Determination of Vitamin E in Multivitamin Products by Gas-Liquid Chromatography

By F. P. MAHN, V. VISWANATHAN, C. PLINTON, A. MENYHARTH, and B. Z. SENKOWSKI

The determination of vitamin E in multivitamin preparations has presented considerable analytical problems. Reports on the gas-liquid chromatography of this vitamin have shown that a linear response with concentration can be obtained. This investigation deals primarily with the quantitative analysis of alpha-tocopheryl acetate utilizing a hydrogen-flame detector. The influence of gas flow rates on detector response and retention times is demonstrated. The conditions for the assay of vitamin E in multivitamin preparations are outlined. A suitable internal standard employed in this study was dotriacontane. The precision of the proposed procedure under the conditions studied is ± 2–3 percent.

SINCE A CONSIDERABLE number of multivitamin preparations contain vitamin E (alpha tocopherol or its esters), a specific and reproducible method of determination is desirable. A comprehensive review of the literature has been made by Kofler *et al.* (1) pertaining to existing methodology. The most popular procedure for the analysis of alpha tocopherol in vitamin preparations is the Emmerie-Engel (2) reaction utilizing the iron-bipyridyl complex. However, this method is susceptible to many interferences, especially vitamin A, which is present in most multivitamin products. In addition, the use of the colorimetric procedure requires a hydrolysis step to be carried out if esters of tocopherol are present. A publication by Fisher *et al.* (3) employed a hydrogenation step prior to colorimetry of alpha tocopherol in order to minimize the interference due to vitamin A. A review of titrimetric and colorimetric methods for the assay of vitamin E in pharmaceutical products was made by Lehman (4).

It would appear that the tremendous versatility of GLC should make this technique the one of choice for the quantitative determination of vitamin E. The majority of reports in the literature, concerning the GLC of vitamin E, emphasized the qualitative separation of the various isomers of the tocopherols (1, 5, 6, 7, 8). Most of the investigators reported that the best separations of the tocopherols were achieved using columns prepared with SE-30 on diatomaceous earth.[1] In this laboratory good results were obtained using a column containing 10% SE-30 on Aeropak 30, 100/120 mesh. However, the silanization of the diatomite aggregates[2] did not significantly change the results of the vitamin E assay, when this high percentage of liquid phase was used. Libby and Sheppard (9) have reported on the quantitative response of alpha, gamma, and delta tocopherol as well as alpha tocopheryl acetate, utilizing an argon ionization detector. Pillsbury *et al.* (10) in a later report, have applied GLC to vitamin E in pharmaceuticals and quantitatively analyzed for vitamin E, utilizing a hydrogen-flame detector. Comparison was also made with the Emmerie-Engel method.

This investigation deals primarily with the quantitative determination of alpha tocopheryl acetate by GLC, as carried out in this laboratory for quite some time on a number of different multivitamin preparations. In order to compensate for column characteristics, instrumental variations, and sample introduction technique, an internal standard was employed. Dotriacontane (11), a C-32 hydrocarbon was used as an internal standard in this study. It should be mentioned that a C-33 or C-34 hydrocarbon would have been preferable, but were not available commercially. An aluminum oxide column was used in the sample preparation in order to remove some interferences derived from excipients in the various formulations.

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[1] Silanized Gas-Chrom P, Applied Science Laboratories, State College, Pa.; and Celite, Johns-Manville, New York, N. Y.

[2] Chromosorb W, Johns-Manville, New York, N. Y.

This method had previously been submitted, in 1965, to the United States Pharmacopeia for a preliminary collaborative trial.

EXPERIMENTAL

Operational Parameters—The instrument used for this work was a gas chromatograph, (Varian Aerograph model 600 or equivalent), equipped with a hydrogen flame-ionization detector. The column used was a copper coil, 121.9 cm. (4 ft.) long and 3 mm. i.d., packed with 10% SE-30 on Aeropak 30, 100/120 mesh. Prior to use, this packed column was flow conditioned at 280° for 16–20 hr. with a stream of nitrogen. The temperatures were: column, 245°; injector port, 275° with a Pyrex insert; and detector, 275°. The flow rates were: carrier gas, nitrogen, 40 ml./min.; detector gas, hydrogen, 20 ml./min.; and air 300 ml./min. All injections were made using a 10-μl. syringe (Hamilton) with the injection volume being approximately 5 μl. The instrument was operated at a range of 10 and attenuation 32×, or equivalent to result in approximately 50% response of the recorder scale. The recorder used was 0–1 mv. (Texas Instrument) with a pen response of 0.4 sec. and a chart speed of 12 in./hr. All peak areas were measured using either a disk integrator, digital integrator, or the peak height multiplied by the peak width at half height. Under the conditions stated the relative retention time of alpha tocopheryl acetate was 0.85 with respect to the internal standard, dotriacontane, which has a specific retention time of approximately 30 min. A sample chromatogram is shown in Fig. 1.

Reagents and Chemicals—Silicone gum rubber SE-30 and Aeropak 30, 100/120 mesh were obtained from Varian Aerograph. Benzene and tetrahydrofuran were obtained from Fisher Scientific Co. Solvent hexane (petroleum ether) was Merck grade, b.p. 30–60°. Alumina was Woelm neutral. Dotriacontane was purchased from Eastman Organic Chemicals.

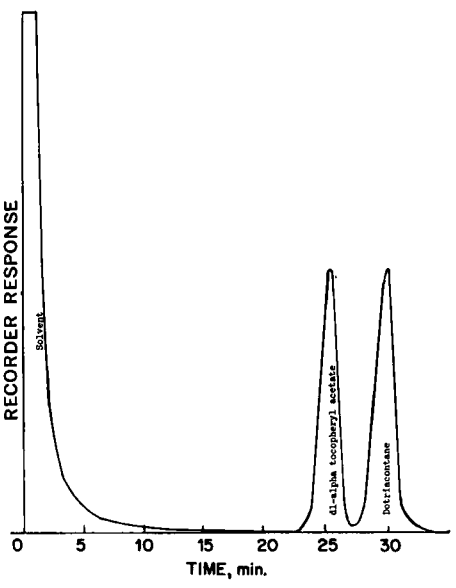

Fig. 1—Sample chromatogram.

Standard Preparation—*Internal Standard*—Accurately weigh 200 mg. of dotriacontane into a 100-ml. volumetric flask. Dissolve and dilute to volume with tetrahydrofuran. Mix well (internal standard stock solution).

Reference Standard—Accurately weigh 100 mg. of reference *dl*-alpha tocopheryl acetate into a 50-ml. amber volumetric flask. Dissolve and dilute to volume with the internal standard stock solution (working standard solution). Standards of alpha tocopherol and alpha tocopheryl succinate are prepared in a similar manner.

Sample Preparation—*A. Multivitamin Tablets or Capsules*—Determine the average tablet weight or capsule fill. Grind the tablets using a mortar and pestle and weigh a quantity of the tablet mass, or capsule fill, equivalent to about 6 mg. of *dl*-alpha tocopheryl acetate, into a 50-ml. centrifuge tube. Add 25.0 ml. of benzene, stopper, and shake, or place into an ultrasonic vibrator bath for 20 min. Centrifuge at high speed for 5 min. Transfer 15.0 ml. of the benzene layer into a glass column, 1.5 cm. in diameter and 30 cm. in length, which has been packed with 8 g. of alumina (Woelm, neutral) deactivated with 10–12% water (w/w). Collect the eluate in a glass-stoppered 50-ml. conical flask. Further rinse the column with an additional 30 ml. of benzene in small portions, allowing each portion to drain to the surface of the alumina, and collect the combined eluates in the 50-ml. conical flask. Evaporate the solution to dryness on a steam bath under a flow of nitrogen. Dissolve the residue in 2.0 ml. of the internal standard stock solution (sample working solution).

B. Gelatin Capsules—In the case where gelatin capsules are encountered, 5 ml. of water is placed into the centrifuge tube with the sample capsules and warmed for 5–10 min. at 50° to dissolve the gelatin. The resulting mixture is cooled, 25.0 ml. of benzene is added, and the procedure described under *A* carried out. Capsules which would not dissolve in water were slit open and the contents extracted with benzene as described.

C. Injectables and Liquids—Injectables and liquid samples are diluted directly with tetrahydrofuran and water is added, if necessary, to obtain a clear homogeneous solution resulting in the alpha tocopheryl acetate concentration of 4 mg./ml. Mix 5.00 ml. of this solution with 5.00 ml. of the internal standard stock solution (sample working solution). If miscibility is poor or interference is encountered, then extraction with 25.0 ml. of benzene is necessary and the procedure described under *A* carried out.

It may be necessary in some formulations to extract several times with benzene in order to quantitatively remove the alpha tocopheryl acetate. In such instances the combined extracts should be concentrated to 25 ml. and subjected to the procedure described under *A*.

D. Multivitamin Cream—Into a 50-ml. glass-stoppered centrifuge tube weigh accurately a sample of the multivitamin cream equivalent to about 10 mg. of alpha tocopheryl acetate. Add 10 ml. of water and heat at 70–80° for 10 min. Cool to room temperature and add 3 g. of sodium chloride. Extract the sample in the centrifuge tube with five 20-ml. portions of solvent hexane,

(Merck, b.p. 30–60°), shaking mechanically. Separate the layers by centrifugation. Collect the combined hexane extracts in a 125-ml. conical flask. Evaporate to a volume of approximately 10 ml. on a steam bath with the aid of a stream of nitrogen. Quantitatively transfer the hexane concentrate, with the aid of a small volume of additional hexane, onto a glass column, 1.5 cm. in diameter and 30 cm. in length which has been packed with 17 g. of alumina (Woelm, neutral) deactivated with 10–12% water (w/w). Adjust the elution rate at 4 ml./min., drain the solvent hexane to the surface of the alumina, and then elute with an additional 175 ml. of solvent hexane. Discard the first 25 ml. of solvent hexane, and collect the remaining eluate in a 250-ml. conical flask. Evaporate the collected hexane to dryness on a steam bath under a stream of nitrogen. Dissolve the residue in a sufficient volume of internal standard stock solution (5 ml.) to result in a concentration of alpha tocopheryl acetate of 2 mg./ml. (sample working solution).

E. Dry Powders—Weigh accurately a portion of the sample equivalent to 100 mg. of alpha tocopheryl acetate into a 50-ml. centrifuge tube. Add 5 ml. of 4*N* sulfuric acid and 5 ml. of 3A alcohol.[3] Swirl and heat in a hot water bath for 5 min. to disperse the beadlets. Cool to room temperature. Extract with 6 × 15 ml. of solvent hexane (Merck, low-boiling 30–60°), shaking mechanically for 5 min., centrifuging, and combining the hexane extracts in a 100-ml. volumetric flask. Dilute to volume with hexane and mix well. Pipet 10.0 ml. of the hexane solution into a small glass-stoppered flask. Evaporate this solution to dryness on a steam bath under nitrogen. The residue obtained is taken up in 5.00 ml. of internal standard stock solution (working sample).

Standard Calibration and Sample Analysis—Chromatography of several 5-μl. injections of working standard solution is required in order to condition the column and to determine the instrument sensitivity and peak retention times.

Five-microliter volumes of the working standard and sample solutions, equivalent to approximately 10 mcg. of alpha tocopheryl acetate are alternately injected into the instrument with the described operational parameters. After the elution of the dotriacontane peak the instrument is ready for the following injection. Duplicate samples and standards are chromatographed and the respective areas are determined from the integrator or by the peak height multiplied by the peak width at half height.

Calculations—Assay for alpha tocopheryl acetate in the preparation.

Determination of response factor for alpha tocopheryl acetate (RF_E).

$$RF_E = \frac{A_E\,(\text{std.}) \times C_D}{A_D\,(\text{std.}) \times C_E}$$

where:

A_E = peak area of tocopheryl acetate in the working standard.
A_D = peak area of dotriacontane in the working standard.
C_D = mg. of dotriacontane per ml. of working standard.
C_E = mg. of tocopheryl acetate per ml. of working standard.

The equation for mg. of alpha tocopheryl acetate per gram of sample is:

$$\frac{A_E\,(\text{spl.}) \times C_D \times F}{A_D\,(\text{spl.}) \times RF_E \times \text{sample weight (g.)}}$$

where:

A = peak area in the working sample.
RF_E = response factor as described above.
C_D = mg. of dotriacontane per ml. of working sample.
F = dilution factor of sample (per ml. of working sample).

Milligrams of alpha tocopheryl acetate per tablet, capsule, *etc.* = mg. of alpha tocopheryl acetate per gram × average unit weight.

RESULTS AND DISCUSSION

It was found necessary to saturate the column daily with the form of vitamin E to be determined in order to attain reproducible results. This conditioning effect also was described by Libby and Sheppard (9) in their initial report. Tocopheryl acetate required minimal daily conditioning to attain constant detector response. Tocopherol and tocopheryl succinate required longer conditioning times as well as periodic checking throughout the day.

In the course of this study a high ratio of liquid phase to inert support was employed in order to overcome any major loss of vitamin E by adsorption, thereby causing low results. Aeropak 30, with a mesh range of 100/120, increased column efficiency, and minimized adsorption.

Since no significant difference was observed in the results obtained with Pyrex or metal columns, copper tubing was selected for this work. Aging of the packed column will be evident by the resolution of the peaks. Usually a new column should be prepared when this resolution becomes poor, or after approximately 1 month of continuous use.

Several solvents were investigated for the dissolution of vitamin E prior to gas chromatography. It was found that acetone and chloroform resulted in poor reproducibility of response per unit quantity of vitamin E. This effect could be attributed to column adsorption. Tetrahydrofuran as well as *n*-hexane (9) appeared to minimize this result. Since the use of water was contemplated in some sample preparations, tetrahydrofuran, because of its water miscibility, was chosen as the solvent.

The effect of carrier gas flow rate with retention time was investigated and a linear relationship obtained. The equation calculated by the method of least squares is $\log t_r = -0.796 \log R_f + 2.38$, where t_r is the retention time in minutes and R_f is the flow rate in ml. per min. The effect of column temperature on retention time also resulted is a straight line following the equation $\log t_r = -0.0160\,Tc + 5.33$, where Tc is the column temperature in °C. Theoretical considerations of retention volume with absolute temperature and

[3] Five parts methanol and 100 parts alcohol, v/v.

TABLE I—DETECTION LIMITS AND RETENTION TIME[a]

Compd.	Limit of Detection, mcg.	Approximate Retention Time, min.	Relative Retention Time
Alpha tocopheryl acetate	0.4	25.5	0.85
Alpha tocopherol	1.2	21.3	0.71
Alpha tocopheryl succinate	1.2	22.5	0.75
Vitamin D_2 (calciferol)	1–2	20.4	0.68
C-32		30.0	1.00

[a] Column: 10% SE-30 on Aeropak 30; length 121.9 cm. (4 ft.), temperature 245°. Flow rate: N_2 = 40 ml./min.; H_2 = 20 ml./min.; air = 300 ml./min.

column loading are discussed in a paper by Sawyer and Barr (12). The detector response per approximately 10.8 mcg. of alpha tocopheryl acetate with the change in hydrogen flow rate at constant air and carrier flow was obtained. The optimum hydrogen flow to carrier gas was in the ratio of 1:2.

In this investigation the level of detectability of the various forms of vitamin E studied, as well as their relative retention times with respect to dotriacontane, are listed in Table I.

The retention times of alpha tocopherol and alpha tocopheryl succinate are quite similar under these conditions. The identification of these forms should be made using the Emmerie-Engel test while gas chromatography would serve as the quantitative assay.

The possibility of vitamin D interference in the determination of vitamin E also was investigated. With the use of a hydrogen-flame detector the ratio of vitamin E and vitamin D, per injection, which would affect the assay results by 5%, are shown in Table II. Since most multivitamin pharmaceutical products, with both vitamin E and D, contain up to 15 mg. of vitamin E per 400 units (10 mcg.) of vitamin D, no interference from vitamin D in the gas chromatographic method would be expected.

The linearity of response with alpha tocopherol, alpha tocopheryl acetate, and alpha tocopheryl succinate is shown in Fig. 2. As can be seen, the linearity of response for alpha tocopherol and alpha tocopheryl succinate is not as good at the lower concentrations. For these two forms it is desirable to work at the 10–15-mcg. range for best linearity. Since the alpha tocopherol and alpha tocopheryl succinate are usually present at higher levels than alpha tocopheryl acetate, an alumina column was not found necessary for tablets and capsules containing these forms of Vitamin E.

TABLE II—EFFECT OF VITAMIN D ON VITAMIN E RESPONSE PER INJECTION

Vitamin E, 10 mcg.	Level of Vitamin D to Cause a 5% Positive Error in Vitamin E Results, mcg.
Alpha tocopheryl acetate	8
Alpha tocopherol	1–2
Alpha tocopheryl succinate	1–2

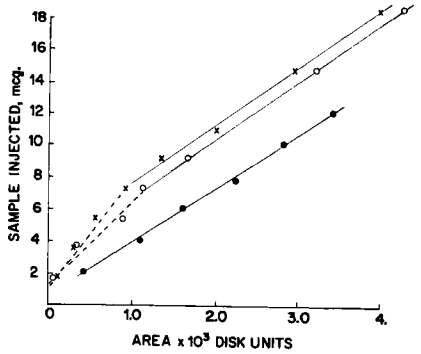

Fig. 2—Linearity of response with concentration. Column: 10% SE-30 on Aeropak 30, length 121.9 cm. (4 ft.); temperature 265°; 5 µl. injected; attenuation 32×. Key: ●, alpha tocopheryl acetate; ○, alpha tocopherol; ×, alpha tocopheryl succinate.

TABLE III—DETERMINATION OF VITAMIN E IN MULTIVITAMIN PRODUCTS

Sample	Label Claim	Found
Tablet		
A	2.00 mg./tablet	2.36 mg./tablet
B	1.00 mg./tablet	1.01 mg./tablet
C	2.72 mg./tablet	3.36 mg./tablet
Liquid	3.33 mg./ml.	3.76 mg./ml.
Cream	5.0 mg./g.	5.60 mg./g.
Capsule		
A	5.00 mg./capsule	5.14 mg./capsule
B	15.0 mg./capsule added	15.2 mg./capsule

A number of products containing tocopheryl acetate were assayed and the results presented in Table III. The area of the chromatographic peaks in a majority of samples was determined with the use of a disk integrator. Where applicable this technique of area measurement was found most suitable. The precision obtained with replicate determinations on manufactured multivitamin tablets of known composition was 101 ± 2.4%.

SUMMARY

A gas chromatographic method for alpha tocopheryl acetate has been developed which, in addition to saving considerable analytical time, also adds to the specificity of the assay for this vitamin.

Subsequent to the preparation of this manuscript a similar procedure was reported (13).

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Keyphrases

Vitamin E analysis—multivitamin products
Dotriacontane—internal standard
GLC—analysis

Technical Articles

Powder Flow Studies IV

Uniformity of Flow: Instrumentation and Applications

By GERALD GOLD, RONALD N. DUVALL, BLAZE T. PALERMO, and JAMES G. SLATER

Use of the recording powder flowmeter for the qualitative evaluation of nonuniform flowing formulations and additional instrumentation for the quantitative measurement of the variation are described. This additional refinement prints out the time, in hundredths of a minute, for preselected weight increments of powder to flow from a hopper. The variation in time is a measure of the uniformity of flow. To illustrate the utility of the instrument, two formulations having similar average flow rates but differing in their uniformity of flow were tableted. The nonuniform flowing formulation tableted on a single-rotary press had a higher coefficient of intertablet weight variation and did not conform to USP standards. Both formulations were tableted on a double-rotary press equipped with induced die feed and low coefficients of variation were obtained in both instances indicating that the die feed mechanism was effective in minimizing intertablet weight variation of an irregularly flowing formulation.

FLUCTUATING FLOW properties of tablet and capsule formulations have been generally overlooked in studying such formulations or at best, evaluated only very subjectively. This has been due, quite largely, to the fact that the two general methods used to evaluate flow of powders, angle of repose measurement, and timed delivery through an orifice, do not distinguish irregularly flowing materials from those which flow smoothly and consistently. For example, Gunsel and Lachman (1) in comparing the flow of formulations prepared with conventionally processed and spray-dried lactose using timed delivery through a funnel found that the formulation having the fastest flow rate exhibited the highest intertablet weight variation.

Another method used to evaluate flow is based on tablet weight variation as actually obtained during tableting trials. Augsburger and Shangraw (2) utilized this method in investigating the effect of various silica-type glidants on the fluidity of direct compression formulations. Increased tablet weight, along with decreased weight variation, indicates improved flowability. This method does give a true picture of the situation under production conditions, but it is time consuming, requiring numerous weighings, and expensive since large quantities of raw material are usually required. Knoechel *et al.* (3) used an instrumented rotary tablet press to evaluate the relative flowability of tableting materials. However, the use of either of these techniques is

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