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Gas-Liquid Chromatography of Fat-Soluble Vitamins[1]

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Abstract

Gas-liquid chromatography (GLC) was found to be useful for analysis of tocopherols and vitamin K_1. Vitamin A preparations showed evidence of alteration and ubiquinones gave no peaks on the chromatogram under the conditions used.

Introduction

A REPORT (8) on the application of GLC to separation of different members of the vitamin D group of compounds prompted us to investigate the possibility of separating other fat-soluble vitamins by this technique. Results obtained with vitamin A, tocopherols, vitamin K_1, and with ubiquinones on columns of SE-30 and QF-1-0065 are reported in this paper.

Experimental

Analyses were carried out on a Barber-Colman Model 10 chromatograph equipped with a 1 cm diode detector containing 80 μc Radium^{226}. The column packing consisted of Gas Chrom P, 100–140 mesh, siliconized according to the procedure of Sjovall et al. (6) and coated with 3% by wt of either SE-30 or QF-1-0065. Gas Chrom P was obtained from Applied Science Laboratories, State College, Pa.; SE-30 from Wilkins Instrument Co., Walnut Creek, Calif.; and QF-1-0065 from Dow Corning Corp., Midland, Mich. The coated support was packed in 6 ft x 1/8 in. glass columns and conditioned at 225C for 48 hr. Materials to be separated were dissolved in chloroform and injected from a Hamilton syringe in a volume of 1–5 μl. The vitamin A and tocopherol preparations used for these studies were supplied by Distillation Products Industries. The vitamin K_1 was obtained from the California Corp. for Biochemical Research, Los Angeles, Calif. and the ubiquinones were kindly provided by W. E. J. Phillips of the Science Branch, Canada Department of Agriculture, Ottawa, Ontario.

Results

Tocopherols. The initial experiments were carried out with tocopherols since at least eight isomers with different biological activities are known to occur in nature and since the best chemical methods of assay are either incomplete (2) or very tedious (1,3). It was found that the *d*-isomers of monomethyl-, dimethyl- and trimethyltocols emerged in that order and were readily separated at 205C on the SE-30 column (*Fig.* 1). The retention times of *d*-gamma- and *d*-beta-tocopherols were too similar to permit resolution on this column and another dimethyltocol, *dl*-zeta$_2$-tocopherol was also eluted at approx the same time. The optical isomers of *dl*-zeta$_2$-tocopherol and of *dl*-alpha-tocopherol appeared to have identical retention times. Alpha-tocopherol acetate was eluted after alpha-tocopherol and these two compounds were cleanly separated. Retention times show in Table I.

The retention times of the tocopherols were also determined on a column of 3% QF-1-0065 on siliconized Gas Chrom P at 195C (Table I). Delta-, beta- and alpha-tocopherols were separated on this column but not as well as on SE-30. The order of elution of gamma- and beta-tocopherol was reversed but their retention times on QF-1-0065 were nearly identical. Alpha-tocopheryl acetate had a greater relative retention time on this column.

Vitamin K_1. The chromatographic behaviour of vitamin K_1 on these columns was also investigated. Two peaks were obtained on the SE-30 column with retention times of 41.7 and 50.3 min and peak areas bearing a ratio of 4:1. On QF-1-0065 the same preparation gave a single peak with a retention time of 27.8 min. It is not known whether the second peak observed on the SE-30 column was due to an impurity in the preparation or whether it resulted from alteration on the column.

Vitamin A. The chromatographic behaviour of all-*trans* retinol and its acetate ester was investigated with the SE-30 column. Since vitamin A has a shorter side-chain than the tocopherols, it was neces-

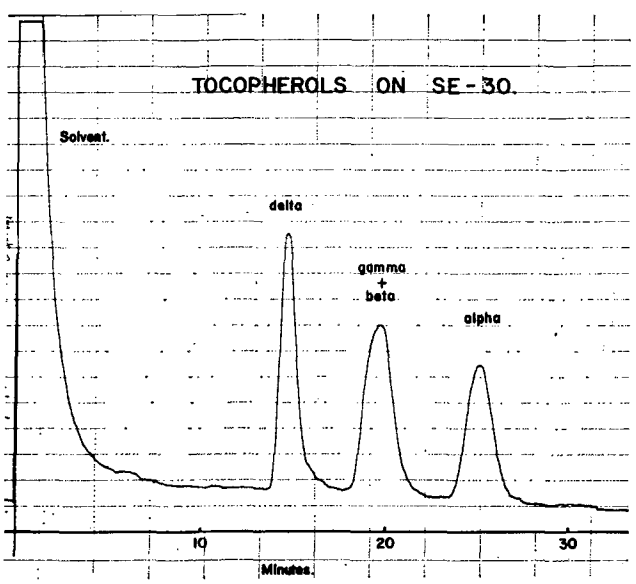

FIG. 1. Gas chromatographic separation of tocopherol isomers. Conditions used for analysis show in Table I. The column load was approx 0.5 μg of each isomer.

[1] Presented at the AOCS Meeting in Toronto, 1962.

TABLE I

Retention Times of Tocopherols and Vitamin K_1

	Position of methyl groups	on 3% SE-30 (min)	on 3% QF-1-0065 (min)
Cholestane		10.0	3.6
Delta-tocopherol	8	14.5	8.2
Beta-tocopherol	5,8	19.2	9.4
Zeta2-tocopherol	5,7	19.5	
Gamma-tocopherol	7,8	19.7	9.3
Alpha-tocopherol	5,7,8	24.4	10.3
Alpha-tocopheryl acetate	5,7,8	29.6	20.4
Vitamin K_1		{ 41.7 { 50.3	27.8

Chromatographic conditions—column temp 205C (SE-30), 195C (QF-1-0065), flash heater 270C, cell bath 220C, cell voltage 1000V, argon pressure at inlet 54 psi, sensitivity 1 x 10^{-9} amps full scale. The retention times of cholestane are given for comparison.

sary to reduce the column temp somewhat in order to separate peaks due to the vitamin preparations from the solvent peak. Both the retinol and its acetate gave rise to several overlapping peaks, suggesting that alterations were occurring on the column (Fig. 2). These multiple peaks were observed at temp as low as 165C. It seemed probable that alteration during chromatography would involve the conjugated systems of double bonds and to check this, samples of vitamin A were hydrogenated either in ethanol at 27C for 4 hr at 47 psi with 5% palladium on calcium carbonate as catalyst or in glacial acetic acid at 23C for 3 hr at 49 psi with Adam's catalyst. This lowered the iodine value (I.V.) from a theoretical of 443 to 55 and 40, respectively, indicating that about one-half double bond/molecule still remained after hydrogenation. The preparation with I.V. 40 gave a single major peak and one very minor peak with retention times of 5.6 and 2.3 min, respectively, under the conditions given for Figure 2. The preparation with I.V. 55 showed an additional minor peak at 4.5 min and both minor peaks were more prominent.

On the QF-1-0065 column, the all-*trans* retinol again gave multiple peaks at column temp ranging from 145–180C while the hydrogenated retinol of I.V. 40 gave a single peak with a retention time of 1.7 min at 181C.

Ubiquinones. Attempts to use these columns for analysis of ubiquinone (coenzyme Q) isomers were unsuccessful. No peaks were observed with either ubiquinone$_{30}$ or ubiquinone$_{45}$ (CoQ_6 and CoQ_9, respectively) and it seems likely that the long side-chain of these compounds prevented their being eluted within a reasonable time period.

Discussion

The results obtained with tocopherols and with vitamin K_1 indicated that GLC was a useful method for analysis of these compounds. Since this work was completed, papers by Wilson et al. (7) and by Nair and Turner (5) have also reported successful separation of these compounds by GLC. Our results are in general agreement with theirs but the conditions used in our experiments made it possible to work at a column temp of 205C rather than at 225–250C.

Our attempts to use GLC for analysis of vitamin A gave unsatisfactory results because multiple peaks were obtained in each case. It seems probable that these result either from decomposition or from *cis-trans* isomerization similar to that observed by Morris et al. (4) for fatty acid esters with conjugated double bonds. It may be possible to find chromatographic conditions which do not cause these alterations but our efforts to achieve this by lowering the temp of the column, flash heater and detector were unsuccessful. It is apparent that hydrogenation of the vitamin A

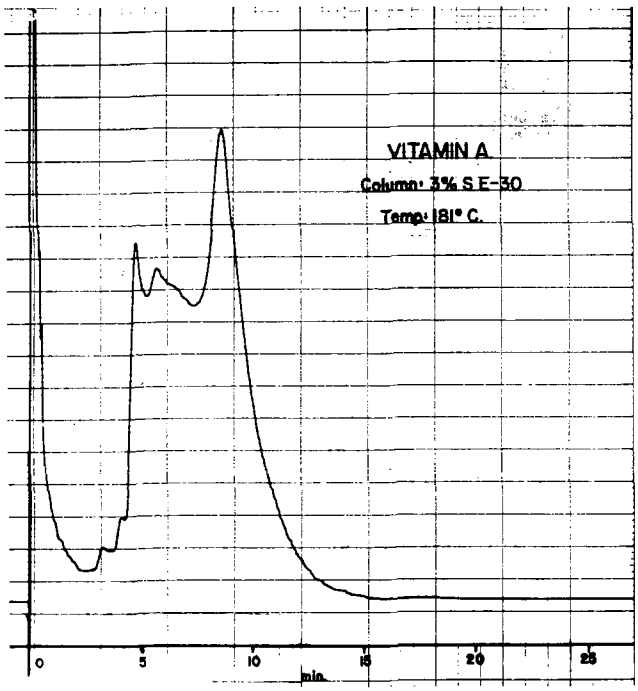

FIG. 2. Gas chromatographic analysis of all-*trans* retinol on 3% SE-30. Column temp 181C, flash heater 243C, cell bath 217C, cell voltage 900V, argon pressure at inlet 43 psi, sensitivity 1 x 10^{-8} amps full scale. Cholestane had a retention time of 53 min under these conditions.

results in a single main peak with a lowered retention time.

The observed order of elution of all-*trans* retinol, tocopherols and vitamin K_1 from gas chromatographic columns is to be expected since they contain 20,27–29 and 31 carbon atoms/molecule, respectively. The failure of ubiquinones$_{30}$ and $_{45}$ to appear on the chromatograms might also be predicted since they contain 39 and 54 carbons, respectively. It also seems very unlikely that vitamin K_2, which contains 41 carbons, would emerge from the columns under the conditions used. A semi-log plot of retention time on SE-30 against number of carbon atoms/molecule, using data obtained for the retinol, tocopherols and vitamin K_1, indicated that similar compounds containing 39–41 carbon atoms might be expected to have retention times ranging from 12–24 hr.

The presence of cholesterol or related plant sterols in extracts may interfere with analysis of tocopherols since, like the tocopherols, they contain 27–29 carbon atoms/molecule. Cholesterol was in fact found to have retention times of 20.8 and 11.0 min on SE-30 and QF-1-0065, respectively. It may therefore be advisable to carry out a preliminary separation of sterol and tocopherol fractions when gas chromatography is to be used for analysis of naturally-occurring mixtures.

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