.8
6	104-109 (7mm)	44.0	---	5.19	90.3

GLC analysis of FR #6 showed about 3.9% high boiling materials.

Table 3

Data for Refractionation of $C_7F_{15}\overset{*}{C}O_2H$

Fr	bp (mm)	Yield (g)	Assay (Area %) $C_7F_{15}CO_2CH_3$
1	67-71 (20)	1.5	89.03
2	71-73 (19)	3.7	97.26
3	72-74 (8)	24.3	97.54
4	62 (4)	13.4	96.08
		42.9 (g)	

Assay for input to refractionation was 94.35% $C_7F_{15}CO_2H$ analyzed as $C_7F_{15}CO_2CH_3$.

Table 4

Specific Activity Determination of FC-143-^{14}C

Solution I 10.56mg/10.0ml µCi/mg	Solution II 25.28mg/10.0ml µCi/mg	Solution III 20.06mg/10.0ml µCi/mg
0.4887	0.4999	0.4966
0.4898	0.5292	0.4922
0.5020	0.5265	0.4965
0.5061	0.5067	0.4982
0.4917	0.5006	0.5007
0.5038	0.5006	0.5084
0.5124	0.5330	0.4948
0.5122	0.5142	0.5061
0.5172	0.5180	0.5041
0.5070	0.5193	0.4973
0.5303	0.5160	0.4999
0.5163	0.5101	0.5098
0.5065±0.0124	0.5145±0.0114	0.5004±0.0056

Comparison of Scintillation Solvents

Aquasol® µCi/mg	MTSS µCi/mg
0.4887	0.5061
0.4898	0.4917
0.5020	0.5038
0.5124	0.5070
0.5122	0.5303
0.5172	0.5163
0.4999	0.5067
0.5292	0.5006
0.5265	0.5006
0.5330	0.5193
0.5142	0.5160
0.5180	0.5101
0.4966	0.4982
0.4922	0.5007
0.4965	0.5084
0.4948	0.4973
0.5061	0.4999
0.5041	0.5098
0.5074±0.0137	0.5068±0.0093

Comparison of 10µl and 50µl Aliquots

10µl µCi/mg	50µl µCi/mg
0.4887	0.5124
0.4898	0.5122
0.5020	0.5172
0.5061	0.5070
0.4917	0.5303
0.5038	0.5163
0.4999	0.5330
0.5292	0.5142
0.5265	0.5180
0.5067	0.5193
0.5006	0.5160
0.5006	0.5101
0.4966	0.4948
0.4922	0.5061
0.4965	0.5041
0.4982	0.4973
0.5007	0.4999
0.5084	0.5098
0.5021±0.0109	0.5121±0.0101

Overall average = 0.5071±0.0115µCi/mg

Table 5

Thin-Layer Chromatography Systems for FC-143-^{14}C

Plate No.	Solvent System[a]	R_f[b] of FC-143-^{14}C
1	100 chloroform 100 acetone	0.03
2	100 chloroform 100 methanol 2 acetic acid[c]	0.67
3	100 butanol 10 water 10 acetic acid[c]	0.60
4	150 chloroform 50 methanol 5 ammonium hydroxide[c]	0.27
5	100 chloroform 35 methanol 5 ammonium hydroxide[c]	0.30

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

[b] R_f is of major (>98%) peak on the thin-layer chromatography plate.

[c] Acetic acid and ammonium hydroxide were concentrated.

Figure 1

Pathway for Synthesis of FC-143-^{14}C

$C_7H_{15}\overset{*}{C}OCl$ —ECF / HF—> $C_7F_{15}\overset{*}{C}OF$
R-3256-B

Prepared by
Pathfinder Laboratories

$C_7F_{15}\overset{*}{C}OF$ —One-Plate Distillation—> $C_7F_{15}\overset{*}{C}OF$
I

I + NaOH —1) Heat, 2) H_2SO_4—> $C_7F_{15}\overset{*}{C}O_2H$
II

II —Fractional Distillation—> —Refractionation—> $C_7F_{15}\overset{*}{C}O_2H$
> 95% III
Assay

III + NH_3 —FC-80—> $NH_4\overset{*}{C}O_2C_7F_{15}$
FC-143-^{14}C[a]

*-Denotes position of Carbon-14

[a] FC-143-^{14}C, Ammonium perfluorooctanoate

Figure 2

Thin-layer Radiochromatogram of
FC-143-^{14}C, Plate No. 1

SGF Uniplate: 100 chloroform
100 acetone

Total CPM on Plate = 99,892

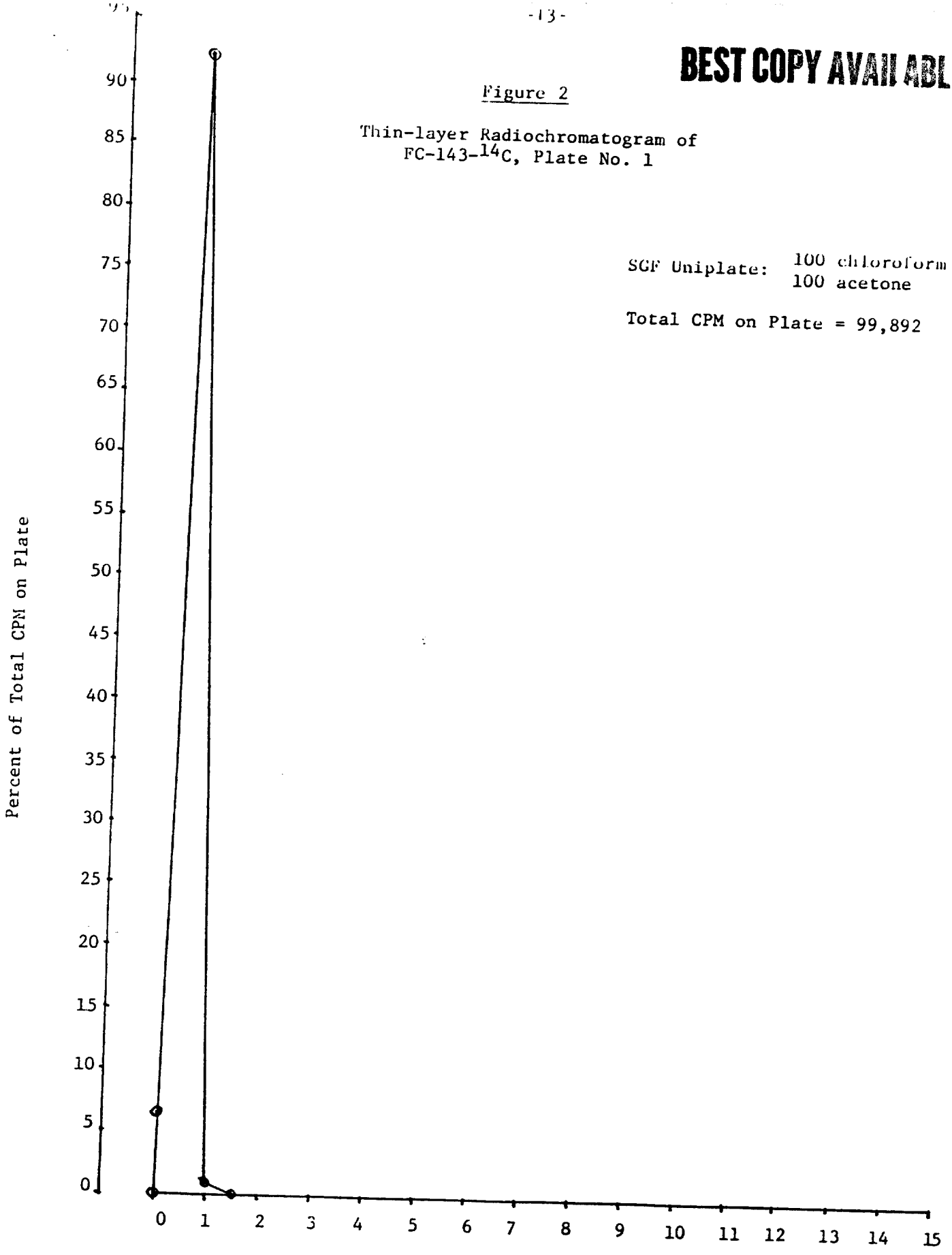

Figure 3

Thin-layer Radiochromatogram of
FC-143-^{14}C, Plate No. 2

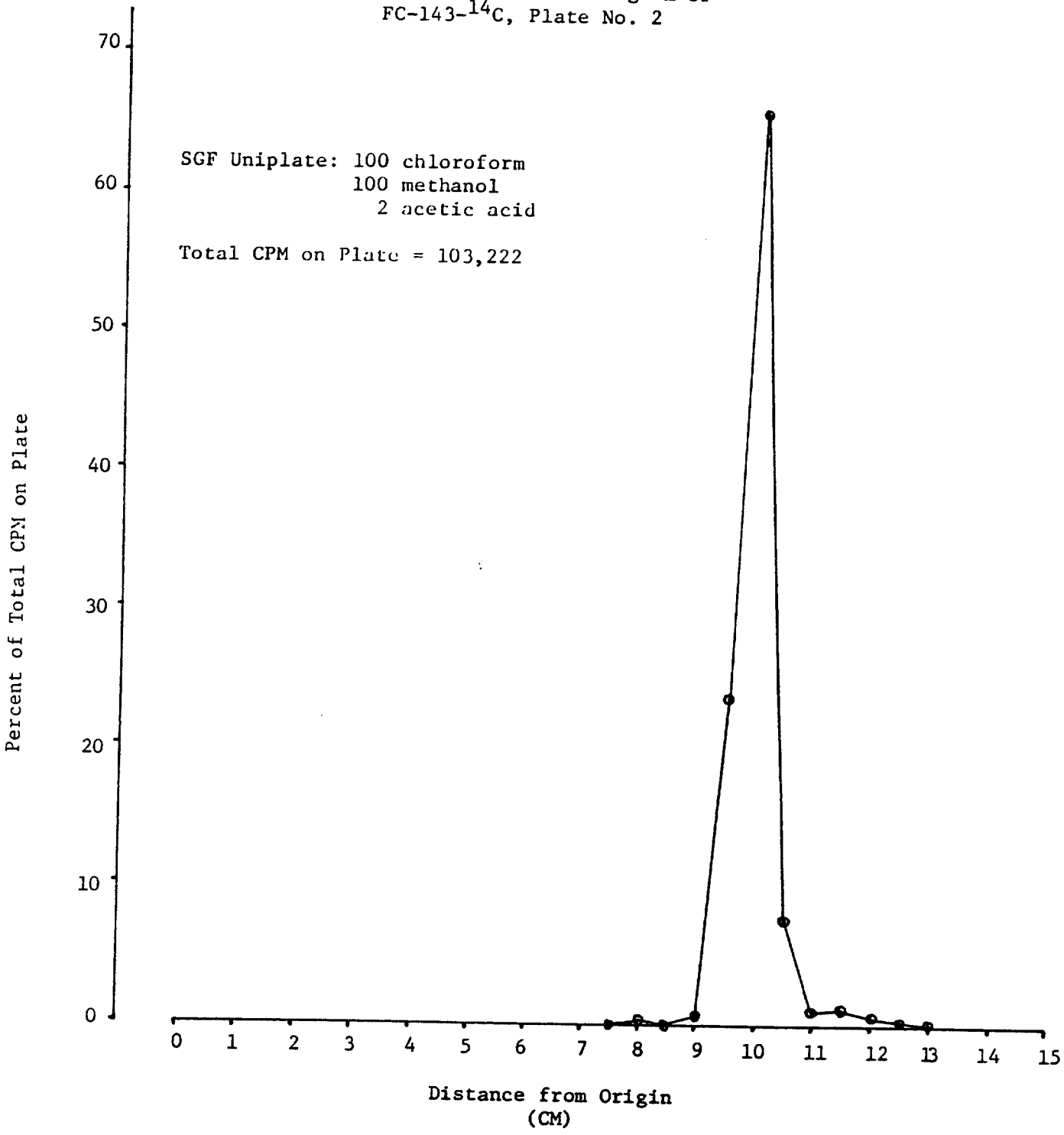

Figure 4

Thin-layer Radiochromatogram of
FC-143-^{14}C, Plate No. 3

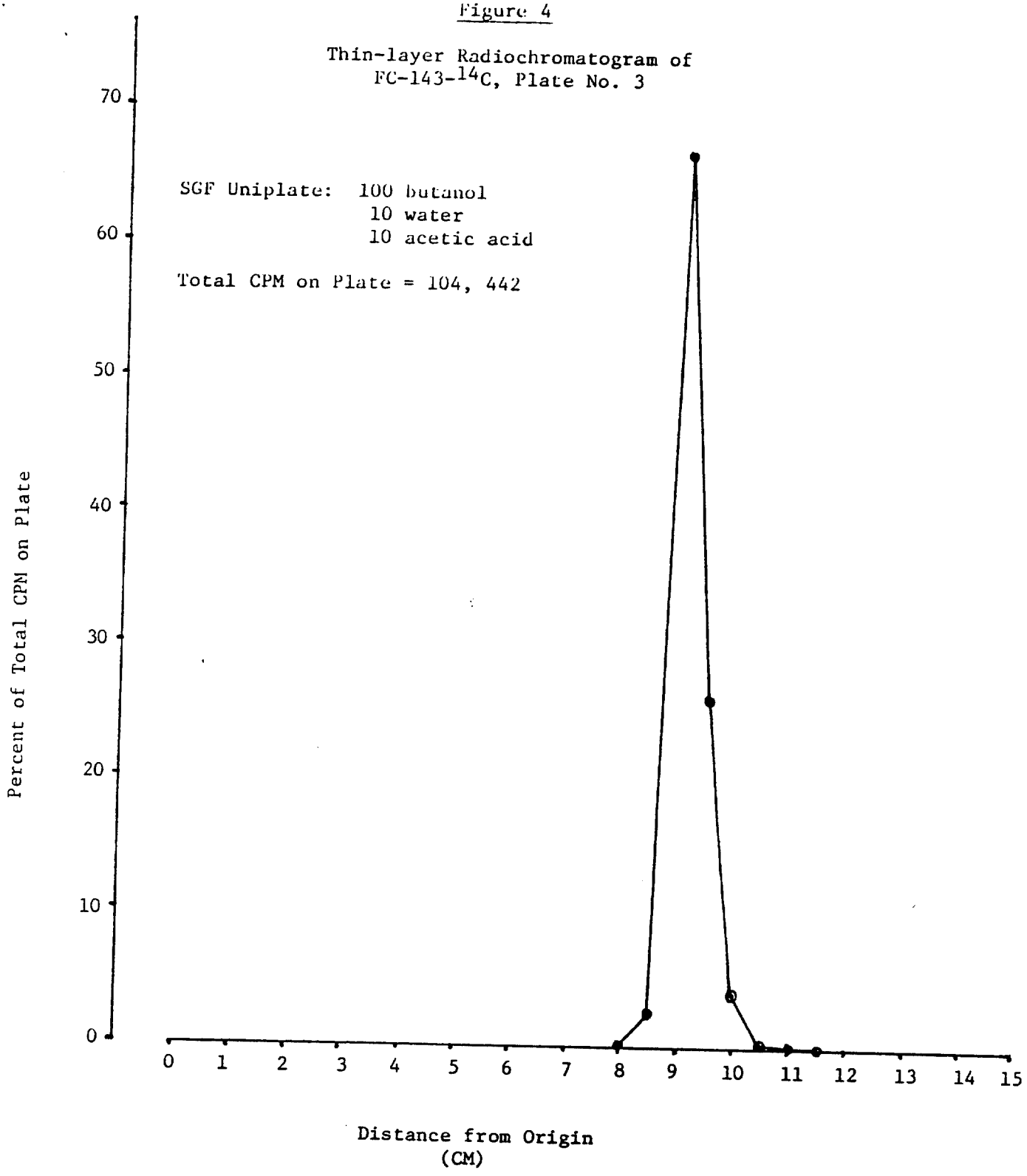

Figure 5

Thin-layer Radiochromatogram of
FC-143-^{14}C, Plate No. 4

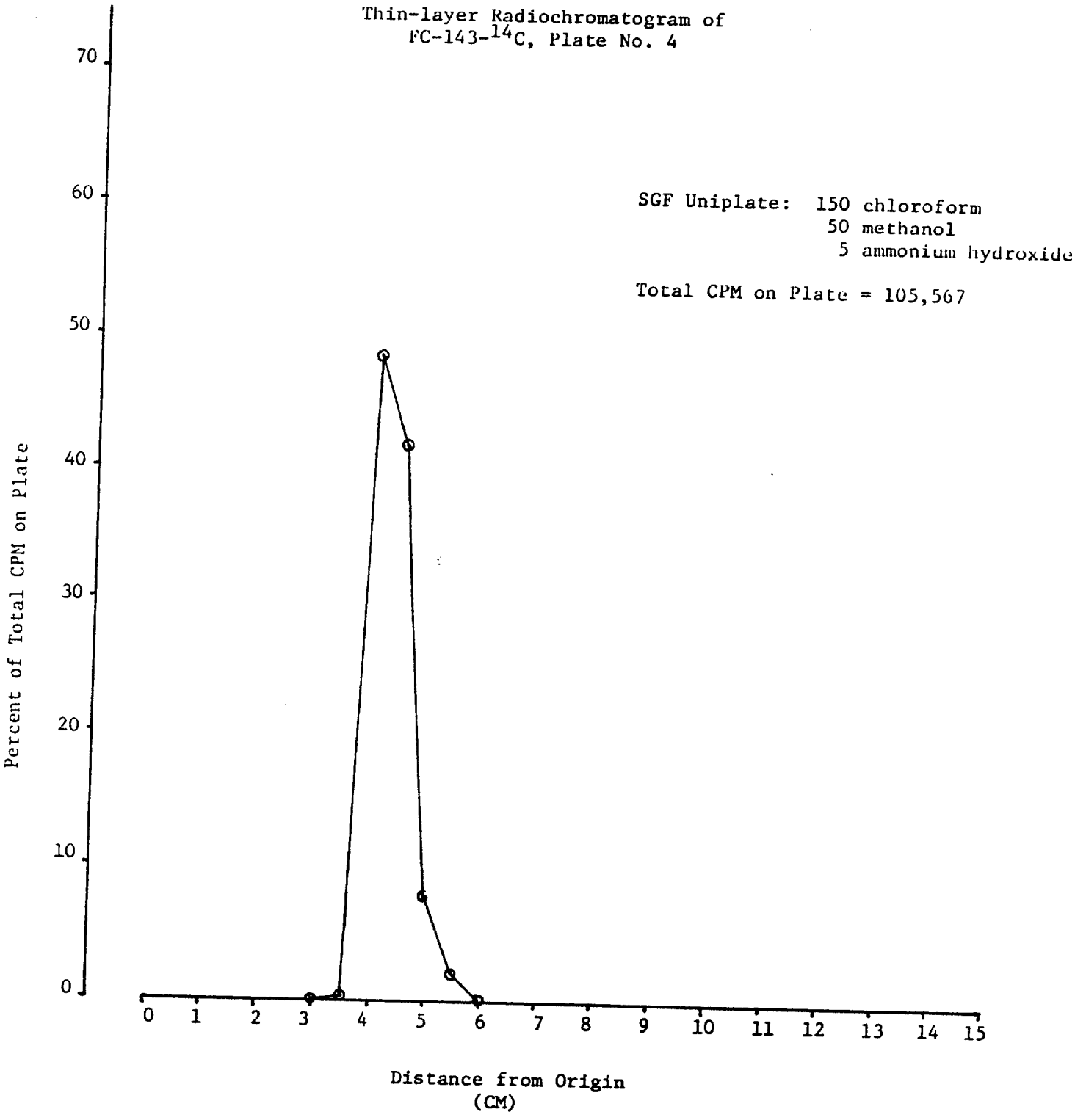

Figure 6

Thin-layer Radiochromatogram of
FC-143-^{14}C, Plate No. 5