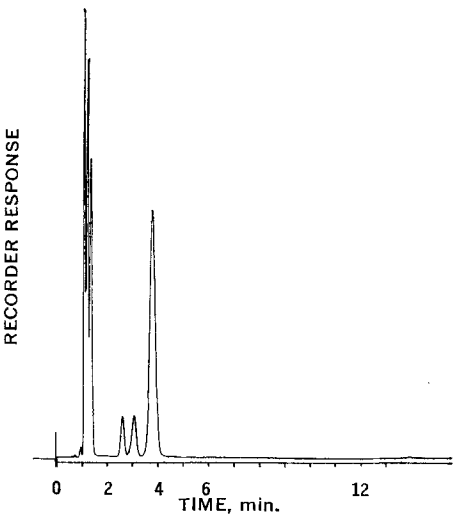

Fig. 3—Chromatogram of dehydroxylated terpin hydrate in alcohol solution on a Carbowax 20M column.

on a Carbowax 20 M column. The resultant chromatogram, shown in Fig. 3, indicates the low retention times for the reaction products which include terpinene, terpineol, and eucalyptol. The distillate also was subjected to etherification using the conditions stated under *Method B* and the chromatogram produced showed the absence of any significant peaks after a retention time of 4 min.

Dodecyl alcohol was selected as an internal standard based on a similarity in molecular weight to terpin hydrate, reactivity to form a trimethylsilyl ether, and ready availability of the material in a high state of purity. General use of the internal standard method has the advantage of minimizing sampling variation as a source of error in gas chromatographic procedures.

Terpin hydrate and dodecyl alcohol exhibited a linear response between the ratios of 0.5 to 3.0 (DDA/TH standard) in the range of 70 mg. to 200 mg. of either compound. An analogous study with the TMS ethers indicated a linear response between essentially the same ratios as the parent compounds. Average values for the relative response factor F were determined to be 1.75 for method A and 0.64 for method B.

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Determination of Terpin Hydrate in Elixirs by Gas Chromatography

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A previous paper describes the determination of terpin hydrate by gas chromatographic analysis. The method has been extended to the assay of terpin hydrate in official elixirs after isolation of the drug in relatively pure form. The procedure is considered to be an improvement over existing methods in specificity and reproducibility.

TERPIN HYDRATE is used extensively as an ingredient in the preparation of cough suppressing elixirs. The most common formulations are terpin hydrate elixir, terpin hydrate and codeine elixir, and terpin hydrate and dextromethorphan hydrobromide elixir which are official in N.F. XII (1).

Literature reports on determinations of terpin hydrate in elixirs are confined to gravimetric (2–4) and colorimetric (5–7) procedures. Spectrophotometric assays in the visible region are due to the formation of molybdenum blue, a reduction product of the phosphomolybdic acid reagent.

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A previous paper by the authors deals with gas chromatographic analysis of terpin hydrate by two techniques (8). A method is presented for the isolation of terpin hydrate from the elixirs in relatively pure form and application of the gas chromatographic procedures developed for the drug substance.

EXPERIMENTAL

The gas chromatograph, columns, chromatography conditions, reagents, and terpin hydrate reference standard employed are described in a previous paper (8).

Isolation of Terpin Hydrate—Transfer 10.0 ml. of the elixir into a separator, add 25 ml. of saturated sodium acetate solution, shake for 1 min.,

and allow to stand for 30 min. with occasional shaking. Extract with four 25-ml. portions of chloroform, filtering each portion through chloroform-saturated cotton into a beaker. Concentrate the solution to about 10–15 ml. by warming on a steam bath with the aid of a current of air, and evaporate the remaining solvent without heat using a gentle current of air.

Method *A*

Internal Standard Solution—Dissolve about 1 Gm. of dodecyl alcohol, accurately weighed, in 100.0 ml. of absolute alcohol.

Procedure—Dissolve the residue from the elixir extract in about 15 ml. of absolute alcohol and transfer quantitatively to a 50-ml. volumetric flask containing 10.0 ml. of internal standard solution. Dilute to volume with absolute alcohol, and mix. A 0.5-μl. portion of this solution was injected into the chromatograph.

Calculation—The quantity of terpin hydrate N.F. in the elixir, in mg. per ml., is calculated by the formula:

$$\frac{H \times (C \times V) \times F \times 1.104}{10}$$

in which

H = $\frac{\text{peak ht. of terpin hydrate}}{\text{peak ht. of internal std.}}$

C = concentration, in mg. per ml., of internal standard solution,

V = volume, in ml., of internal standard solution taken,

F = relative response of equal weights of dodecyl alcohol (DDA) and terpin hydrate (TH) standard as determined by peak height measurement (DDA/TH),

1.104 = factor to convert anhydrous terpin hydrate to terpin hydrate N.F.

The per cent recovery of terpin hydrate is obtained by the expression

$$\text{terpin hydrate found } \frac{(\text{mg./ml.}) \times 100}{17}$$

in which the denominator represents the theoretical amount of terpin hydrate, in mg. per ml. of official elixirs.

Method *B*

Internal Standard Solution—Dissolve about 2 Gm. of dodecyl alcohol, accurately weighed, in 100.0 ml. of pyridine.

Procedure—Dissolve the residue from the elixir extract in about 15 ml. of pyridine and transfer quantitatively to a 50-ml. volumetric flask containing 10.0 ml. of internal standard solution. Dilute to volume with pyridine, and mix. Transfer 1.0 ml. to a screw-cap vial, add 0.3 ml. of hexamethyldisilazane, 0.2 ml. of trimethylchlorosilane, stopper the vial, and shake vigorously for 1 min. Allow to stand for 1 hr. and inject 0.4 μl. of supernatant liquid into the chromatograph.

Calculation—The formulas given in method *A* may be used for calculation of terpin hydrate recoveries. However, for this procedure H is the ratio of peak heights for the corresponding trimethylsilyl ethers of terpin hydrate and dodecyl alcohol, and F is the relative response determined for the TMS ethers.

Table I—Analysis of Terpin Hydrate in Elixirs

Elixir	Recoveries, % Method *A*	Method *B*
Terpin hydrate[a]	99.7 ± 0.22[c]	99.8 ± 0.27[c]
Terpin hydrate[b]	100.2	102.0
	100.9	101.1
Terpin hydrate and codeine[b]	102.4	103.1
	103.3	103.3
Terpin hydrate and dextromethorphan HBr[b]	102.6	100.9
	103.4	101.4

[a] Prepared in this laboratory according to N.F. XII. [b] Commercial products. [c] Based on 10 determinations.

RESULTS AND DISCUSSION

Isolation of terpin hydrate in the elixirs is achieved by salting the material out of solution and subsequent extraction with chloroform. This has been found to be necessary due to the similarity of terpin hydrate solubility in water and chloroform. Several saturating agents were investigated and sodium acetate was selected on the basis of reproducibility of initial assays. Recoveries obtained for terpin hydrate, terpin hydrate and codeine, and terpin hydrate and dextromethorphan hydrobromide elixirs through the addition of saturated sodium acetate solution are summarized in Table I. Satisfactory results may be obtained by the addition of a saturated magnesium sulfate solution. However, on occasion it has been observed that gel formation occurs during the prescribed waiting period. This effect may be eliminated by adding 1–2 ml. of water and gentle mixing.

Care must be exercised in the evaporation of solvent following extraction of terpin hydrate with chloroform. Terpin hydrate is fairly volatile and can be vaporized under the influence of excess heat or air. The preferred handling is to concentrate the solution and remove the last portion of chloroform without the application of heat.

Chromatograms produced by injections of sample solutions are basically the same as those obtained from previous gas chromatographic analyses of terpin hydrate. One extraneous peak is noted at a retention time of about 2 min. This peak has been assigned to a component in orange oil by preparation and injection of an alcoholic solution of orange oil U.S.P. and does not interfere with the dodecyl alcohol or terpin hydrate peaks which have a much longer retention time.

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