Synthesis and Characterization of N-Ethyl FOSE-^{14}C

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Synthesis Conducted by:	F.E. Behr
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Summary

The synthesis of a 10.0 g lot of 2-N-ethylperfluorooctanesulfamido ethanol (N-Ethyl FOSE-^{14}C), L-4545, is described (carbon α to sulfur atom is labeled). The specific activity was determined to be 0.483 ± 0.020 μCi/mg. Radiochemical purity determination showed the N-Ethyl FOSE-^{14}C to be at least 98% pure. The N-Ethyl FOSE was found suitable for metabolism studies.

Introduction

A series of experiments has been started to investigate the metabolism of N-Ethyl FOSE-^{14}C. To facilitate these experiments, carbon-14 labeled N-Ethyl FOSE was synthesized and characterized. The synthetic pathway illustrated in Figure I is described.

A. Synthesis of N-Et FOSE-^{14}C (L-4545).

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

The fractional distillation of crude cell drainings from cell run R-3256-B has been previously described (1). GLC[a] analysis showed 98.15% $C_8F_{17}SO_2F$.

II. Amidation of $C_7F_{15}{}^*CF_2SO_2F$ (Step 2).

Into a tared 250 ml three-necked round-bottomed flask was added $C_7F_{15}{}^*CF_2SO_2F$ (69.0 g). The flask was fitted with an inlet and exit gas bubbling assembly, a glass dip tube, condenser, thermometer and a drying tube. Anhydrous ethylamine was introduced to the heated fluorochemical at 60°C until excess $C_2H_5NH_2$ was observed at the exit side of the reaction apparatus. A total of 46.3 g of $C_2H_5NH_2$ was lost from the cylinder mass. The reaction mixture was stirred at 70°C for 1.5 hrs. Dry nitrogen gas was used to de-gas the reaction mixture. The reaction mixture was treated by the addition of sulfuric acid (10%, 75 ml). The mixture was heated at 50°C for 0.25 hrs and the upper aqueous phase was discarded (pH 4-5). Additional sulfuric acid (10%, 75 ml) was added and the mixture was stirred at 70°C for 0.25 hr. Upon cooling, the acidic aqueous phase (pH <1, Congo red paper) was decanted from the solid fluorochemical phase. The fluorochemical phase was washed with deionized water until the pH of the wash solution was 4.5 to 5.0. Four water washes were needed. The fluorochemical was heated at 70°C for 0.25 hr at reduced pressure (vacuum pump) to remove residual water. A crude yield of 67.7 g (95.2%) was obtained.

III. Reaction of N-Ethyl perfluorooctanesulfonamide with Ethylene Chlorohydrin (Step 3).

Into a pre-tared 200 ml three-necked round-bottomed flask, which had been equipped with a distillation head and condenser, thermometer, an additional funnel and a magnetic stirring bar, was placed the crude radioactive sulfonamide (67.7 g) from Step 2; ethylene glycol (33.9 ml, RM[b] 3017) and 25% sodium methoxide in methanol (36.6 g) was added to the ethylene glycol-sulfonamide mixture. Methanol (10 ml) was used to wash the addition funnel. The mixture was heated to 55°C to effect the formation of sodium N-ethyl perfluorooctanesulfonamide and to start the distillation of methanol. The residual methanol was removed with water aspirator vacuum and, finally, the mixture was heated at 70°C and 3-4 mm vacuum. The mixture was cooled to 48°C, the vacuum was broken and anhydrous powdered sodium carbonate (3.8 g), ethylene chlorohydrin (17.0 ml) and ethylene glycol (33.0 ml) were added rapidly. A vigorous reaction occurred as the mixture was heated to 60°C.

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[a] Quality Control Manual called for 96.0% min.

[b] RM = Raw Material.

After the initial exothermic reaction had subsided, the mixture was heated first to 90°C for 1.0 hr and then the temperature of the mixture was raised to 115°C for 5 hrs. The mixture was cooled to 80°C and the mixture was washed successively with water, brine (three times) and the residual water was stripped by vacuum at 75°C. Crude alcohol (72.7 g) was vacuum distilled to yield two fractions: FR #1, 57.3 g, b.p. $_{3.5}$ 142-144° and FR #2, 7.6 g, b.p. $_{5.4}$ 146-148°. GLC analysis showed FR #1 and #2 to be > 99.9%

$$C_8F_{17}SO_2N\begin{matrix}C_2H_5\\CH_2CH_2OH\end{matrix}$$ (see reference 2).

The non-distillable material (5.0 g) was discarded as radioactive waste material. FR #1 and #2 were combined and melted to give N-Et FOSE[a] (Step 3) in 64.9 gm yield. A ten gram sample was taken from the total sample and was assigned the following designation: L-4545, Carbon-14 labeled N-Et FOSE.

B. Specific Activity Determination of N-Ethyl FOSE-^{14}C.

Three standard solutions of N-Ethyl FOSE-^{14}C[b] (L-4545 see Synthesis Section) were prepared by weighing I 17.47 mg, II 37.55 mg, and III 25.08 mg into three 10 ml class A volumetric flasks[c]. The volumes were adjusted to 10 ml with methanol and the flasks were mixed by inverting by hand. Calibrated micropipettors[d] 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®[e] were added. The remaining vials were prepared with 2.5 ml of methanol and 7.5 ml of MTSS[f]. Analyses of three standard solutions allow comparison of three primary weighings. Analyses of 10 µl and 50 µl aliquots from each solution allow comparison of the precision of the micropipettors. The use of two different scintillation solvents allows comparison of solubility. (MTSS is toluene based and Aquasol® is a water gel.) 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 1. The overall average specific activity based on the 36 replicates (12 from each weighing) was 0.483±0.020 µCi/mg. The mean µCi/mg found for each of the primary weighings was within 1.2% of the overall mean. The mean µCi/mg between Aquasol® and MTSS were within 4.1%, and the mean µCi/mg between 10 µl and 50 µl aliquots were within 0.7%.

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[a] 2-N-Ethylperfluorooctanesulfonamido ethanol.

[b] Riker Isotope Number 468.

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

[d] L/I Micropippetor, Lab Industries, Berkeley, CA.

[e] New England Nuclear, Boston, Mass.

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

C. Radiochemical Purity Analysis of N-Ethyl FOSE-^{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" 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 mixed by shaking and cooled in the dark. 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. Column Chromatography System

A 14 mm column was packed to 21 cm with a slurry of silicic acid[c] in chloroform. A one ml aliquot of N-Ethyl FOSE-^{14}C test solution (see next section C-III) was applied to the column. The column was eluted successively with 200 ml of chloroform, 200 ml of 1:1 chloroform-methanol, and 200 ml of methanol. The three fractions were evaporated to dryness, reconstituted in 200 µl of 1:1 chloroform-methanol and streaked on three separate 5 x 20 cm plates. The plates (No. 6, 7, and 8) were developed in the same solvent system as Plate No. 5.

III. Solution Used for Radiochemical Purity Analysis

The solution used to streak the thin-layer plates was prepared by placing 26.16 mg of N-Ethyl FOSE-^{14}C (Riker Isotope Inventory Number 468) 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 to each plate. One ml of this solution was applied to the silicic acid column.

IV. Results and Discussion

Since the chemical identity of the N-Ethyl FOSE-^{14}C is well established by synthesis and gas chromatography (see Section A, this report), unlabeled N-Ethyl FOSE was not co-chromatographed with the

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

[b] Supelco Inc., Bellefonte, Pennsylvania.

[c] Unisil, activated silicic acid 100/120 mesh, Clarkson Chemical Company, Inc., Williamsport, Pennsylvania.

N-Ethyl FOSE-^{14}C. The results of the thin-layer radiochemical purity analysis are shown in Table 1 and Figures 1-5. Small amounts of impurity (<2%) were present either at the origin or just before the N-Ethyl FOSE-^{14}C peak in Plates No. 1-5. The impurities were separated by the column; 99.2% of the radioactivity recovered was eluted with chloroform, 0.7% with 1:1 chloroform-methanol and 0.1% with methanol. Plate No. 6 is a radiochromatogram of the chloroform fraction. The N-Ethyl FOSE-^{14}C peak contained 99.3% of the total radioactivity recovered from the plate. Thus, this analysis indicates a radiochemical purity of 98.5% (99.3% of the total in the fraction [99.2%] = 98.5%). The presence of small amounts of impurities is demonstrated for the other two column fractions by Plates No. 7 and 8. No attempt was made to identify these minor impurities. Overall, the radiochemical purity of N-Ethyl FOSE-^{14}C is at least 98%. This is in agreement with the GLC analysis (see Section A, this report). The N-Ethyl FOSE-^{14}C is suitable for metabolism studies.

Acknowledgement

The authors gratefully acknowledge the gas chromatographic analysis of the carbon-14 labeled cell drainings and N-Ethyl FOSE-^{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 $C_8H_{17}SO_2F$.

References

1. Johnson JD and Behr FE: Synthesis and Characterization of FC-95-^{14}C (Report) November 2, 1979.

2. Analytical Report Number 14557.

Table 1

Specific Activity Determination of N-Ethyl FOSE-^{14}C

Solution I	Solution II	Solution III
17.47 mg/10.0 ml	37.55 mg/10.0 ml	25.08 mg/10.0 ml
µCi/mg	µCi/mg	µCi/mg
0.4854	0.5048	0.4828
0.4832	0.4986	0.4848
0.4811	0.5000	0.4853
0.4822	0.4533	0.4749
0.4827	0.4749	0.4709
0.4790	0.4635	0.4853
0.4935	0.4864	0.5065
0.5008	0.4953	0.4955
0.5080	0.4900	0.4946
0.4297	0.4843	0.4833
0.4958	0.4920	0.4916
0.4100	0.4773	0.4939
0.4776±0.0287	0.4850±0.0154	0.4875±0.0096

Aquasol®	MTSS	10 µl	50 µl
µCi/mg	µCi/mg	µCi/mg	µCi/mg
0.4854	0.4822	0.4854	0.4935
0.4832	0.4827	0.4832	0.5008
0.4811	0.4790	0.4811	0.5080
0.4935	0.4297	0.4822	0.4297
0.5008	0.4958	0.4827	0.4958
0.5080	0.4100	0.4790	0.4100
0.4986	0.4533	0.4986	0.4864
0.5048	0.4749	0.5048	0.4953
0.5000	0.4635	0.5000	0.4900
0.4864	0.4843	0.4533	0.4843
0.4953	0.4920	0.4749	0.4920
0.4900	0.4773	0.4635	0.4773
0.4828	0.4749	0.4828	0.5065
0.4848	0.4709	0.4848	0.4955
0.4853	0.4853	0.4853	0.4946
0.5065	0.4833	0.4749	0.4833
0.4955	0.4916	0.4709	0.4916
0.4946	0.4939	0.4853	0.4939
0.4931±0.0087	0.4735±0.0225	0.4818±0.0123	0.4849±0.0251

Overall average = 0.4834±0.0195

See text (Section B) for description of sample preparation.

List of Tables and Figures

Table 2

Thin-Layer Chromatography Systems for N-Ethyl FOSE-^{14}C

Plate No.	Solvent System[a]	[b] R_f of N-Ethyl FOSE-^{14}C
1	100 chloroform 100 acetone	0.67
2	100 chloroform 100 methanol 2 acetic acid[c]	0.77
3	100 butanol 10 water 10 acetic acid[c]	0.73
4	150 chloroform 50 methanol 5 ammonium hydroxide[c]	0.80
5	100 chloroform 35 methanol 5 ammonium hydroxide[c]	0.70

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

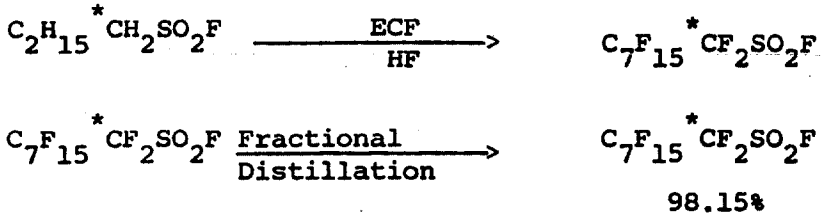
[b] R_f is of major (>98%) peak on the thin-layer chromatography plate.

[c] Acetic acid and ammonium hydroxide were concentrated.

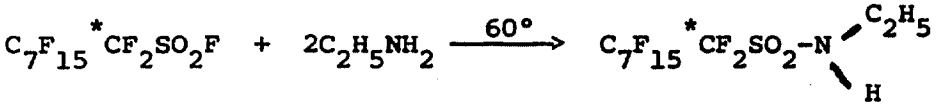
Figure 1

Synthetic Pathway for Synthesis of N-Ethyl FOSE-^{14}C[a]

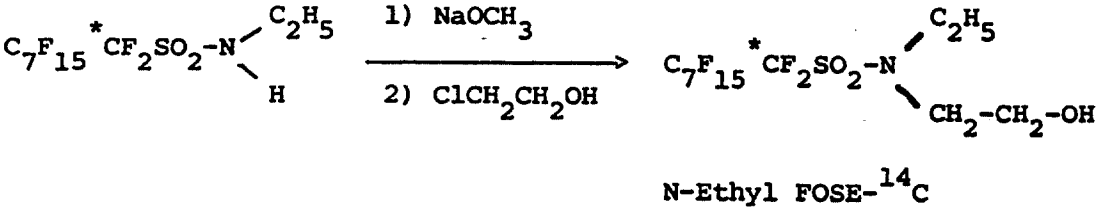
Step 1



Step 2



Step 3



N-Ethyl FOSE-^{14}C

*Denotes position of carbon-14 label.

[a]N-Ethyl FOSE is 2-N-ethylperfluorooctanesulfonamido ethanol.

Figure 2

Thin-layer Radiochromatogram of N-Ethyl FOSE-^{14}C, Plate No. 1

SGF Uniplate: 100 chloroform
100 acetone

Total CPM on Plate = 88,572

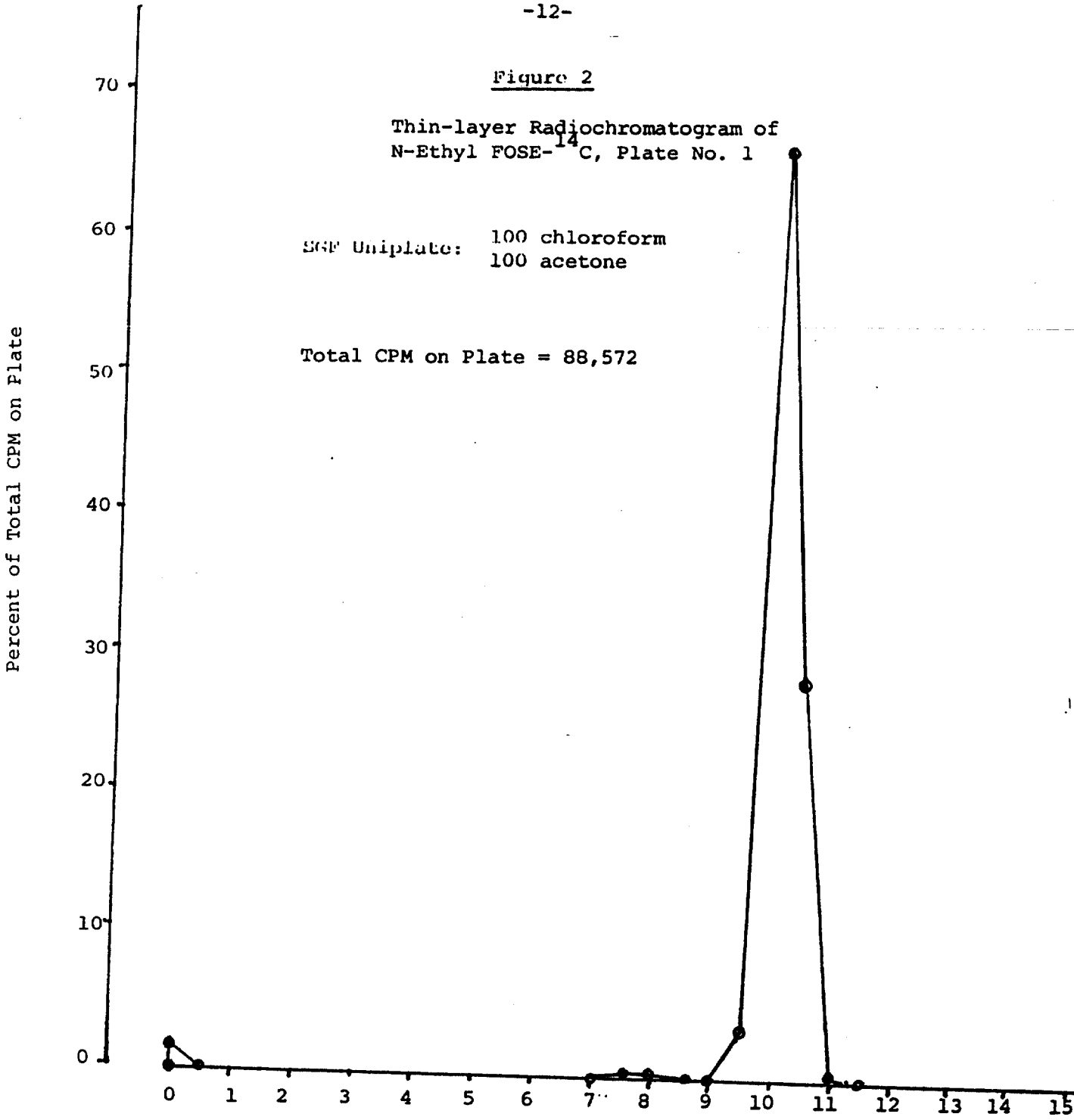


Figure 3

Thin-layer Radiochromatogram of
N-Ethyl FOSE-^{14}C, Plate No. 2

SGF Uniplate: 100 chloroform
100 methanol
2 acetic acid

Total CPM on Plate = 82,187

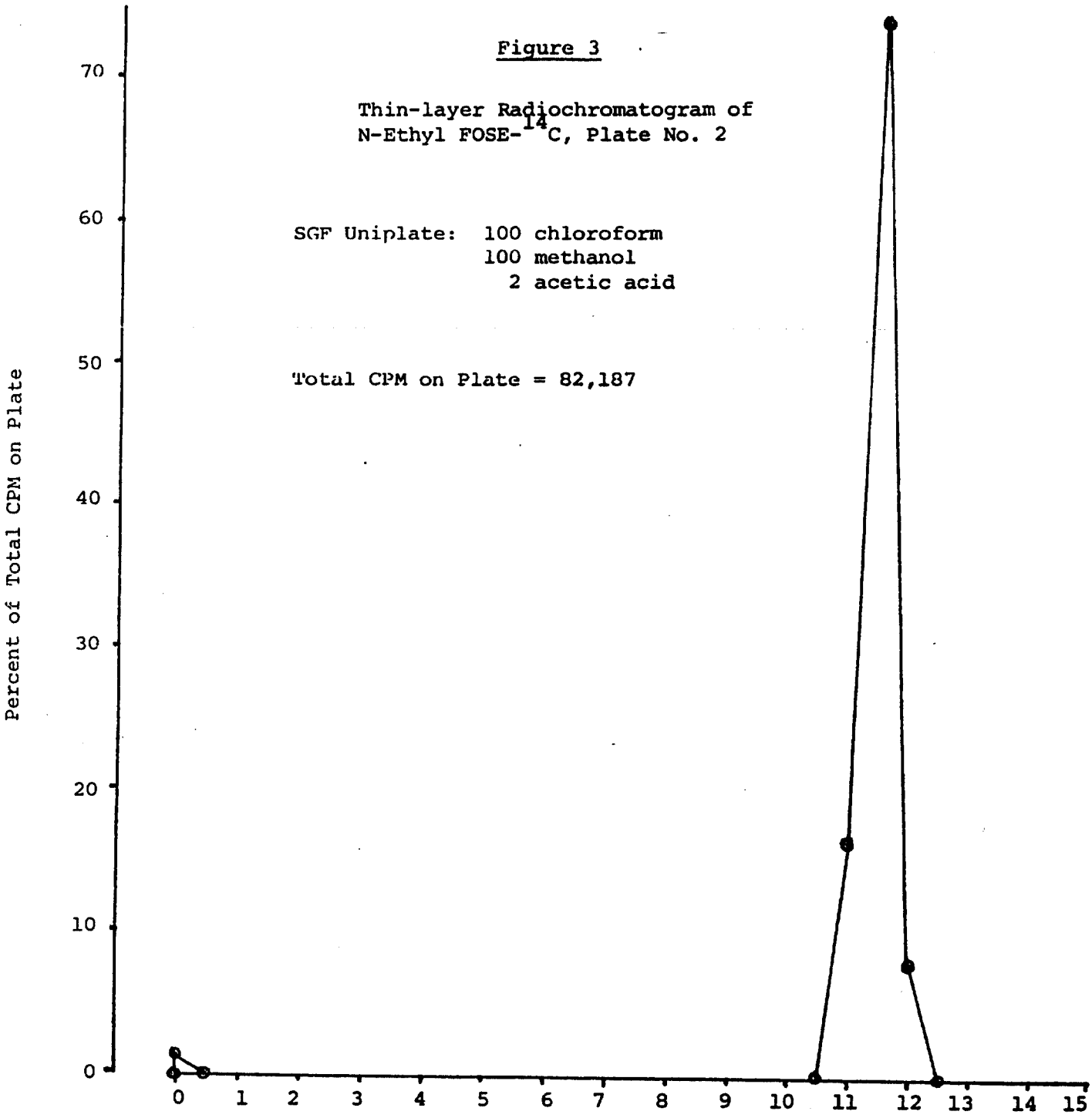


Figure 4

Thin-layer Radiochromatogram of
N-Ethyl FOSE-^{14}C, Plate No. 3

SGF Uniplate: 100 butanol
10 water
10 acetic acid

Total CPM on Plate = 89,696

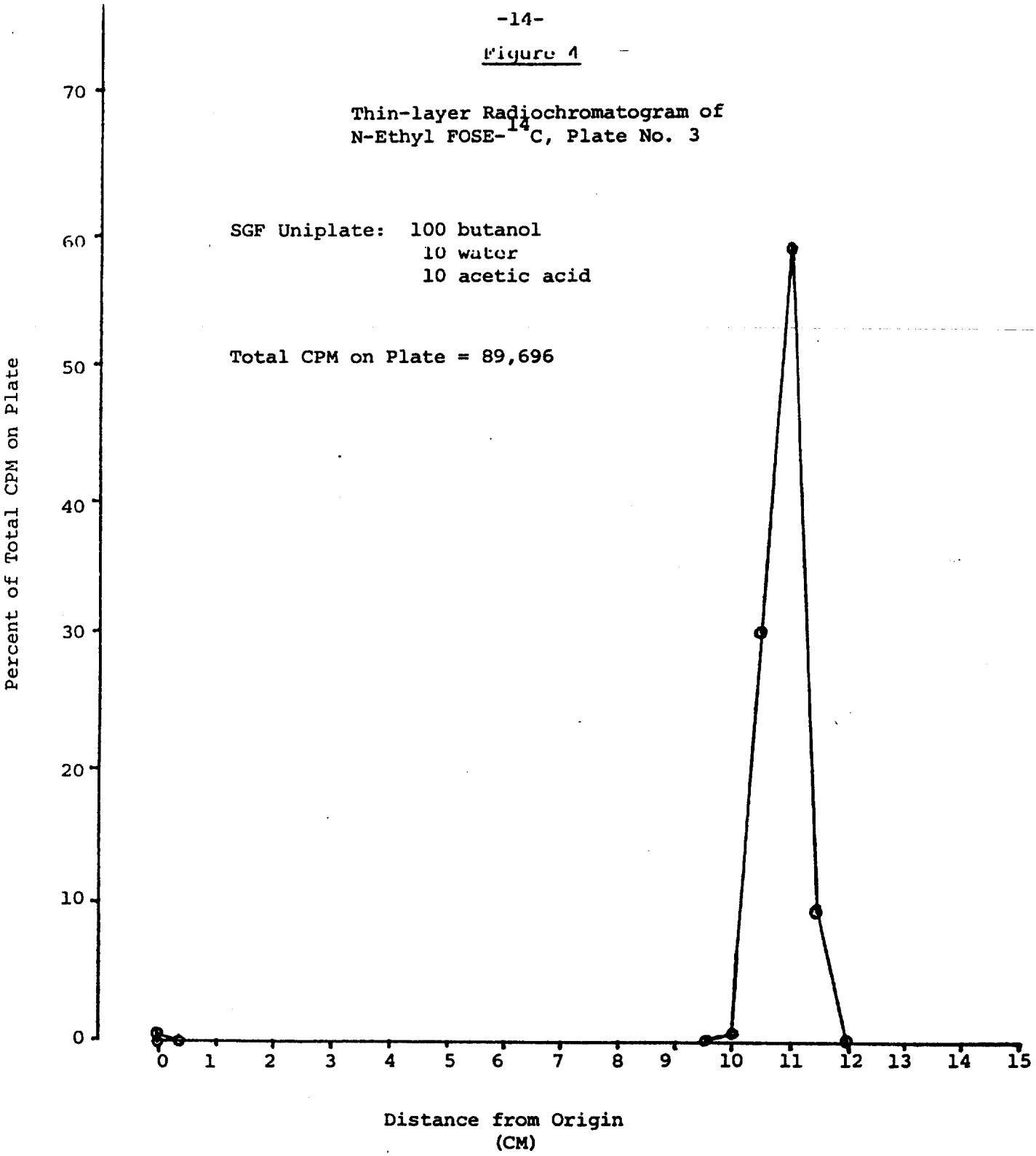


Figure 5

Thin-layer Radiochromatogram of
N-Ethyl FOSE-^{14}C, Plate No. 4

SGF Uniplate: 150 chloroform
50 methanol
5 ammonium hydroxide

Total CPM on Plate = 91,164

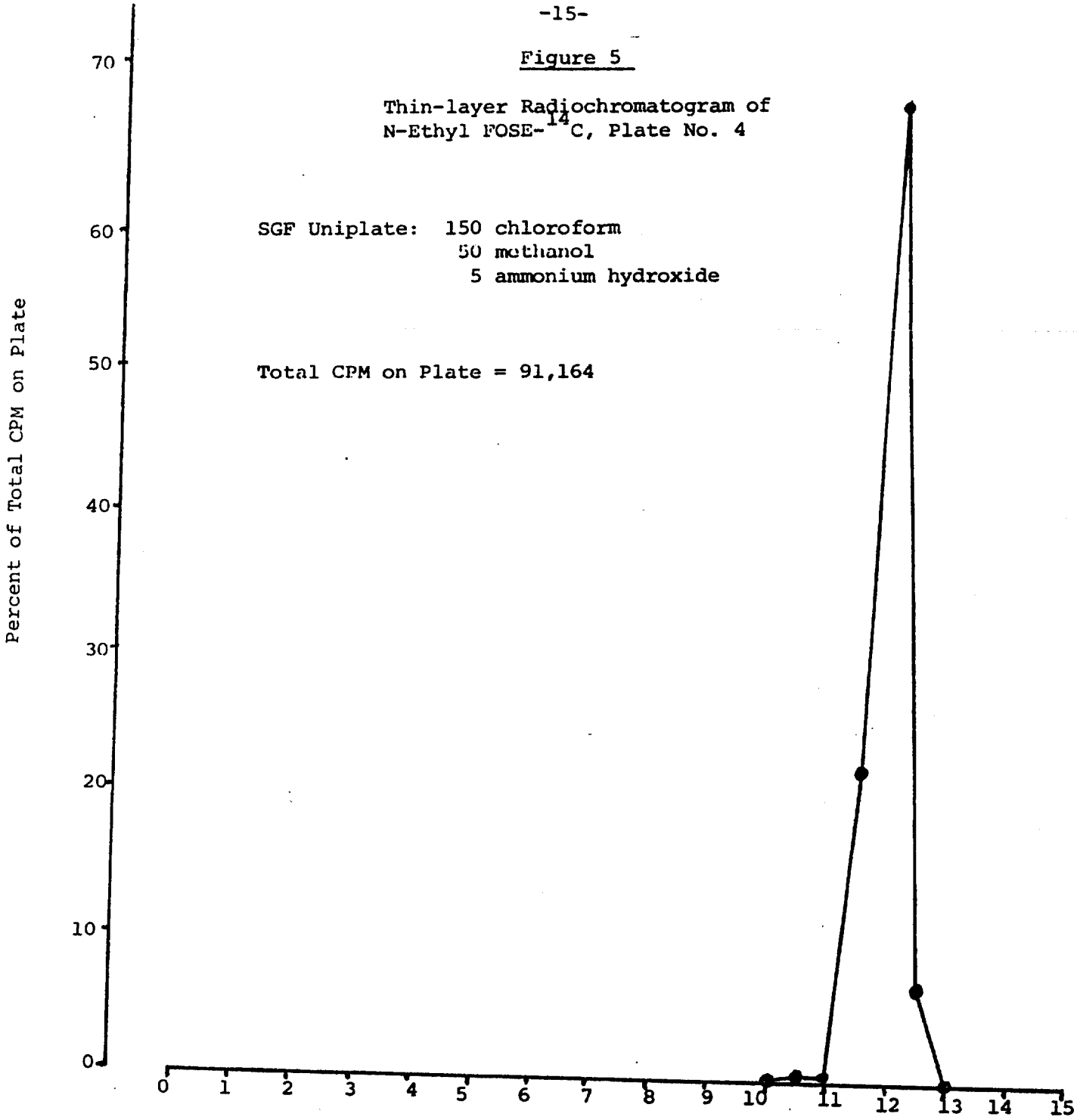


Figure 6

Thin-layer Radiochromatogram of
N-Ethyl FOSE-^{14}C, Plate No. 5

SGF Uniplate: 100 chloroform
35 methanol
5 ammonium hydroxide

Total CPM on Plate = 90,961

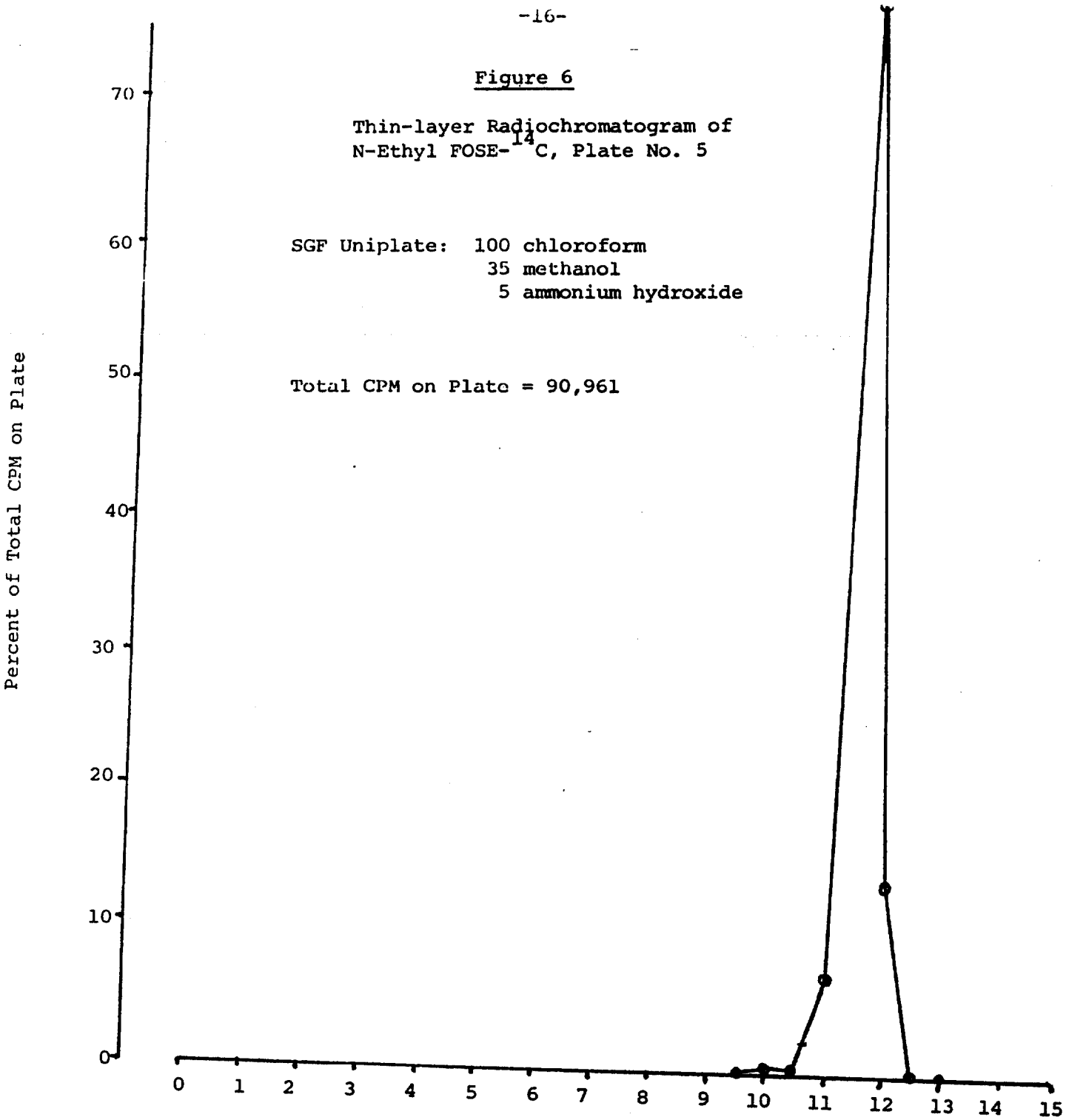


Figure 7

Thin-layer Radiochromatogram of Chloroform column fraction
N-Ethyl FOSE-^{14}C, Plate No. 6

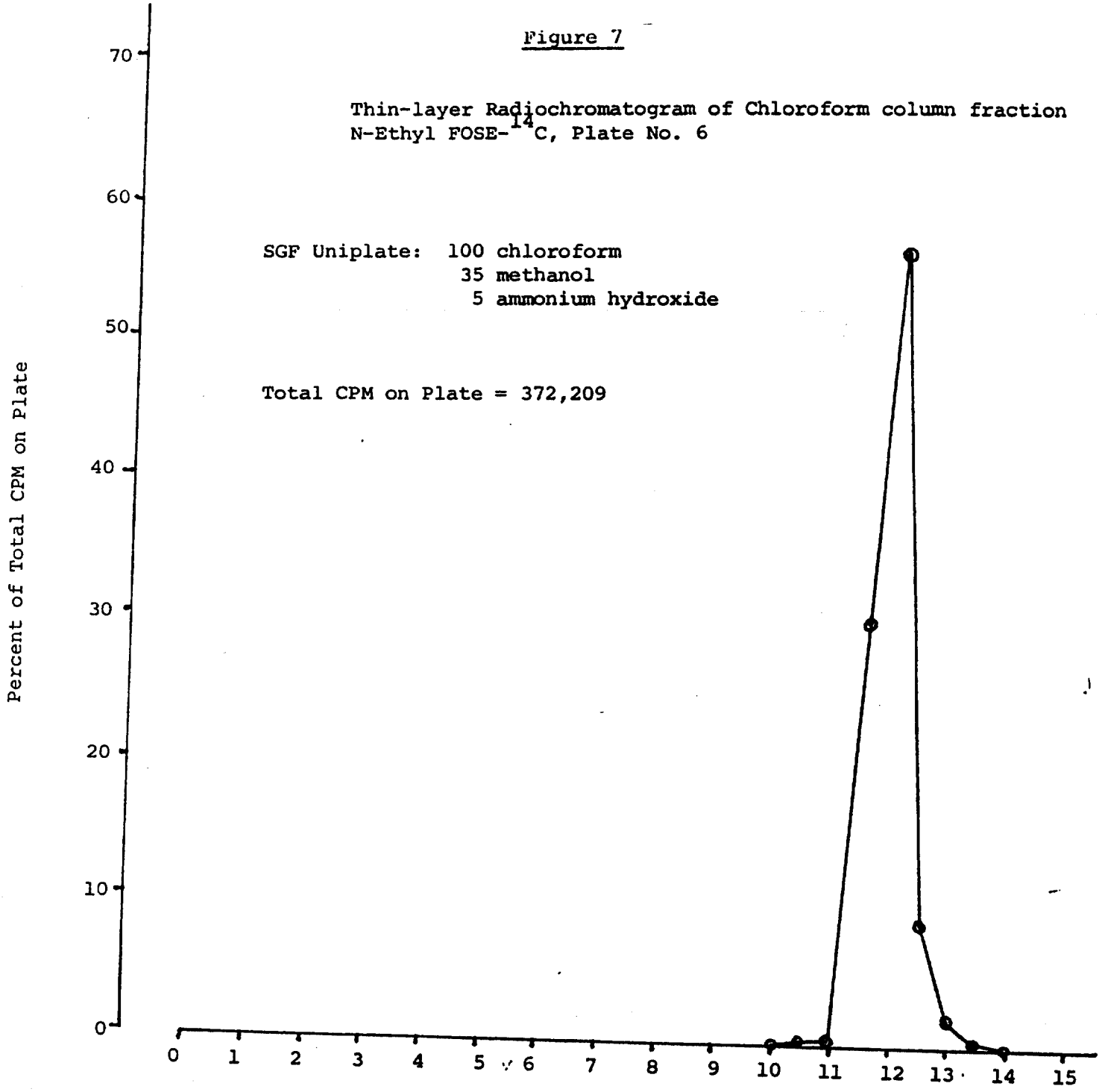


Figure 8

Thin-layer Radiochromatogram of 1:1 Chloroform-Methanol Column Fraction of N-Ethyl FOSE-^{14}C, Plate No. 7

SGF Update: 100 chloroform
35 methanol
5 ammonium hydroxide

Total CPM on Plate = 2,594

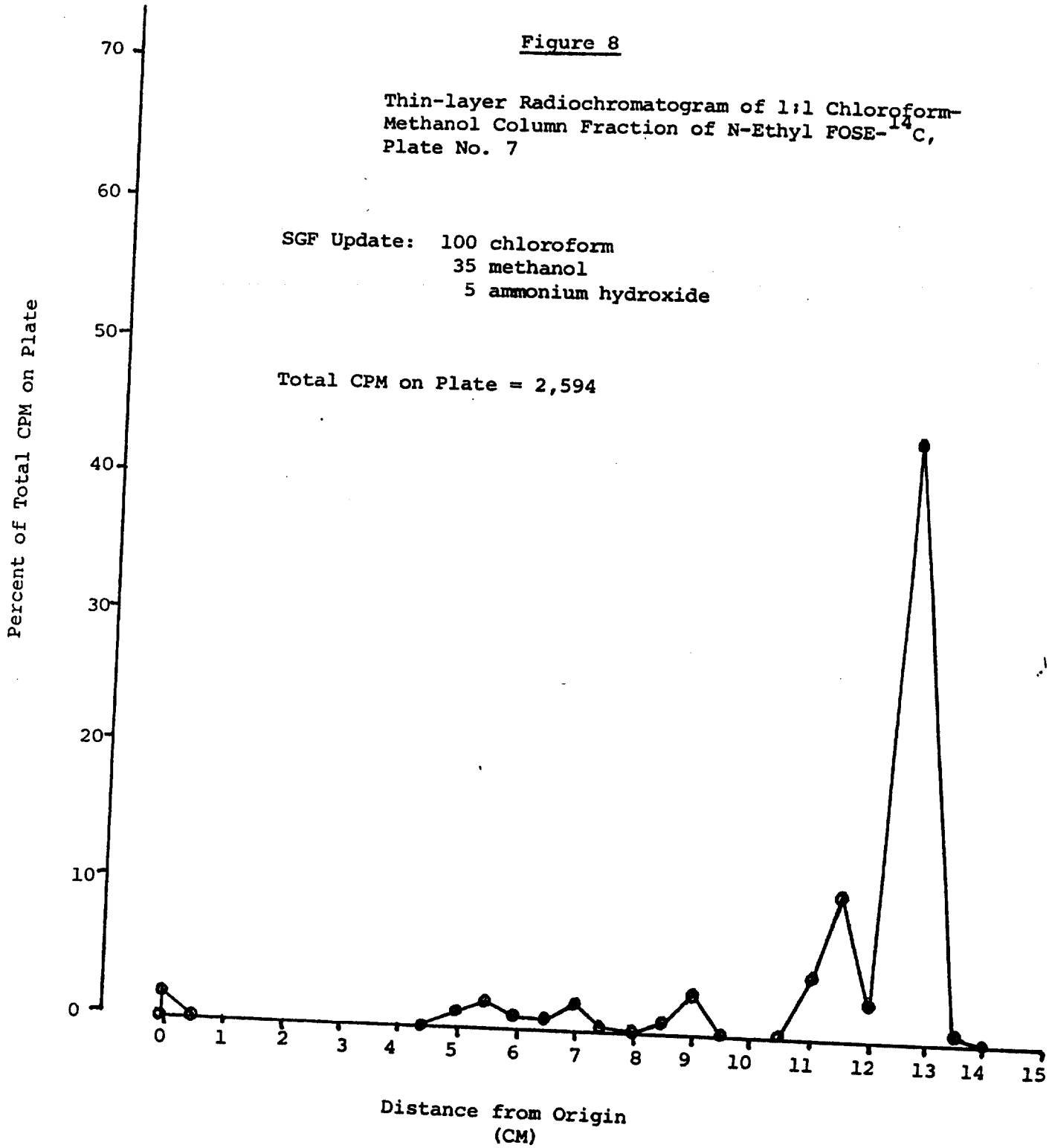
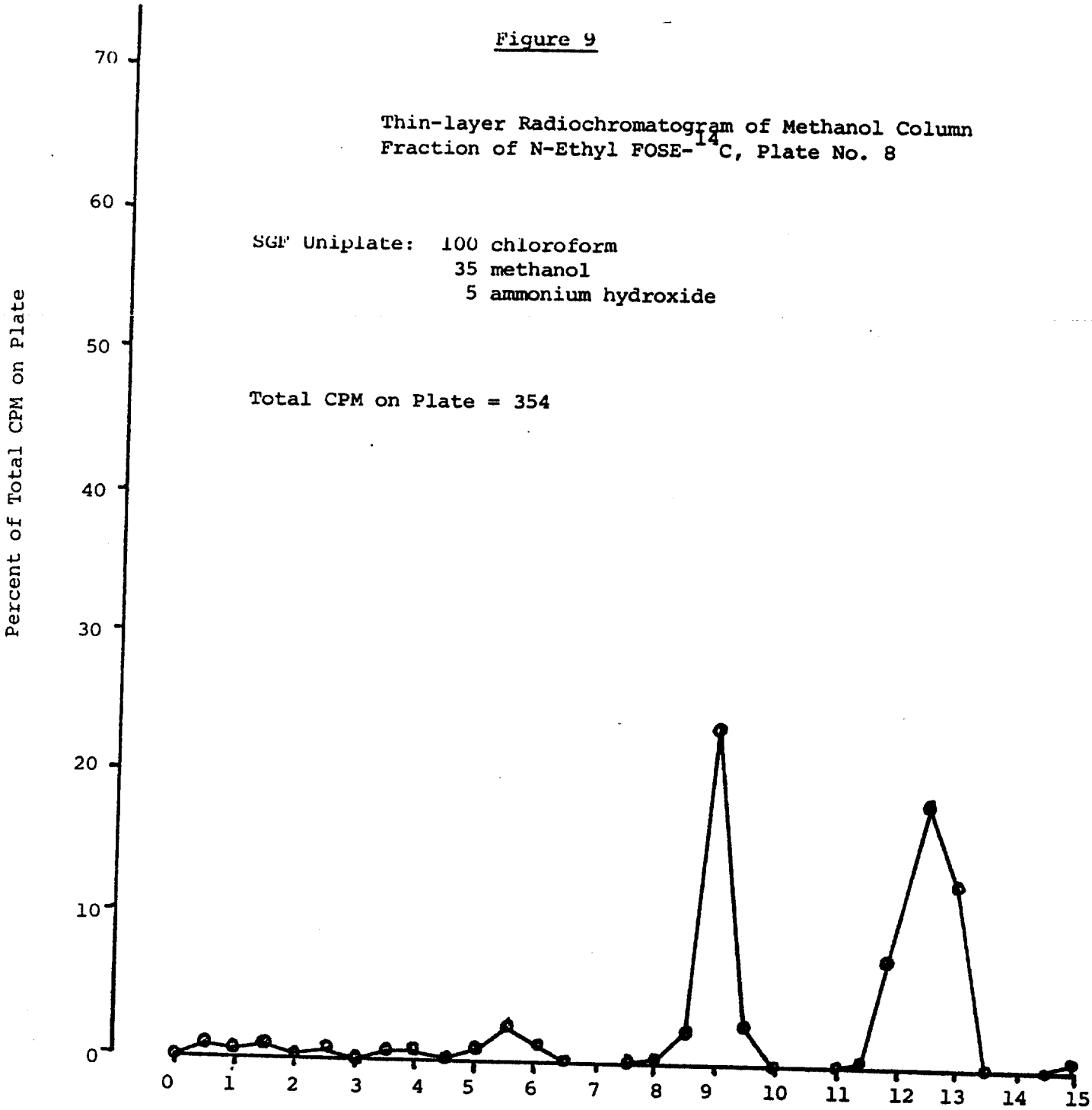


Figure 9

Thin-layer Radiochromatogram of Methanol Column Fraction of N-Ethyl FOSE-^{14}C, Plate No. 8

SGF Uniplate: 100 chloroform
35 methanol
5 ammonium hydroxide

Total CPM on Plate = 354



Distribution List

M.T. Case	218-2
S.F. Chang	218-2
G.J. Conard	218-2
H.E. Freier	201-1S
L.I. Harrison	218-2
J.D. LaZerte	236-1
L.J. Magill	223-6S
R.A. Nelson	218-1
R.E. Ober	218-2
R.A. Prokop	236-3B
A.L. Rosenthal	230-3
F.A. Ubel	220-2E
P. Venkateswarlu	236-3A
H.A. Vogel	236-3B
Drug Metabolism Central File	218-2
Tech Communications	201-2CN