while it gave cleaner extracts, the efficiency of extraction for acetaminophen was significantly lower. Acetaminophen and the internal standard partitioned nearly identically to one another. Absolute recovery of acetaminophen was 80–90%, and recovery relative to a matrix-free standard was 109%.

Linearity was evaluated by spiking drug-free serum with 10 standard solutions over the range of 20 ng/ml–20 μg/ml. The correlation coefficient obtained was 0.9997. Reproducibility was determined by spiking six different drug-free serum samples. The relative standard deviation at 2 μg/ml was 3.0%; at 200 ng/ml, it was 5.1%.

Reversed-phase chromatography with electrochemical detection is a useful means of quantitating acetaminophen metabolites in blood, urine, and liver homogenates. Since all major metabolites are more polar than the free drug or oxidize at significantly more positive potentials, they do not interfere in the present method.

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Extraction and TLC Separation of Food, Drug, and Cosmetic Dyes from Tablet-Coating Formulations

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Abstract □ A rapid method for extraction of dyes from tablet-coating formulations is described. The dyes were released from their lakes by treatment with concentrated phosphoric acid and dissolved in methanol. After being made alkaline with ammonium hydroxide, the mixture was centrifuged to obtain a clear supernate for application to the TLC plate. With ethyl acetate–methanol–water–concentrated ammonium hydroxide (150:40:35:5) on silica gel, 20 dyes were separated sufficiently to confirm their presence in the coating formulation.

Keyphrases □ Dyes, various—TLC determination in tablet-coating formulations □ TLC—determination, various dyes in tablet-coating formulations □ Tablet-coating formulations—TLC determination of various dyes

The widespread use of coloring agents in pharmaceutical preparations, combined with stringent and changing regulations on their use, makes a simple and rapid method for identifying the components of a colorant mixture highly desirable. TLC has been used extensively for this purpose, and several systems for the separation of dyes were published (1–4). If the dyes are components of a formulation, a suitable preparative scheme must be employed before the sample is applied to the thin-layer plate. Since tablet-coating formulations are relatively simple[1], published systems for extracting dyes from foods and drugs (5–7) are unnecessarily complex.

The aim of this work was to develop a combination of preparative scheme and TLC system that could be applied uniformly to various formulations.

[1] Formulations contained one or more lakes, titanium dioxide, and one or more of the following: sucrose, povidone, hydroxypropylcellulose, sodium benzoate, alcohol, and water.

Table I—Summary of R_f Values

Dye	R_f
FD&C Yellow No. 5	0.06
FD&C Green No. 3	0.07
FD&C Red No. 2[a] (amaranth)	0.07
FD&C Blue No. 1	0.16
D&C Blue No. 4	0.17
FD&C Yellow No. 6	0.22
FD&C Red No. 40	0.22
FD&C Red No. 33	0.23
FD&C Red No. 4	0.24
FD&C Violet No. 1[a]	0.26
FD&C Red No. 7	0.27
D&C Green No. 5	0.31
FD&C Red No. 3 (erythrosine sodium)	0.31
FD&C Red No. 28	0.33
FD&C Orange No. 4	0.36
D&C Red No. 19	0.36
D&C Yellow No. 10	0.37
D&C Yellow No. 11	0.77
D&C Red No. 36	0.81
D&C Green No. 6	0.85

[a] Use no longer permitted in the United States.

EXPERIMENTAL

Extraction of Dyes—Ten drops (~0.4 ml) of phosphoric acid (85%) were placed in a 50-ml centrifuge tube, and an appropriate amount of tablet-coating liquid (up to 1 ml), pure dye or lake, was added. The contents of the tube were mixed by swirling intermittently for 5 min. Methanol, 10 ml, was added, and the tube was shaken for 1 min. Then 1 ml of concentrated ammonium hydroxide (29%) was added, and the tube again was shaken for 1 min. Solids were removed by centrifuging, and 10 μl of the supernate was applied to the thin-layer plate.

TLC Plates—Silica gel G[2] plates, 20 × 20 cm, 0.25 mm thick, were

[2] E. M. Reagents, E. Merck, Darmstadt, West Germany.

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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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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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