Inexpensive cartridge for the collection of radioactive methyl esters from gas–liquid chromatographs

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SUMMARY Glass wool was substituted for anthracene in glass cartridges designed for the collection of methylated fatty acids in the effluent stream from a gas–liquid chromatograph. Inexpensive cartridges that gave the same results as those filled with anthracene were obtained.

KEY WORDS gas–liquid chromatography · fatty acid methyl esters · radioactive · collection · glass wool · anthracene · scintillation counting

METHYL ESTERS of radioactive fatty acids in the effluent stream from a gas–liquid chromatograph[1] can be conveniently collected by using cartridges filled with anthracene or *p*-terphenyl in conjunction with an appropriate fraction collector (1, 2). Such a method is particularly useful for the collection of samples of low radioactivity because the cartridges can be counted repeatedly in a liquid scintillation counter for long enough periods to assure reliable values. The cost of these cartridges, however, may be prohibitive if they are to be used for an extensive series of assays.

We have modified the collection method by filling the glass cartridges (Tri-carb cartridges No. 6001039 for Tri-carb Fraction Collector, Packard Instrument Company, Inc., La Grange, Ill.) with glass wool (Pyrex brand, fine grade) to about 5 mm from either end. After the collection the glass wool is extruded into a low-potassium glass vial containing 14 ml of scintillator solution (0.3 g of *p*-bis[2-(5-phenyloxazolyl)] benzene and 5.0 g of 2,5-diphenyloxazole in 1 liter of reagent grade toluene) and the empty cartridge is also dropped into the same vial for counting. With chromatograph and collector arm at 210°C, the methyl esters condensed as completely on the glass wool at room temperature as on anthracene at room temperature (Table 1). To check whether further passage of hot carrier gas would volatilize any of the deposit, we applied a mixture of methyl esters, to which linoleate ^{14}C had been added, directly to a glass-wool cartridge which was then held in the effluent stream. No radioactivity was lost during a heating period of 3 min.

Table 1 shows the method to be as satisfactory as that using anthracene for collection of fatty acid methyl esters from a standard mixture or from liver or plasma lipids.

[1] We used a Beckman GC-2A instrument with an 8 ft × 1/4 inch I.D. column packed with 15% diethylene glycol succinate polyester on acid-washed Chromosorb W. The column temperature was 210°C, and the helium gas flow was 113 ml/min.

TABLE 1 DISTRIBUTION OF RADIOACTIVE COUNTS OF CERTAIN GAS CHROMATOGRAPHIC FRACTIONS COLLECTED ON CARTRIDGES FILLED WITH ANTHRACENE OR GLASS WOOL

	Anthracene				Glass Wool			
	Standard†				Standard			
Fraction*	1	2	Liver	Plasma	3	4	Liver	Plasma
				% of total counts				
1	1.1	1.0	0.0	0.0	0.8	1.0	0.1	0.0
2	4.0	4.1	3.9	0.0	3.1	4.6	4.3	0.0
3	1.2	1.0	16.5	1.3	1.2		16.6	1.2
4	87.1‡	87.5‡	4.7	0.7	90.1‡	89.8‡	4.1	1.2
5	3.1	2.9	8.6	0.6	2.3	1.9	8.7	0.7
6	2.2	2.0	44.4§	2.2	1.9	1.8	38.9§	2.2
7	1.3	1.5	16.7‖	55.2	0.7	0.9	21.9‖	57.0
8	—	—	3.5	5.3	—	—	3.7	6.4
9	—	—	1.8	1.6	—	—	1.7	1.3
10	—	—	—	5.4	—	—	—	3.8
11	—	—	—	16.3	—	—	—	13.4
12	—	—	—	11.3	—	—	—	12.6
				Total counts per 5 min				
	6050	5208	8262	20,650	4917	7975	10,450	16,899
Ratio of sum of counts collected on glass wool: sum of counts collected on anthracene after correction for amounts of total sample applied					1.0	1.11	1.02	1.17

* The fractions are not comparable between standard, liver, and plasma. They were arbitrary cuts taken to test the method.

† Applied Science Laboratories, Inc., State College, Pa. Mixtures K104, H105, methyl palmitate, arachidonate, and linoleate-^{14}C. Standard run no. 1 has a different specific activity from run nos. 2, 3, and 4 which are the same.

‡ Methyl linoleate. Radioactivity in other fractions of the standard represent contamination.

§ ‖ These two fractions were difficult to duplicate because of the different column conditions in the two assays; however, the sum of 6 and 7 collected on anthracene was 61.1% of the total and that on glass wool was 60.8% of the total counts.

The fractions are arbitrary cuts taken for purposes of comparison. The total recovery and percentage distribution of counts between fractions agree, within experimental error, for the two types of cartridges. Fractions collected on glass-wool cartridges and counted in toluene may give a slightly higher efficiency than when anthracene cartridges are used for collection and counting.

Cartridges filled with glass wool exert no back pressure and give a smooth baseline on the chromatograms, in contrast to the effects of those filled with crystals. In addition to being considerably less expensive initially than the crystal-filled cartridges, the cartridges and vials may be reused, if they contain little radioactivity, after being washed twice in petroleum ether–alcohol and rinsed in acetone followed by hot water.

Individual fractions collected on glass wool may be recovered for chemical characterization or other work.

The authors are grateful to Mr. Pete Miljanich of the Department of Nutritional Sciences, University of California, Berkeley, for advice in the technical aspects of collecting the fractions and preparation of the manuscript.

This work was supported by PHS Research Grant No. AM 04309 from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, U.S. Public Health Service.

Manuscript received 5 November 1965; accepted 10 January 1966.

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