prepared using an automatic TLC plate coater[3]. Plates were dried at 40° and activated by heating at 105° for 15 min just prior to use.

Solvent—Ethyl acetate–methanol–water–concentrated ammonium hydroxide (150:40:35:5) was mixed and transferred to the chromatographic chamber just prior to use.

Chamber—A paper-lined glass tank, ~30 × 9 × 27 cm, was saturated with solvent just prior to use.

RESULTS AND DISCUSSION

Essentially complete extraction of dyes from the tablet-coating formulation was indicated by the lack of color in the centrifuged solid residue. Development of the thin-layer chromatogram required about 50–60 min.

The R_f values were not affected by applying all dyes in combination or by varying quantities applied from 0.1 to 10 μg. Other components of the tablet-coating formulations had no effect.

Average R_f values for the 20 dyes are shown in Table I. The spots were generally compact horizontal bands that were visually distinct even when the R_f values were very close. Distinction was aided by hue differences even among dyes of the same color group. The possibility of discriminating among all dyes of a given color group was confirmed by applying mixtures. Any of several similar published systems (2, 3) probably could be used to resolve any possible confusion.

The method has been applied successfully to more than a dozen different color-coating formulations to date.

[3] Camag model 21 602, Muttenz, Switzerland.

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Collaborative Study of a GLC Method for Vitamin E

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Received March 9, 1978, from the *Division of Nutrition, Food and Drug Administration, Washington, DC 20204.* Accepted for publication May 4, 1978.

Abstract □ The official GLC method of the Association of Official Analytical Chemists (AOAC) for determining vitamin E was modified and collaboratively studied for the National Formulary (NF). The internal standard hexadecyl hexadecanoate (cetyl palmitate) was substituted for the dotriacontane used in the AOAC method, and some other minor changes were made. Eleven samples, representing all types of NF formulations and NF bulk materials, were analyzed by 11 laboratories. The coefficients of variation of the reproducibility and repeatability were 4.5 and 2.4%, respectively, for all laboratories and samples. The values were 3.4 and 1.6%, respectively, when the one laboratory statistically determined to be an outlier was excluded. The coefficients of variation of reproducibility and repeatability for α-tocopheryl acid succinate were 2.1 and 1.5%, respectively. All of these values lie within the 5% limit required by the NF.

Keyphrases □ Vitamin E—GLC analysis, collaborative study of 11 samples by 11 laboratories □ GLC—analysis, vitamin E, collaborative study of 11 samples by 11 laboratories

Interest in GLC as compendial or official methodology for the quantitative measurement of α-tocopherol, α-tocopheryl acetate, and α-tocopheryl acid succinate has developed during the past 10 years. Sheppard *et al.* (1) conducted a collaborative study of a GLC method for determining vitamin E and demonstrated the superiority of GLC over the colorimetric method (2, 3). An external calibration procedure was used for calculating the quantity of specific vitamin E isomers in pharmaceutical preparations. The Association of Official Analytical Chemists (AOAC) adopted the GLC method (4, 5).

BACKGROUND

An extensive collaborative study of the GLC method for determining vitamin E was initiated in April 1970 by the Pharmaceutical Manufacturers Association Quality Control Section (PMAQCS) at the request of the National Formulary (NF) for a better, more specific compendial assay for vitamin E and vitamin E decavitamin preparations (6). A preliminary intralaboratory GLC study comparing the dotriacontane internal standard method with the AOAC external standard method showed no significant difference in the results. Since the two calibration methods were apparently equally valid, the less complicated internal standard method was chosen for the PMAQCS study.

The GLC method was again demonstrated to be more specific and rapid than the compendial colorimetric methods (2, 3). The internal standard calibration method used in the PMAQCS study gave improved precision over that of the AOAC method (1), which used an external calibration technique. On the basis of the PMAQCS study, the AOAC adopted the GLC method with the dotriacontane internal standard as a primary calibration method; the external standard method became an alternative calibration method (7).

Concurrently, the NF staff was studying the PMAQCS collaborative study results and planned to include a GLC method based on that study in NF XIV (8). However, this GLC method was the subject of much discussion and debate. Therefore, the NF decided to adopt the AOAC method as an interim method until a generally agreed-upon GLC method could be studied collaboratively. The NF incorporated the AOAC method, without the external calibration alternative, into NF XIV (8).

A meeting of persons from domestic and foreign industry, the AOAC, and the Food and Drug Administration was held in 1974 to resolve various issues and to agree upon methodology. Hexadecyl hexadecanoate (cetyl palmitate) was chosen to replace dotriacontane as the internal standard. Electronic integration was considered mandatory. Other minor modifications were made in the AOAC method, and the revised method was then converted to NF monograph form and circulated to the attendees for approval. The method was then collaboratively studied in 1975. The results of that study are presented here.

EXPERIMENTAL

Method—The collaborators were instructed to follow the official AOAC method (7) with the following changes: column temperature, 245–265°; and inert carrier gas flow, adjusted so that the α-tocopheryl acetate peak appears about 20 min after sample injection.

The internal standard solution is prepared by dissolving 500 mg of

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hexadecyl hexadecanoate in n-hexane in a 500-ml volumetric flask and diluting to volume with n-hexane.

The column is conditioned for 1 hr at 300° with no carrier gas flow. The column is then cooled, and the carrier gas flow is begun and maintained for 4 hr.

Tablets and capsules are prepared according to Ref. 7. Bulk materials are treated the same as the standards. Other preparations are diluted with internal standard solution so that the final concentration is 1 mg of vitamin E/ml.

The instrument is calibrated by injecting 2 μl of standard solution(s).

Retention times, relative to the internal standard, are: α-tocopherol, 0.53; α-tocopheryl acid succinate, 0.54; and α-tocopheryl acetate, 0.62.

Collaborative Study—Eleven samples and instructions were sent to 11 laboratories in the United States and the Federal Republic of Germany. All collaborators were experienced in GLC analysis of vitamin E and were familiar with the official AOAC method. Nine industrial laboratories plus this laboratory and the USP Drug Research and Testing Laboratory participated. The collaborators were instructed to analyze the samples in duplicate, using a single injection. "Duplicate," as used in the study, is a second subsample carried through the entire method, terminating with a single GLC analysis.

The samples[1] were NF formulations and bulk materials especially selected and prepared for the study to be representative of the many situations that confront the quality control, formulary, or regulatory chemist performing a routine analysis. The samples were coded in this laboratory and were packed and shipped by the NF. The shipment also included the reference and internal standards furnished by the NF.

Sample 1 was a practice sample, *dl*-α-tocopheryl acetate, 400 IU of vitamin E/capsule. Sample 2 was a vitamin E capsule, *dl*-acetate, 200 IU. Sample 3 was a vitamin E capsule, *d*-acetate, 400 IU. Sample 4 was a vitamin E capsule, *d*-alcohol, 400 IU. Sample 5 was *dl*-α-tocopheryl acetate, pharmaceutical grade, 500-mg ampuls.

Sample 6 was *dl*-α-tocopherol, pharmaceutical grade, 600-mg ampuls. Sample 7 was *d*-α-tocopheryl acetate, 94.0 g of vitamin E/100 g. Sample 8 was *d*-α-tocopheryl acid succinate, minimum assay 97%. Sample 9 was *d*-α-tocopherol, mixed tocopherols, minimum assay 671 mg/g. Sample 10 was an unknown, and Sample 11 was an unknown, 489 mg of α-tocopheryl acetate.

Samples 1–9 were labeled as to content claim; Samples 10 and 11 bore only sample numbers. Sample 10 was a blind duplicate of Sample 4. For Sample 10, the analyst was required to identify the tocopheryl isomer or isomers present and to determine quantitatively the amount of the specific isomer present.

The GLC method, instructions to collaborators, and sample number label information were sent under separate cover to each collaborator.

RESULTS AND DISCUSSION

The analytical results are summarized in Table I. The statistical summary is presented in Table II. Repeatability as used here relates to within-laboratory measurements, and reproducibility refers to between-laboratory measurements.

A two-way analysis of variance for the data from all laboratories and all samples was performed. A significant difference ($p < 0.01$) was found among laboratories. A significant ($p < 0.01$) laboratory × sample interaction also was found, because the majority of the laboratories did not obtain consistent results for all samples. For some samples, a laboratory obtained higher results in comparison to other laboratories; for other samples, the same laboratory obtained lower results in comparison to other laboratories.

A one-way analysis of variance was performed for each sample, both with and without Laboratory 5 (statistically determined to be an outlier). When this laboratory was excluded, a significant difference (at least $p < 0.05$) was found among laboratories for each sample. When Laboratory 5 was included, there were no significant ($p > 0.05$) differences among laboratories for Samples 2, 3, 5, and 10. In every instance, the coefficients of variation for repeatability and reproducibility were either equal or smaller when Laboratory 5 was excluded from the analysis.

A two-way analysis of variance was performed to compare the duplicate samples, 4 and 10, and a significant difference ($p < 0.05$) was found among laboratories. When Laboratory 5 was excluded, there was a significant

Table I—Collaborative Results (International Units) of the GLC Determination of Vitamin E[a]

Sample	Laboratory										
	1	2	3	4	5	6	7	8	9	10	11
1	385, 382	360, 364	385, 390	404, 402	400, 401	377, 393	394, 393	382, 384	409, 400	357, 363	387, 391
2	221, 207	188, 187	202, 203	202, 203	177, 244	196, 193	212, 201	204, 206	213, 209	202, 194	201, 204
3	294, 326	299, 299	309, 305	290, 303	329, 266	297, 296	324, 324	307, 309	334, 318	289, 288	309, 316
4	271, 262	289, 282	302, 302	327, 322	263, 286	274, 272	275, 265	292, 295	297, 310	284, 277	304, 315
5	1000, 982	910, 910	952, 970	970, 969	990, 910	935, 949	955, 939	960, 1000	929, 932	942, 937	958, 968
6	1021, 1069	1004, 1004	1004, 997	1048, 1045	890, 902	1000, 986	999, 988	1017, 1016	1000, 975	1011, 1031	990, 1014
7	983, 949	897, 871	970, 961	952, 958	950, 880	945, 947	901, 959	950, 953	940, 929	959, 959	958, 969
8	1030, 1039	985, 1003	1000, 1039	1047, 1038	970, 939	972, 993	1004, 1005	989, 1008	995, 1002	1003, 998	984, 1023
9	687, 707	708, 726	764, 787	810, 799	659, 600	731, 739	757, 766	748, 763	761, 759	729, 744	758, 757
10	275, 274	300, 310	298, 262	329, 328	332, 269	277, 274	306, 306	293, 294	308, 307	286, 292	305, 315
11	491, 486	486, 490	515, 510	494, 487	457, 431	489, 479	512, 516	487, 489	500, 524	507, 494	494, 497

[a] Duplicate analyses.

[1] Sample 1 was purchased on the open market. Samples 2–4 and 10 were provided by R. P. Scherer, Detroit, Mich. Samples 5 and 6 were obtained from Hoffmann-La Roche, Nutley, N.J. Sample 7 was given by General Mills Chemicals, Minneapolis, Minn. Samples 8, 9, and 11 were provided by Distillation Products Industries, Rochester, N.Y.

Table II—Statistical Summary of Collaborative Results of GLC Analysis of Vitamin E

Statistic	Sample[a] 1	2	3	4	5	6	7	8	9	10	11
All laboratories											
Mean	385.9	203.0	306.0	289.4	953.1	1000.5	945.5	1003.0	738.9	297.3	492.5
Reproducibility	15.1	16.0	16.3	19.4	27.2	41.2	28.5	26.4	47.6	20.0	20.4
CV, %	3.9	7.9	5.3	6.7	2.9	4.1	3.0	2.6	6.4	6.7	4.2
Repeatability	3.0	16.0	15.8	7.2	20.5	14.2	18.0	15.6	15.8	15.8	8.7
CV, %	0.8	7.9	5.2	2.5	2.2	1.4	1.9	1.6	2.1	5.3	1.8
Without Laboratory 5											
Mean	384.4	202.5	306.8	290.8	953.4	1011.0	948.5	1007.8	749.8	297.0	497.4
Reproducibility	15.1	8.4	13.6	19.4	25.7	23.4	25.8	21.4	31.0	18.6	12.6
CV, %	3.9	4.1	4.4	6.7	2.7	2.3	2.7	2.1	4.1	6.2	2.5
Repeatability	3.2	4.6	8.7	5.5	11.9	14.6	10.5	14.8	10.0	8.8	7.0
CV, %	0.8	2.3	2.8	2.6	1.3	1.4	1.1	1.5	1.3	3.0	1.4

[a] For all laboratories and samples, the mean was 601.4, the reproducibility was 27.1 with a coefficient of variation of 4.5%, and the repeatability was 14.5 with a coefficient of variation of 2.4%. When Laboratory 5 was eliminated, the mean was 604.5, the reproducibility was 20.6 with a coefficient of variation of 3.4%, and the repeatability was 9.8 with a coefficient of variation of 1.6%.

($p < 0.01$) laboratory × sample interaction. Six of the laboratories obtained consistent results for both samples. Four of the five remaining laboratories obtained results that were considerably higher for Sample 10, while one laboratory reported considerably higher results for Sample 4. Because of the significant laboratory × sample interaction, no significant difference ($p < 0.05$) was found between Samples 4 and 10.

One laboratory failed to identify properly the isomer present in Sample 10. It was learned that the procedure had not been followed correctly. Therefore, in this instance, the procedure was not at fault. All other laboratories identified the isomer present in Sample 10. The required isomer identification was properly carried out for all other samples by all laboratories.

CONCLUSION

Even with the data of the poorest performing laboratory included, coefficients of variation of 4.5% for reproducibility and 2.4% for repeatability are within the 5% required by NF. The laboratory exhibiting the poorest performance can be eliminated statistically, and the resulting coefficients of variation are 3.4 and 1.6% for reproducibility and repeatability, respectively. The reproducibility of 2.1% and the repeatability of 1.5% for the α-tocopheryl acid succinate are exceptionally gratifying since this compound is suspected of breaking down during GLC analysis and would be expected to exhibit larger coefficients of variation. The method, as collaboratively studied, appears to meet the requirements for an NF compendial method.

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Absence of Povidone-Iodine-Induced Mutagenicity in Mice and Hamsters

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Received November 3, 1977, from the *Department of Industrial Hygiene and Toxicology, BASF Aktiengesellschaft, D-6700 Ludwigshafen/Rh., Federal Republic of Germany.* Accepted for publication May 19, 1978.

Abstract □ Povidone-iodine USP was tested for mutagenicity in mice by the dominant lethal assay or micronucleus test and in Chinese hamsters by the bone marrow test. None of the three tests revealed any evidence of mutagenic effect.

Keyphrases □ Povidone-iodine—evaluated for mutagenicity in mice and hamsters □ Mutagenicity—povidone-iodine evaluated in mice and hamsters □ Anti-infectives, topical—povidone-iodine, evaluated for mutagenicity in mice and hamsters

According to Wlodkowski *et al.* (1), povidone-iodine blocked growth of the DNA polymerase-deficient *Escherichia coli* strain whereas no mutagenic effects were found with *Salmonella typhimurium* in the same (Ames) test. In the fluctuation test, povidone-iodine was mutagenic only for *S. typhimurium* T 1530 and not for *S. typhimu-*

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