Determination of Sucrose Polyesters by High Performance Gel Permeation Chromatography

C. GRANT BIRCH and **FRED E. CROWE**, Miami Valley Laboratories,
The Procter and Gamble Company, Cincinnati, Ohio 45247

ABSTRACT

Sucrose polyester (SPE) in feces and diets is determined by freeze-drying the samples, extracting with ethyl ether, and subjecting the extracts to high performance gel permeation chromatography with tetrahydrofuran as solvent. SPE elutes as a single peak before other components of the extract, and is quantitated by a refractive index detector. The relative standard deviation for samples containing 11-60% SPE (dry weight basis) is 2%. The SPE is a mixture of sucrose hexa-, hepta-, and octa-esters with C_{16} and C_{18} fatty acids.

INTRODUCTION

Esters of polyhydric alcohols and long chain fatty acids have attracted interest as potential components of special-purpose diets for humans (1-3). One of the materials that has been so studied is sucrose polyester (SPE), a mixture of esters made up of sucrose esterified with 6, 7, and 8 molecules of fatty acid, predominantly C_{16} and C_{18} saturated and unsaturated acids. SPE has been found (1,2) not to be absorbed from the digestive system when it is administered orally to the rat. In those experiments, the measurement of absorption was accomplished by using SPE labled with ^{14}C, or by measuring the total content of fatty acids in the feces. Neither of these methods is suitable for use with human subjects. Therefore, the analytical method described here was developed. It relies on gel permeation chromatography, and can be used for determining SPE in human diets and in feces from the rat, dog, or human.

EXPERIMENTAL PROCEDURES

Sucrose Polyesters

The SPE samples used in this work were prepared by the base-catalyzed transesterification of sucrose and fatty acid methyl esters. The composition of a typical SPE is shown in Table I.

TABLE I

Typical Composition of Sucrose Polyester (SPE)

Percent of sucrose in SPE:	14.7%
Percent of TFA[a] in SPE:	91.5%
SPE distribution (by TLC):	
% SE_8 =	54%
% SE_7 =	41%
% SE_6 =	5%
	100%
Ester fatty acid distribution (by gas chromatography):	
C_{16}	10.4%
C_{18}	59.0%
$C_{18}(1=)$	17.6%
$C_{18}(2=)$	12.3%
$C_{18}(3=)$	0.5%
	99.8%
Molecular weights (as oleates):	
SE_8	2458
SE_7	2193
SE_6	1929

[a]TFA = total fatty acid.

Sample Preparation and Extraction

The samples to be analyzed were freeze-dried, weighed, homogenized in a blender, and aliquoted for assay, except that dog feces were mixed with half their weight of water and homogenized before freeze-drying.

Lipids were extracted from the dried samples with ethyl ether. The quantities and procedures differed slightly for different materials. Rat feces (0.3 g) and human diets (1 g) were extracted 3 times with 8 ml ether by agitating in a Vortex mixer, centrifuging, and drawing off the solvent with a pipet. Ten-gram samples of dog or human feces were extracted for 10 hr in a Soxhlet extractor with 250 ml ether. (The larger samples were necessary to obtain reproducibility in the subsequent aliquoting.) The extracts from either procedure were evaporated to dryness under a stream of nitrogen, and the residues were taken up in tetrahydrofuran (THF) to give solutions containing ca. 25 mg residue per ml. The THF solutions were centrifuged to prevent clogging of the chromatographic column with undissolved material which resulted from unknown matrix components having greater solubility in ethyl ether than THF. No SPE was in the residue.

Gel Permeation Chromatography

THF (Curtin Matheson Scientific, Cincinnati, OH, reagent grade) was drawn directly from the reagent bottle through a 50 μ frit, and delivered by a pump (Milton Roy Model 711-31, Laboratory Data Control, Riviera Beach, FL) past a 2000 psi pressure relief valve (PRV 2X2, Glenco Scientific, Inc., Houston, TX) into a loop injector (SVOV-6-IXC20, Glenco Scientific, Inc.), where 200 μl of sample solution was introduced into the solvent and carried into the chromatographic system which comprised in series two 7.6 mm ID x 30 cm columns packed with 500 Å pore-size μStyragel® and one 7.6 mm ID x 30 cm column packed with 1000 Å pore-size μStyragel® (Waters Associates, Inc., Milford, MA). The emergence of solute peaks in the efflu-

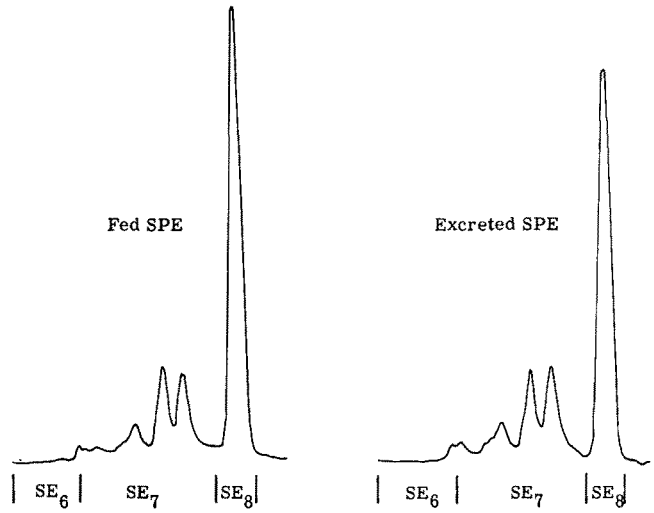

FIG. 1. Densitometer scans of fed and excreted sucrose polyester separated by TLC (70:30:1 hexane, diethyl ether, acetic acid) and charred with 25% w/v H_2SO_4. Ester identification based on comparison of R_f with standards.

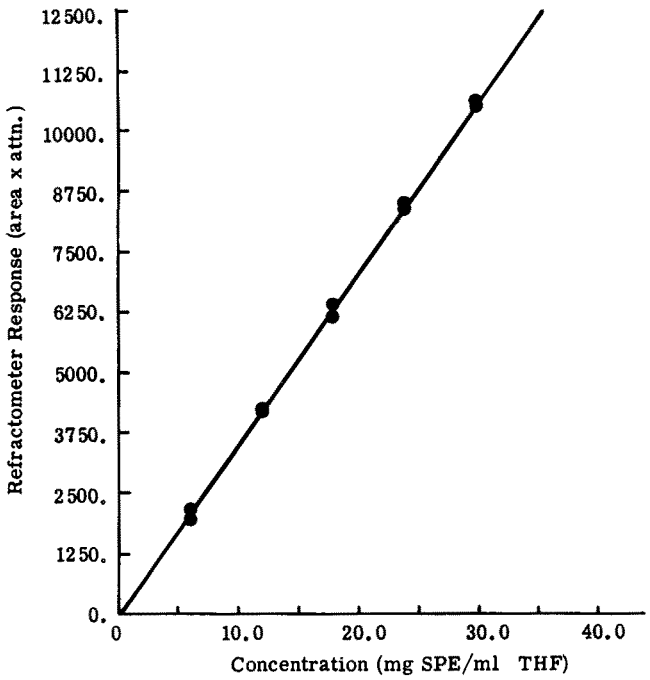

FIG. 2. Typical calibration curve of gel permeation chromatography apparatus. Dots represent experimental points and line is least-square plot. SPE = sucrose polyester, THF = tetrahydrofuran.

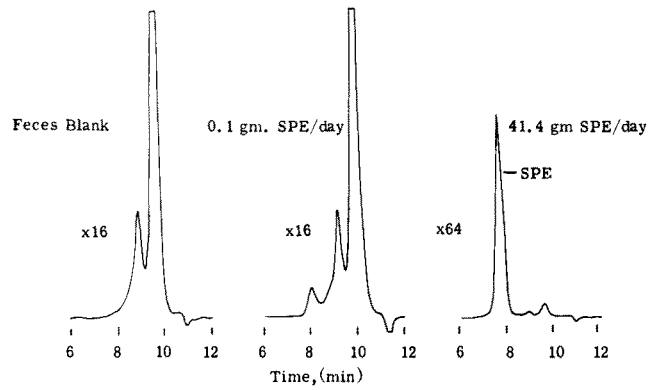

FIG. 3. Chromatograms obtained for extracts of human feces collected during baseline and sucrose polyester (SPE) feeding periods. Flow rate of 2.8 ml/min used.

ent was detected by a low-dead-volume differential refractometer (R401, Waters Associates, Inc.), used with a static THF reference. Peaks were displayed and integrated on a strip-chart recorder. Flow rates ranged from 1.5 to 2.8 ml/min, depending on the sample matrix. The entire system operated at ambient temperature.

The chromatographic equipment was calibrated daily

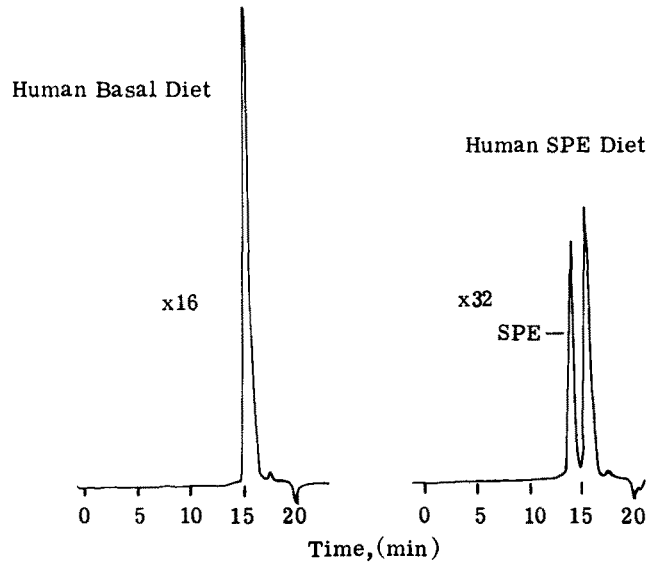

FIG. 4. Chromatograms of basal and sucrose polyester (SPE) diet extracts. Flow rate of 1.5 ml/min used.

with the SPE that had been used for the feeding studies. The quantity of SPE in each unknown sample was then read from a standard graph of peak area vs. concentration. All unknowns were analyzed in duplicate.

Thin Layer Chromatography (TLC)

The hexa-, hepta-, and octa-esters of sucrose were resolved by TLC on plates coated with 250 μ layers of Silica Gel G (Analtech, Inc., Wilmington, DE) developed with hexane:ethyl ether:acetic acid (70:30:1). The spots were made visible by spraying with 25% sulfuric acid and heating 20 min at 250 C. Densitometry scans were made with a visible reflectance densitometer (Model 900, Nester-Faust, Wilmington, DE) operated in the dual-beam mode.

RESULTS AND DISCUSSION

To establish that the analytical method was appropriate, it was shown that the SPE was not altered by passage through the animals or by the analytical method itself. Several samples of feces from animals that had been fed with SPE were extracted by the above procedures; the extracts were chromatographed and the SPE peaks were collected as they emerged from the column. These samples of recovered SPE were compared by TLC with the SPE that had been fed. All samples were identical. A typical pair of scans is shown in Figure 1.

To determine which μStyragel® pore size was best suited for isolating SPE, columns packed with 100-, 500-, 1000-, and 10,000-Å pore sizes were evaluated. The 500- and 1000-Å columns were equally effective in segregating SPE from other components of fecal and diet extracts. They did

TABLE II

Precision and Accuracy of Method (Dog Feces)

Sample	Percent of sucrose polyester in freeze-dried feces		Percent recovery	Relative SD (n=5) (%)
	Average of five daily assays	Expected[a] result		
1	0	0	-	-
2	11.6	11.4	102	3.4
3	23.6	22.9	103	2.3
4	35.2	34.3	103	1.8
5	45.9	45.7	100	2.4
6	50.7	50.6	100	2.7
7	59.6	59.6	100	2.2

[a]Samples prepared by mixing known amounts of samples 1 and 7.

not separate the sucrose hexa-, hepta-, and octa-esters from each other.

Figure 2 shows the response of the detector to known amounts of SPE. The response was linear, and the least squares line passed through the origin. The relative standard deviations of the data were ca. 1%. The variance of the least squares slope between calibrations was also ca. 1%.

Figure 3 compares chromatograms of extracts of feces from human subjects whose diets had contained zero, low, and high levels of SPE. No co-eluting peak from the fecal matrix was detected, even with the detector set for high sensitivity. Identical results were found with dog and rat feces.

The presence of triglycerides in the extracts of human diets made these samples more difficult to analyze, since the retention times for triglyceride and SPE are similar. Reducing the flow rate through the chromatographic column to 1.5 ml/min (4) resolved these two materials so that the SPE could be quantitated (Fig. 4).

The extraction of SPE from diet or fecal matrices was judged quantitative since repeated extractions with ether or other solvents gave no additional SPE. Moreover, when [carboxy-^{14}C]SPE was added to feces samples and then extracted as specified above, $>99\%$ of the ^{14}C was extracted.

The accuracy, linearity, and precision of the method were determined in two ways. First, various known amounts of SPE were added to blank samples of feces and diet, which were then analyzed. Second, a sample of dog feces that had been found by analysis to contain $59.6 \pm 1.3\%$ SPE was blended with blank feces to give mixtures having SPE concentrations ranging from 11 to 60%. Analyses of both kinds of samples resulted in similar data for precision and accuracy, as summarized in Table II. Results were linear throughout this concentration range. The relative standard deviation of analyses of the same samples on five different days was 2%, and the relative standard deviation on any one day was 1% (n = 10).

The practical lower limit of the method is 10 mg SPE/g dried sample with a relative error of ca. 10%. Qualitatively, ca. 200 μg of SPE per gram of dried sample can be detected.

In conclusion, this method offers a quick and accurate way for determining SPE in diets and feces. It can be applied over a wide concentration range, and it gives linear response. The simplicity of the method makes it amenable to automation.

ACKNOWLEDGMENTS

The assistance of C. Kuehlthau and E. Hollenbach with sample preparation and N. Artman with the manuscript is greatly appreciated.

REFERENCES

1. Mattson, F.H., and G.A. Nolen, J. Nutr. 102:1171 (1972).
2. Mattson, F.H., and R.A. Volpenhein, Ibid. 102:1177 (1972).
3. Mattson, F.H., and R.A. Volpenhein, J. Lipid Res. 13:325 (1972).
4. Kirkland, J.J., Editor, "Modern Practice of Liquid Chromatography," Wiley Interscience, New York, NY, 1971, p. 22.

[Received January 7, 1976]