A New Internal Standard for GLC Determination of Monoglycerides and Propylene Glycol Esters

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Monoheptadecanoin (monomargarin) has been used as an internal standard for the gas-liquid chromatography (GLC) determination of monoglyceride and propylene glycol ester emulsifiers. The compound is expensive and difficult to prepare. Octadecyl glyceryl ether (batyl alcohol), a less expensive commercially available material, has been found to be acceptable as an internal standard for this determination.

KEY WORDS: Emulsifiers, gas-liquid chromatography, monoglycerides, propylene glycol esters, shortenings.

Monoglyceride and propylene glycol emulsifiers can be determined by gas liquid chromatography (GLC) of their trimethylsilyl ethers (1,2). This determination requires the presence of an internal standard which has a retention time in the chromatogram which does not overlap any of the peaks which are to be measured. Monoheptadecanoin, since it does not occur in natural fats and oils in significant quantity, has been useful for this purpose. However, because of its scarcity, it is quite expensive to purchase in pure form. The synthesis of the compound is difficult because it involves the regioselective monoacylation of heptadecanoic acid to a primary hydroxyl group on glycerine. This can be accomplished by the use of acetonide-protected glycerol. However, after the protecting group is removed, the 1-monoglyceride is susceptible to acyl shift or ester interchange under basic conditions and/or elevated temperatures. A search for a less costly material was therefore initiated.

MATERIALS AND METHODS

Gas-liquid chromatographic analyses of monoglycerides and propylene glycol esters were performed on a Varian 6000 Gas-liquid Chromatograph equipped with a 7′ × ⅛″ stainless-steel column packed with 10% OV-1 dimethyl polysiloxane on 80/100 mesh Chromosorb W. Eluted peaks were analyzed using a flame ionization detector.

Reagents. Chloroform, potassium hydroxide, and pyridine were A.R. Grade. Pyridine solution was dried over the potassium hydroxide pellets prior to use. Hexamethyldisilazane (HMDS) and chlorotrimethylsilane (CTMS) were a specially purified grade obtained from Pierce Chemical Company, Rockford, IL. d,l-Batyl alcohol was obtained from Aldrich Chemical Company, Milwaukee, WI, and was used without further purification. Other internal standards were synthesized according to the procedures described later in this section.

Preparation of internal standards. The internal standard (1.0 g) was weighed to the nearest 0.1 mg into a 500 mL volumetric flask and diluted to the mark with chloroform. The flask was stoppered and well-mixed. The internal standard solution contains 2 mg/mL. Caution: (d,l)-batyl alcohol is labeled as an irritant dusting material. Precautions must be taken to avoid inhalation.

Sample preparation. Melted, well-mixed shortening or emulsifier was weighed into a 2-dram vial with Teflon-lined cap — 0.15 g (150 mg) or 0.02 g (20 mg), respectively. Sample weights were not exactly the weight shown but were weighed to the nearest 0.1 mg. Internal standard solution (5.00 mL) was added using a volumetric pipet. This corresponds to an addition of 10.0 mg solid internal standard. The chloroform solution was evaporated in a stream of nitrogen with gentle heat (less than 80°C). Dry pyridine (0.5 mL) was added and the mixture was gently heated to dissolve the residue. HMDS (0.5 mL) and CTMS (0.25 mL) were added using 2-mL tuberculin syringes. The vial was capped and placed on a mechanical shaker for 20 min. The solution (1.0 microliter) was injected into the instrument.

Chromatograph conditions. Gas flow rates (all gases were chromatography grade) were helium through column: 25 mL/min; hydrogen to flame ionization detector (FID): 25 mL/min; and compressed air to FID: 325 mL/min. Temperatures were injection port: 300°C; column: 245°C; and detector: 280°C.

Peak assignments. Peaks were identified by their retention times which were measured from the time the solvent started to elute to the maximum height of the component peak. Emulsifier component peaks eluted in the following order: i) propylene glycol monopalmitate (PGMP), ii) propylene glycol monostearate (PGMS), iii) monopalmitin (GMP), iv) internal standard, v) monoolein (GMO), and vi) monostearin (GMS). Many very small peaks were observed but were ignored in calculations of concentration. Typical chromatograms of emulsifier and emulsified shortening are shown in Figures 1 and 2, respectively. These assignments were confirmed with authentic standards. Glycerides were obtained from Nu-Chek-Prep Inc., Elysian, MN. Propylene glycol ester stan-

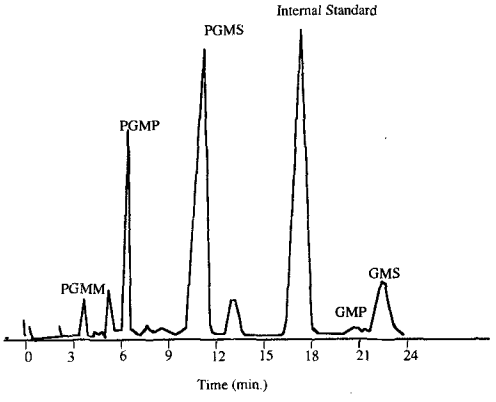

FIG. 1. Gas-liquid chromatogram of a propylene glycol ester emulsifier.

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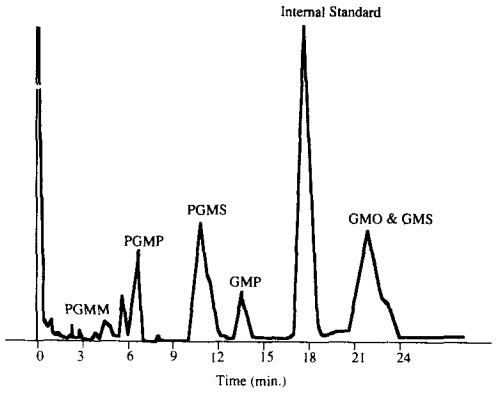

FIG. 2. Gas-liquid chromatogram of a shortening containing propylene glycol esters and monoglycerides.

dards were synthesized at Anderson Clayton Research Center, Richardson, TX. A control sample of known concentration was run before each day's samples to check for accuracy of quantitation.

Monoheptadecanoin (monomargarin). 2,2-Dimethyl-1,3-dioxolane-4-methanol (Solketal, Aldrich Chemical Co., 71.8 g., 0.54 mol) was dissolved in 200 mL chloroform along with 50 g (0.185 mol) heptadecanoic acid and 1.5 g (0.0079 mol) p-toluenesulfonic acid. The mixture was heated to reflux in a flask equipped with a water separator/condenser and magnetic stirrer. The reaction was continued for 12 hr at which time no additional water separation was observed. Sodium bicarbonate (1.5 g, 0.018 mol) was added and the mixture extracted with three 100 mL portions on a Buchi rotary evaporator. The solid residue was dissolved in 200 mL 2-methoxyethanol along with 100 g boric acid. The mixture was heated at reflux temperature for 45 min with stirring. The reaction mixture was cooled and then poured into 1,000 mL diethyl ether. The ether solution was extracted with three 100 mL portions of 10% sodium carbonate and then several portions of 5% sodium chloride solution until the washings were neutral to pH paper. Layer separation of these extractions was facilitated by gentle warming of the solution on a steam bath. The ether solution was dried over 3A° molecular sieves, heated to boiling on a steam bath, and filtered through a Buchner funnel. The filter paper was washed with two 100 mL portions boiling diethyl ether. Evaporation of the diethyl ether yielded 37.5 g (50%) monoheptadecanoin. GLC analysis under standard conditions showed a single peak at 17.88 min.

1,2-dihydroxybutyl-4-palmitate. 1,2,4-Butanetriol (28 g, 0.25 mol) was dissolved in 200 mL chloroform with 18.8 g (0.32 mol) acetone and 1.5 g p-toluenesulfonic acid. Initially the mixture appeared to be heterogeneous. The mixture was heated at reflux temperature in a flask equipped with a water separator/condenser and a magnetic stirrer. After 48 hr, the mixture appeared clear and homogeneous. Hexadecanoic acid (50 g, 0.2 mol) was added and the mixture refluxed for an additional 48 hr. Sodium bicarbonate (1.5 g) was added, the mixture washed three times with water, and the solvent removed on a rotary evaporator. The residue was dissolved in 200 mL 2-methoxyethanol with 50 g boric acid. The mixture was refluxed for 30 min and then partitioned between 50 mL water and 2,000 mL diethyl ether. The organic phase was extracted with three 100 mL portions 10% sodium carbonate followed by several portions of water until the washings were neutral to pH paper. After drying over 3A° molecular sieves, solvent was evaporated to yield 61.2 (87%) of a white amorphous solid. Analysis by GLC showed an intense peak approximately the same retention time as monoheptadecanoin but also showed several smaller peaks which would interfere with monoglyceride and propylene glycol monoester (PGME) analysis. No attempt was made to further purify the material.

RESULTS AND DISCUSSION

Monoheptadecanoin had been traditionally prepared in our laboratories using the synthetic process described herein. Heptadecanoic acid starting material was very expensive (~$40 for 25g) to purchase and the synthesis, although short, required extensive solvent extraction, washing, and separation steps. The procedure was therefore tedious and time-consuming. However, the price for purchase of monoheptadecanoin was $40/g.

An initial attempt was made to prepare a structural isomer of monoheptadecanoin having identical functionality but requiring a much less expensive (~$6/25g) starting material, hexadecanoic (palmitic) acid. Unfortunately, the process required the same tedious procedure, although layer separation in all the solvent extraction steps was much cleaner than for monoheptadecanoin, resulting in a much improved yield. However, GLC analysis indicated that impurities were present in the material which would interfere with the determination of monoglycerides and propylene glycol monoesters. Consequently, no attempts were made to further characterize or purify this material.

TABLE 1

Analysis of Total Monoglycerides by GLC

Trial	% Monoglyceride	
	Monoheptadecanoin	d,l-Batyl alcohol
1	9.38	9.93
2	15.86	17.03
3	18.67	19.07
4	9.25	8.92
5	1.85	1.90
6	3.23	3.31
7	4.99	5.15
8	4.93	4.80
9	3.68	3.84
10	15.51	14.01
11	16.88	16.76
12	9.18	9.04
15	1.91	1.88
14	4.86	4.83
15	4.82	4.76
16	4.81	4.79
17	9.88	9.67
18	17.73	17.28
19	18.67	18.42
20	8.27	9.08
21	1.85	1.95
22	3.33	3.33
23	4.04	3.83
	t = 1.149	t (0.975, 22) = 2.074

TABLE 2

Analysis of Propylene Glycol Monoesters by GLC

Trial	% PG Monoester	
	Monoheptadecanoin	d,l-Batyl alcohol
1	66.89	66.94
2	57.90	57.20
3	56.96	56.17
4	73.59	72.24
5	13.04	12.60
6	0.00	0.00
7	6.85	6.76
8	6.87	6.54
9	2.72	2.69
10	53.41	48.87
11	56.04	55.63
12	73.35	74.00
13	14.18	13.15
14	9.69	9.73
15	6.78	6.73
16	6.67	6.71
17	70.92	71.77
18	59.45	59.78
19	56.92	57.20
20	74.49	75.01
21	13.01	13.12
22	0.46	0.44
23	2.87	2.71
	$t = 1.529$	$t(0.975, 22) = 2.074$

d,l-3-Octadecyloxy-1,2-propanediol (d,l-batyl alcohol) was observed to be identical to monoheptadecanoin in molecular weight and similar in functionality. The notable difference is that the alkyl chain in the terminal position is attached through an ether linkage rather than an ester group. This could be viewed as an advantage since interesterification and 1,2-acyl shift reactions during synthesis or sample preparation are precluded. Stability in chloroform solution would also be expected to be greater for the same reason. The material is available commercially for only $44/10g, only 10% of the cost of monomargarin. The only disadvantage is that the material is an irritant, and solutions must be prepared with proper ventilation. GLC analysis indicated that the compound had essentially the same retention time as monoheptadecanoin and no interfering impurities were present.

Paired comparisons were carried out for a series of monoglyceride-containing shortenings (Table 1) and propylene glycol ester emulsifiers and shortenings that contained these emulsifiers and monoglycerides (Table 2). Each sample was run under identical conditions in duplicate using monoheptadecanoin and batyl alcohol as internal standards. Statistical analysis of the paired data indicated no significant difference between the results at the 95% confidence level. Previous experience with the method using monomargarin as a standard demonstrated approximately the same level of uncertainty. Residual error is most probably a function of the derivatization process. Modification of the method to utilize a derivatizing reagent such as N(t-butyldimethylsilyl)-N-methyltrifluoroacetamide (3), because of its high stability to hydrolysis, may improve reproducibility of the method.

Because of the above evidence, it was concluded that 3-octadecyloxy-1,2-propanediol should be used as an internal standard in preference to monoheptadecanoin because of its commercial availability and relative low cost.

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