Gas Chromatographic Assay for α-Tocopheryl Acetate in Multivitamin Products

By P. B. BOWMAN and W. E. WEST

A gas chromatographic procedure for α-tocopheryl acetate in soft elastic capsules, liquid formulations, and compressed tablets is described. The procedure compares favorably with the NF and USP dipyridyl procedures with respect to speed (10 *versus* 5 per day per analyst), precision (1.2 percent coefficient of variation *versus* 1.5 percent), and specificity. Unlike the dipyridyl procedures, the α-tocopheryl acetate is separated completely from other tocopherols.

THE NEED for a more precise, specific, and rapid assay for α-tocopheryl acetate (vitamin E) led to the development of a gas chromatographic procedure that met these requirements. The procedure is applicable to soft elastic capsules, liquid formulations, and coated compressed tablets.

Considerable work has been done on the gas chromatography of the tocopherols (3–7), but it has been concerned primarily with the separation of the various tocopherols and their determination in natural products. In 1966, at the 80th annual meeting of the Association of Official Analytical Chemists, Pillsbury *et al.* described a gas chromatographic procedure for vitamin E in pharmaceuticals (8, 11). Since the chromatography has been studied in the above references, this paper will be concerned with the procedural aspects of the assay.

EXPERIMENTAL

Instrument—An F & M model 402 gas chromatograph[1] equipped with a flame-ionization detector and U-shaped glass columns, 2 M. long × 3 mm. i.d., was used for this work. The columns were packed with 3% SE-30 on 100/120 mesh Gas Chrom Q.[2] The carrier gas was helium, at a flow rate of approximately 50 ml./min. and hydrogen and air flows were adjusted for maximum flame response. The column oven was operated isothermally at 235°, the flash heater at 275°, and the detector at 300°. The overall sensitivity of the instrument was 64 × 10^{-11} amp. full scale. The recorder was equipped with a model 227 Disc chart integrator[3] that was used for all peak area measurements.

Reagents and Chemicals—Chloroform, USP, anhydrous sodium sulfate, A.R., anhydrous diethyl-ether (no further purification required), 3-(*p*-methoxyphenyl) - 2,4,5 - triphenylcyclopentadieneone,[4] *d*-α-tocopheryl acetate, NF type 6-100,[5] and dry *d*-α-tocopheryl acetate types 667 and 333-S.[6]

The internal standard solution was 3-(*p*-methoxyphenyl)-2,4,5-triphenylcyclopentadieneone, approximately 1 mg./ml. in chloroform. This material was selected on the basis of its retention time (1.6 relative to α-tocopheryl acetate) and thermal stability.

The reference solution was prepared by dilution of a stock solution of the working standard of *d*-α-tocopheryl acetate in chloroform to a final concentration of approximately 1 mg./ml. An aliquot of this solution was evaporated to dryness then the residue dissolved in an equal volume of internal standard solution.

The surfactant solution used in the assay of coated compressed tablets was prepared according to the procedure of Ames and Tinkler (9).

Sample Preparation—Soft elastic capsules were prepared for assay by processing for 5 sec. with 50.0 ml. of internal standard solution at high speed in a blender. (The blender was equipped with a screw-cap jar and Teflon gaskets.) Enough capsules were processed to give a final concentration of α-tocopheryl acetate of approximately 1 mg./ml. The sample for chromatography was filtered by withdrawal through a fluted filter paper.

Liquid formulations were assayed by mixing a volume of the formulation containing 10 mg. of α-tocopheryl acetate with 5 ml. of surfactant solution in a 30-ml. glass-stoppered centrifuge tube, and shaking for 10 min. with 10.0 ml. of internal standard solution. The mixture was centrifuged if necessary, to break any emulsions formed. A portion of the chloroform layer was withdrawn for chromatography.

The assay of coated compressed tablets required the following preparation prior to chromatography: A number of tablets (color coating first removed by water washing) equivalent to approximately 50 mg. of α-tocopheryl acetate was introduced into a 125-ml. glass-stoppered conical flask with five 5-mm. glass beads and 25 ml. of surfactant solution. The mixture was shaken on a mechanical shaker until the tablets were completely disintegrated (1–3 hr.). If desired, the samples can be allowed to soak overnight before shaking. Ten grams (1 teaspoon) of granular anhydrous sodium sulfate and exactly 50.0 ml. of internal standard solution were added to the flask. The flask was stoppered tightly and shaken again for 10 min. on the mechanical shaker. A portion of the liquid was transferred to a 30-ml. glass-stoppered centrifuge tube, centrifuged, and a portion of the chloroform

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[1] F & M Scientific Corporation, Avondale, Pa.

[2] Applied Science Labs, State College, Pa.

[3] Disc Instruments, Inc., Santa Ana, Calif.

[4] Aldrich Chemical Co., No. M-2450, Milwaukee, Wis.

[5] Working standard, assayed according to NF XII, ceric sulfate titration, used as 983 mg./Gm. Distillation Products Industries, Rochester, N. Y.

[6] Type 667 potency = 667 IU/Gm. and 333-S = 333 IU/Gm., Distillation Products Industries, Rochester, N. Y., used in coated compressed tablets.

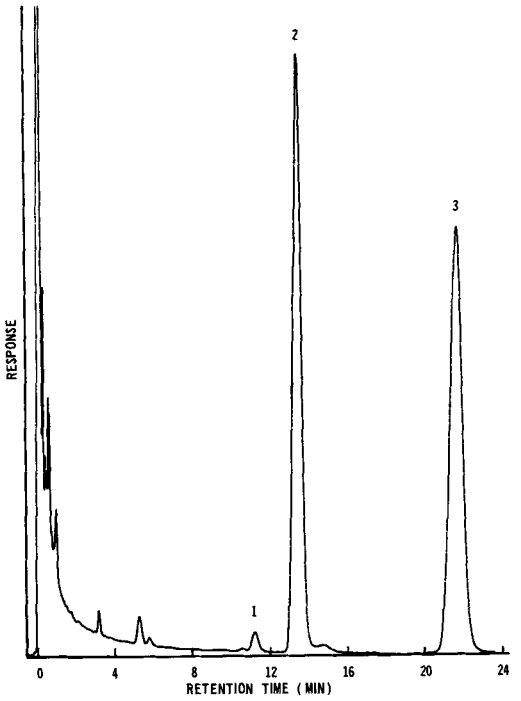

Fig. 1—Chromatogram of α-tocopheryl acetate from a lot of coated compressed tablets. Key: 1, impurity in internal standard; 2, α-tocopheryl acetate; 3, internal standard. The slopes of the integrator trace before and after the α-tocopheryl acetate were determined at 12.5 and 15.5 min., respectively. The peak width was taken at 1.6 min.

layer withdrawn and filtered through a fluted filter paper. The filtrate was then used for chromatography.

The volume of solution injected was adjusted to give nearly full-scale response for the α-tocopheryl acetate peak; this required between 1–2 mcg. of α-tocopheryl acetate. It was found necessary to saturate the column with one or two injections of reference solution at the beginning of each day's run. At least two additional injections of reference solution were made before any samples were run. Reference solution was then injected at intervals, usually after every third sample.

Calculations—The potency of α-tocopheryl acetate in the product was calculated according to the following formula:

$$\text{potency} = \text{Ru} \cdot \frac{\text{Cr}}{\text{Rr}} \cdot \text{X}$$

where

Ru = area of α-tocopheryl acetate peak in sample/area of internal standard peak
Rr = area of α-tocopheryl acetate peak in reference/area of internal standard peak
Cr = concentration of α-tocopheryl acetate in mg./ml in the reference solution
X = dilution factor

RESULTS AND DISCUSSION

Chromatography—An actual chromatogram of a sample of a production lot of coated compressed tablets is shown in Fig. 1. The small peak after the α-tocopheryl acetate peak was not identified. This peak was not considered in the integration; since it was present in the working standard as well as the products. In some cases a base line correction was required, particularly in the soft capsule formulations in which the injected oil bled from the column at an appreciable rate. This correction was done with the drift corrector No. 1290 supplied with the integrator, according to the manufacturer's directions.

Earlier work on the tocopherols (3–7) suggested a number of column packings that were applicable to these compounds and several of these were examined. However, the desired specificity was obtained only with SE-30 on 100/120 mesh Gas Chrom Q. It was found in our laboratories that

TABLE I—RECOVERY OF α-TOCOPHERYL ACETATE FROM VARIOUS MULTIVITAMIN PREPARATIONS

No.	Added, mg.	Found, mg.	% Recovery
	Soft Elastic Capsule[a]		
1	37.35	37.23	99.7
2	37.35	37.42	100.1
3	37.35	37.38	100.1
4	37.35	37.80	101.2
5	37.35	38.22	102.3
	Mean Recovery		100.7
	S.D.		1.1
	Liquid Formulation[b]		
1	8.76	8.73	99.6
2	8.76	8.97	102.4
3	8.76	8.70	99.3
4	8.76	9.00	102.7
5	8.76	8.76	102.3
6	8.76	8.70	99.3
	Mean Recovery		100.9
	S.D.		1.6
	Coated Compressed Tablets		
Formulation 1[c]			
1	55.50	55.76	100.5
2	55.63	55.39	99.6
3	56.53	56.83	100.5
4	56.15	55.23	98.4
5	55.22	55.92	101.3
6	56.20	57.36	102.1
	Mean Recovery		100.4
	S.D.		1.1
Formulation 2[d]			
1	38.78	38.90	100.3
2	37.30	37.41	100.3
3	39.29	39.34	100.1
	Mean Recovery		100.2
	S.D.		0.1
Formulation 3[c]			
1	38.72	37.98	99.6
2	37.51	38.56	102.7
3	38.28	38.33	100.1
	Mean Recovery		100.8
	S.D.		1.3
Formulation 4[c]			
1	112.15	112.90	100.7
2	112.24	111.81	99.6
3	119.50	122.50	102.5
	Mean Recovery		100.9
	S.D.		1.5

[a] *d*-α-Tocopheryl acetate NF; [b] *dl*-α-tocopheryl acetate; [c] *d*-α-tocopheryl acetate type 667; [d] *d*-α-tocopheryl acetate type 333-S.

TABLE II—COMPARISON OF THE GAS CHROMATOGRAPHIC AND NF XII ASSAYS FOR α-TOCOPHERYL ACETATE IN MULTIVITAMIN PREPARATIONS

Sample	Formulation	Gas Chromatographic	NF XII	Theory
1	SEC[a]	7.47 mg./cap.	7.43 mg./cap.	7.65 mg./cap.
2	SEC	7.58	7.32	
3	SEC	7.46	7.43	
4	SEC	7.63	7.32	
5	SEC	7.46	7.35	
6	SEC	7.48	7.30	
7	SEC	7.53	7.30	
8	Liquid[b]	8.65 mg./ml.	8.58 mg./ml.	10.10 mg./ml.
9	Liquid	9.06	8.61	
10	Liquid	9.39	8.85	
11	CCT[c]	10.97 mg./tab.	10.59 mg./tab.	11.03 mg./tab.
12	CCT	10.94	10.88	
13	CCT	10.70	10.73	
14	CCT	11.30	10.51	
15	CCT	11.39	10.88	
16	CCT	11.43	11.03	
17	CCT	11.12	11.01	
18	CCT	7.93	7.04	7.35
19	CCT	7.73	6.99	7.35
20	CCT	23.70	21.47	22.00

[a] Soft elastic capsule. [b] Liquid formulation. [c] Coated compressed tablet.

there was considerable variation in the performance of different lots of this packing, even among the pretested lots; hence some selectivity may be required to find a satisfactory lot of the material. The best indications of a good lot of support were found to be column efficiency (> 700 theoretical plates per foot for α-tocopheryl acetate)[7] and retention time (12–14 min. at the prescribed temperature and flow rate).

Mass spectral and infrared examination of α-tocopheryl acetate eluted from the column indicated that no decomposition occurred during chromatography. The infrared spectrum of the chromatographed material was compared with that of a sample of the same material that had not been chromatographed. No significant differences were visible. The mass spectrum of the chromatographed material showed the molecular ion at *m/e* 472 and major peaks at *m/e* 430, 207, and 165. These peaks are consistent with the structure of the molecule.

Although no concentration limits were established, the chromatographic response was linear over the range from 0.1 to 4 mcg. of α-tocopheryl acetate which bracketed the concentration ranges of the assay procedure.

Recovery—Recovery of α-tocopheryl acetate was determined to be quantitative (> 99%) for each of the three formulations. For soft elastic capsules, recovery was determined by replicate assays of the same fomulation without α-tocopheryl acetate but using chloroform that contained both internal standard and the theoretical quantity of α-tocopheryl acetate. These results are listed in Table I. Although this was not the ideal procedure for a recovery study, it proved to be a practical approach. For the liquid formulation, a similar procedure was followed; the organic phase again contained the internal standard and the α-tocopheryl acetate. The results are shown in Table I. In this case, it was impossible to dissolve the α-tocopheryl acetate in the blank formulation.

Recovery from coated compressed tablets was determined by assaying tablets without α-tocopheryl acetate, but with the label amount of the appropriate dry form weighed into the flask before addition of the surfactant solution. These results are in Table I. Recovery of the α-tocopheryl acetate from the dry material alone was also determined and found to be quantitative.

Precision—Assay precision was determined for each type of formulation by replicate assay of a single lot of the material. In each case, the coefficient of variation (percent standard deviation) was less than 1.2%. According to Lehman (10), the interlab coefficient of variation was 1.52% in a collaborative study of the dipyridyl method (1, 2). The latter procedure suffers from the lack of a recognized standard for α-tocopherol, thus requiring the use of a somewhat arbitrary absorptivity and careful timing of the reaction before measuring the absorbance.

Comparison of the Gas Chromatographic and Dipyridyl Procedures—The results of the gas chromatographic procedure on several lots of each type of formulation are listed in Table II. For comparison, the results obtained by the NF procedure and the label potency are listed.

It should be noted that the gas chromatographic results are consistently higher than those of the NF procedure. The USP procedure gave results similar to those obtained by gas chromatography. The NF method requires a column chromatographic separation to eliminate the interference of vitamin A, whereas this is accomplished by a hydrogenation step in the USP procedure. The low results obtained by the NF procedure can probably be attributed to loss of α-tocopherol during the column chromatography step.

An experienced analyst in our laboratory was able to complete three assays for α-tocopheryl acetate in a working day using the NF XII procedure. Using the USP hydrogenation, five assays could be completed. The gas chromatographic procedure permits the assay of at least 10 samples a day. An analyst operating two chromatographs could double this daily output and still have sufficient time to prepare samples and perform the calculation of results.

[7] Theoretical plates per foot $= \left(\frac{\text{retention time}}{\text{width at half height}}\right)^2 \frac{5.5}{\text{column length}}$

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Keyphrases

Vitamin (multiple) dosage forms
α-Tocopheryl acetate—analysis
GLC—analysis
Mass spectrometry—identity

Determination of Terpin Hydrate by Gas-Liquid Chromatography

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A gas chromatographic procedure has been developed for the quantitative determination of terpin hydrate. The procedure may be applied to any of the NF dosage forms containing this compound. The terpin hydrate is extracted with chloroform from a saturated sodium chloride solution and chromatographed as intact terpin on a hydrogenated castor oil column. Degradation products of terpin hydrate, other terpenes, and formulation excipients do not interfere. The method is specific, rapid, and accurate. A coefficient of variation of less than 1 percent was determined from replicate analyses.

METHODS FOR THE ANALYSIS of terpin hydrate, which have been proposed and described in the literature, include precipitation with mercury salts (1, 2), esterification with 3,5-dinitrobenzoyl chloride (3), and spectrophotometric determinations (4, 5). These methods lack specificity. They do not distinguish terpin hydrate from its decomposition products, and are not satisfactory for many formulations.

A method for the determination of terpin hydrate is presented in "Official Methods of Analysis"(6). In this procedure terpin hydrate is converted by acid dehydration to a mixture of terpenes which is reacted with molybdo-phosphotungstic acid to produce a blue reduction product which is measured spectrophotometrically. Any mixture of terpenes, treated similarly, will result in comparable color formulations.

The gas chromatographic (GLC) procedure presented in this paper is specific for the intact terpin molecule. Degradation products, other terpenes, and formulation excipients do not interfere with the quantitation of terpin hydrate by the procedure described.

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After this manuscript was submitted, a paper by L. Kurlansik, C. Damon, and E. F. Salim was published on the gas chromatographic determination of terpin hydrate (7).

METHOD

Reagents—(*a*) *Internal Standard Solution*—Prepare a chloroform solution containing 5.5 mg. of 3-tert-butylphenol per ml. of chloroform. (*b*) *Reference Preparation*—Dry terpin hydrate NF ($C_{10}H_{20}O_2 \cdot H_2O$) at 60° in vacuum at 10 mm. mercury for 3 hr. Determine any residual moisture in the terpin hydrate reference material by Karl Fischer, and use on the completely anhydrous basis for preparation of the reference solution. Transfer an accurately weighed portion of the anhydrous terpin ($C_{10}H_{20}O_2$), approximately 158 mg., into a 200-ml. volumetric flask. Add exactly 25.0 ml. of the internal standard solution and dilute to volume with chloroform.

Sample Preparation—Transfer an accurately measured portion of the formulation containing approximately 170 mg. of terpin hydrate NF ($C_{10}H_{20}O_2 \cdot H_2O$) into a 250-ml. separator. Add about 80 ml. of saturated sodium chloride solution and mix. Extract the terpin hydrate with two successive 50-ml. portions of chloroform. Collect the chloroform extracts in a 200-ml. volumetric flask, add exactly 25.0 ml. of the internal standard preparation, and dilute to volume with chloroform.

Chromatography—Chromatograph aliquots of the sample and reference preparation. Under typical circumstances the following instrument conditions were found to be satisfactory.

Chromatograph—F & M 402 with flame ionization detector. Column—Glass 3 mm. i.d. × 1.2 meters, 5% hydrogenated castor oil[1] on 80–100 mesh

[1] Castorwax, the Baker Castor Oil Co., Bayonne, N. J.