Determination of Brominated Vegetable Oils in Soft Drinks by Gas Liquid Chromatography

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Abstract

A method is described for the qualitative and quantitative determination of brominated vegetable oils in soft drinks. The procedure involves treatment of the brominated oils with sodium methoxide followed by GLC analysis of the resulting methyl esters using methyl pentadecanoate as internal standard. Recoveries on known amounts of these oils ranged between 93.7% and 102.4%. The technique, applied to several commercial soft drinks, has shown the content of brominated oil to be in the range 10–45 mg per 10 fluid oz of drink.

Introduction

Brominated vegetable oils (olive, corn, sesame and cottonseed oils) are widely used in the soft drink industry as dispersing agents for the flavoring citrus oils. They also give the drink a cloudy appearance thus simulating the natural fruit juice.

Although these brominated oils are classified as safe by the U.S. Food and Drug Administration (1) and presently permitted by the Canadian Food and Drug Directorate (2), recent work in our laboratories has indicated the toxicity of brominated cottonseed oil when fed to rats (3). It was therefore important to develop an analytical method for their detection and estimation in soft drinks and it was considered that a method based on gas liquid chromatography (GLC) would provide the most information.

Considerable difficulty has been experienced in GLC examination of long chain polybrominated esters. Stein (4) claimed success with methyl 9,10-dibromostearate but Landowne and Lipsky (5), and Koehler et al. (6) found evidence for its decomposition under GLC conditions to products containing no bromine. Hanahan (7) has also reported the decomposition of secondary iodides of long chain compounds during GLC.

On the other hand, α-brominated and α-fluorinated carboxylic esters have been successfully chromatographed by Rogozinski (8) and by Gershon and Renwick (9) respectively. 9,10-Dichlorostearate also appears to chromatograph more easily (10–12) and preparative GLC was used by Daniels et al. (10) in the isolation of this compound. The behavior of the tetrachlorostearate was not reported by any of these workers (10–12).

In this investigation two approaches were considered: direct GLC of the brominated methyl esters after methanolysis of the brominated oils and GLC of derivatives of the brominated esters after methanolysis of the oils.

Materials and Methods

Commercial samples of brominated olive, sesame and cottonseed oils were obtained from Abbott Laboratories. Laboratory samples of brominated olive and corn oils were prepared by bromination in anhydrous ether at 0 C as described by Sgoutas and Kummerow (13). Samples of 9,10-dibromostearate and 9,10;12,13-tetrabromostearate were also prepared from high purity methyl oleate and methyl linoleate, purchased from Applied Science Labs, Inc. Several brands of orange flavored soft drinks were purchased from local stores.

Solvents

All solvents were distilled prior to use except for petroleum ether (40–60) which was used as received for TLC. Methanol was dried using magnesium turnings (14), and benzene by distillation from sodium wire.

Thin Layer Chromatography

Thin layer chromatography (TLC) was carried out on 250 μ layers of Silica Gel G using petroleum ether-diethyl ether (85:15) as solvent. Components were visualized by exposure to iodine vapor or by spraying with 50% sulfuric acid and charring at 110 C for 15 min.

Gas Liquid Chromatography

All quantitative measurements reported were made on a Perkin Elmer 800 gas chromatograph equipped with dual columns and a differential hydrogen flame ionization detector. Columns were 3 ft long, ⅛ in. o.d. stainless steel, packed with 3% JXR on Gas Chrom Q, 110–120 mesh (Applied Science Labs, Inc.). The temperature was programmed at 10 C/min from 150 C to 270 C with an injection port temperature of 250 C. Helium used as carrier gas had an ambient flow rate of 40 ml/min. Peak areas were calculated from disc integrator tracings, and retention times are quoted as carbon numbers (C.No.) (15).

In preliminary investigations on the GLC behavior of the dibromo- and tetrabromostearates, analyses were also carried out on a Wilkens Auto-prep A-700 (modified for analytical work) and an F & M 5750, both employing flame ionization detection. On the former, operated at 200 C with injection port and detector temperatures of 210 C, three different columns were used: 5% SE30 on Chrom W 60–80, 3% JXR on Gas Chrom Q 100–120 and 6% BDS on Anakrom ABS 70–80. All columns were 3 ft, ⅛ in. o.d. stainless steel unless otherwise stated.

The F & M 5750, temperature programmed from 150–320 C with injection port and detector temperatures of 250 C, was equipped with columns of 3% OV17 and 3% JXR, each on Anakrom ABS 80–90. A glass column, 5 ft, ¼ in. o.d., packed with the same JXR phase was also used with this instrument.

TABLE I
Acid-Catalyzed Methanolysis of Brominated Oils

Brominated oil	Wt. taken (mg)	Wt. calculated (mg)	Recovery, %
Olive	19.8	19.4	98.0
	33.4	32.8	98.1
Sesame	24.2	23.2	96.0
	48.0	46.4	96.5

TABLE II

Base-Catalyzed Methanolysis of Brominated Oils

Brominated oil[a]	Amount taken (mg)	Amount calculated (mg)	Recovery %	Fatty acid composition[b] 16:0	18:0	DiBr	Tetra Br
Olive (L)	26.7	26.5	99.4				
	46.7	45.7	97.9				
Olive (A)	20.4	19.8	97.0	8.1	5.2	77.0	9.7
	33.0	33.6	102.0				
Sesame (A)	26.9	26.1	97.0	5.8	4.8	41.1	48.3
	52.7	50.0	94.9				
Cottonseed (A)	24.5	25.1	102.4	13.8	2.1	18.6	65.5
	38.8	38.4	99.0				
Corn (L)	30.3	28.4	93.7	6.4	1.6	20.8	71.2
	54.0	52.8	97.9				
Average recovery, %			98.1				

[a] Symbols L and A represent the source of the brominated oil: L, laboratory prepared; A, commercial sample.

[b] All figures quoted are area per cent. Small amounts of unsaturated C_{18} esters are included under 18:0.

Spectroscopy

Infrared spectra (IR) were run on a Perkin Elmer 221 and nuclear magnetic resonance spectra (NMR) on a Varian A-60A spectrometer.

Treatment of Brominated Oil with Sodium Methoxide

Brominated oil (1–50 mg), with methyl pentadecanoate (1–12 mg) as internal standard, was refluxed for 1 hr with sodium in anhydrous methanol (30 ml, 1%) and anhydrous benzene (15 ml). The reaction mixture was then diluted with water, acidified and extracted with diethyl ether. The latter was dried over anhydrous sodium sulfate, concentrated on a rotary evaporator under vacuum at 40 C and analyzed by GLC.

Determination of Brominated Oil in Soft Drinks

Methyl pentadecanoate (0–10 mg) dissolved in methanol was added as internal standard to the soft drink (200 ml) which was then saturated with sodium chloride and extracted with diethyl ether (3 × 75 ml). This extract was dried over sodium sulfate and evaporated under vacuum. The residue was treated with sodium methoxide as described above and finally analyzed by GLC.

Results and Discussion

GLC of the Dibromo- and Tetrabromoesters

Preliminary experiments indicated that acid-catalyzed methanolysis (sulfuric acid-methanol-benzene) of the brominated oils produced methyl esters with no significant loss of bromine, and this was confirmed by recovering authentic samples of the brominated esters unchanged after reaction (TLC, GLC and NMR). This observation was however of limited value because of the difficulties encountered in the GLC analysis of the dibromostearate and, particularly, the tetrabromostearate.

On the Wilkens instrument, methyl dibromostearate gave three peaks on all columns. These peaks of carbon number 18.0, 18.5 and 19.0 (SE30), closely resembled those observed by Landowne and Lipsky (5) and by Koehler et al. (6). The tetrabromoester produced a broad hump ranging in carbon number from 18.0 to 19.0.

With the F & M 5750, initially, sharp peaks were observed for the di- and tetrabromoesters of carbon number 23.7 and 28.0 (OV17) respectively, although the baseline between the peaks was very uneven. After repeated injections however (over a two day period) these peaks were gradually replaced by two broad ill-shaped peaks of carbon number 21.6 and 23.5, which were unsuitable for quantitative work. During the same period, the retention time and peak shape of methyl palmitate and methyl stearate were unchanged. Similar decompositions were observed with a stainless steel and a glass column packed with 3% JXR.

The best results were obtained using a PE 800 gas chromatograph which gave two well-defined peaks for the dibromo- and tetrabromoesters, of carbon number 22.6 and 26.3 respectively, and having weight response factors of 1.9 and 3.9, relative to methyl pentadecanoate. Using these factors, known weights of brominated olive oil and brominated sesame oil were determined to within 5% accuracy (Table I). Shortly thereafter a similar type of decomposition occurred as had been observed on the F & M 5750 instrument. The sharp peaks (C. No. 22.6 and 26.3) were gradually replaced by broad humps of approximate carbon number 20.2 and 22.3 respectively. Decomposition was more apparent with the tetrabromoester.

These results suggest a gradual build up on the column or injection chamber of substances, possibly brominated, which render further chromatography

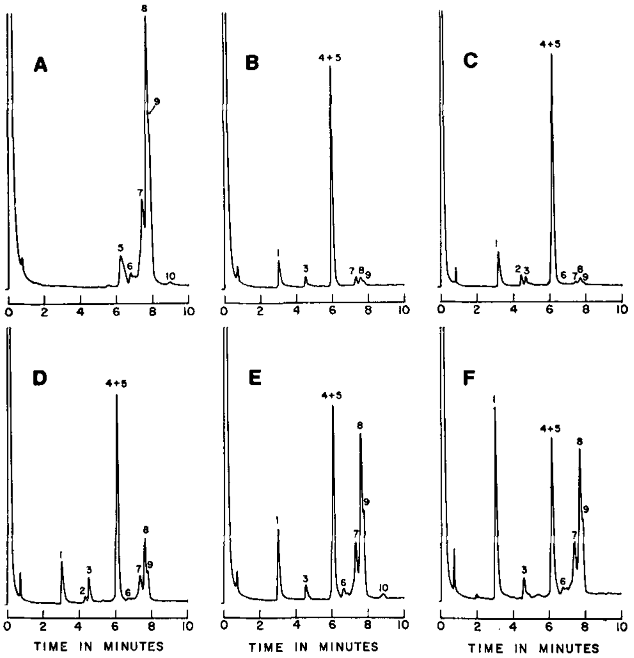

FIG. 1. GLC chromatograms of brominated vegetable oils treated with sodium methoxide. A, methyl tetrabromostearate; B, brominated olive oil (lab prepared); C, brominated olive oil (commercial); D, brominated sesame oil (commercial); E, brominated corn oil (lab prepared); and F, brominated cottonseed oil (commercial). 1, C_{16} methyl ester; 2 & 3, C_{18} methyl esters; 4, methyl dibromostearate derivative; and 5–10, methyl tetrabromostearate derivatives.

TABLE III
Determination of Brominated Oils in Orange Drinks by Base-Catalyzed Methanolysis

Drink	Amount calculated[a] (mg per 10 oz bottle)		Fatty acid composition			
			16:0	18:0	DiBr	Tetra Br
A	29.2	31.0	7.6	6.1	30.7	55.6
B	10.0	10.5	15.8	12.0	14.2	58.0
C	45.0	45.5	6.0	4.7	33.4	55.9
D	45.2	45.5	6.9	3.0	33.0	57.1
E	43.2	46.4	5.5	4.6	43.5	46.4
F	22.8	23.5	6.6	6.4	27.7	59.3

[a] Each result given is the average of two determinations on the same bottle. Two different bottles (or cans) were analyzed with each drink.

TABLE IV
Recovery of Brominated Sesame Oil Added to an Orange Drink

Sample C (ml)	Brominated oil added (mg)	Brominated oil determined (mg)	Recovery %
200	0	31.4	
200	9.8	41.4	100.5
200	19.5	52.7	103.5

of the brominated esters useless. It was noted that the same column, after being used for some time for routine analysis of normal esters, could again be used for the analysis of brominated esters.

Because of the difficulties involved in chromatographing the brominated esters, attention was focused on the formation of derivatives of these esters, which would be stable under GLC conditions. It was found that treatment with sodium methoxide accomplished both ester formation and derivative formation at the same time.

Sodium Methoxide Reaction

On treatment with sodium methoxide, methyl dibromostearate was converted quantitatively to a component of carbon number 20.2 (JXR) with a weight response factor of 1.6. Weight response factors were based on the weights of the original dibromo- and tetrabromoesters. Under similar conditions, the tetrabromoester gave a series of components of carbon number 20.1, 20.9, 21.9, 22.3 and 22.5 (Fig. 1, A), whose total area had a response factor of 2.2. These response factors remained constant throughout the period of analysis. When analyzing a mixture containing both esters, allowance had to be made for the component of carbon number 20.1 (from the tetrabromostearate) which eluted with the dibromoester derivative (C. No. 20.2). This component (C. No. 20.1) amounted to 13% of the total peak area of the remaining components from the tetrabromoester.

The quantitative results from application of this method to some brominated oils are summarized in Table II and the chromatograms obtained are shown in Figure 1. Recovery in most cases was 100 ±5%.

Application to Soft Drinks

The brominated oil content of six orange drinks is summarized in Table III.

All commercial orange drinks analyzed contained sodium benzoate as preservative. Due to the acidity of the drink, this was apparently present (in part or in toto) as benzoic acid which was extracted by the ether. The small amount present in each extract however did not interfere with the determination of the brominated oil.

The ether extract from two samples (C and D, Table III), also contained an unidentified component of carbon number 21.7 (JXR) which eluted where the tetrabromoester derivatives would normally appear. This component was separated from the brominated oil by steam distillation and was shown to disappear from the chromatogram on treatment with sodium methoxide. The residue from steam distillation had a similar brominated oil content to that obtained by direct ether extraction indicating that steam distillation is unnecessary in the determination.

Recovery studies were carried out on sample C to which known amounts of brominated sesame oil had been added. Recoveries were within the limit of experimental error (5%) and are shown in Table IV.

The results summarized in Tables II and III indicate that the method of analysis provides information regarding the identity of the brominated oil as well as giving a quantitative estimation of the amount present.

Thus by comparison of the fatty acid compositions of the standard oils (Table II) with those of the brominated oils in the drinks (Table III) it can be concluded that Samples B and E contain brominated corn oil and brominated sesame oil respectively. The other samples A, C, D and F appear to contain the same brominated oil, the composition of which does not correspond exactly to any of the standard brominated oils. It may be that this is a brominated sesame oil derived from a different source.

Structure of Derivatives

Studies now in progress indicate that the dibromostearate is converted to a methyl octadecenoate with a vinyl bromide grouping (16–18). The tetrabromostearate appears to yield esters possessing vinyl bromide groups, conjugation and one and two methoxy groups. These results will be reported at a future date.

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