

## Determination of Terpin Hydrate by Gas Chromatography

By LEON KURLANSIK, CAROLYN DAMON, and EDWARD F. SALIM

Two gas chromatographic procedures have been developed for the determination of terpin hydrate using dodecyl alcohol as an internal standard. Methods involving direct chromatographic comparison of the two materials as well as formation and measurement of the corresponding trimethylsilyl (TMS) ethers have been tested. Agreement between the methods is  $\pm 1$  per cent.

**T**ERPIN HYDRATE, *cis-p*-menthane-1,8-diol hydrate, has been the subject of several analytical investigations including acetylation (1-3) and aquametry (4). The latter procedure is an example of the application of Karl Fischer reagent to quantitative determination of organic functional groups.

Gas-liquid chromatography has been applied to the separation of terpin hydrate from oils and perfumes (5, 6). Volatility of the compound at relatively low temperatures is conducive to gas chromatographic analysis. Two quantitative methods for the determination of terpin hydrate are presented which incorporate the highly desired essentials of simplicity, specificity, and accuracy. Method *A* is a direct chromatographic comparison of terpin hydrate to dodecyl alcohol as an internal standard and method *B* is the formation and chromatographic measurement of terpin hydrate and dodecyl alcohol as their trimethylsilyl ether derivatives.

### EXPERIMENTAL

**Reagents**—Hexamethyldisilazane (HMDS) (Matheson Coleman & Bell), trimethylchlorosilane (TMCS) (Eastman Kodak), and dodecyl alcohol (Analabs, Inc.) were used without purification.

**Terpin Hydrate Reference Standard**—Terpin hydrate N.F. was recrystallized twice from water-alcohol (5:1). The material was analyzed by differential scanning calorimetry (99.86%) and nuclear magnetic resonance (99.92%). The analytical evaluations are calculated on an anhydrous basis using the water content determined by Karl Fischer titrimetry.

**Apparatus**—An F & M model 810 dual column gas chromatograph with a dual flame ionization detector and a Minneapolis Honeywell recorder was used.

Received March 29, 1967, from the Drug Standards Laboratory, AMERICAN PHARMACEUTICAL ASSOCIATION FOUNDATION, Washington, DC 20037.

Accepted for publication June 6, 1967.

The authors thank Candace Plato and Dr. Arthur Jacobson for analytical evaluations of the terpin hydrate reference standard.

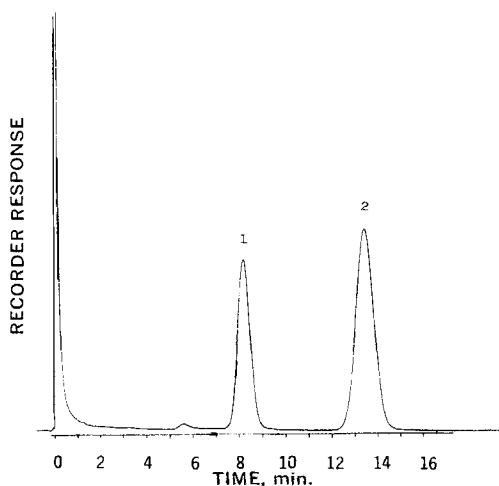


Fig. 1—Chromatogram of an alcohol solution of terpin hydrate and dodecyl alcohol on a Carbowax 20M column. Key: 1, dodecyl alcohol; 2, terpin hydrate.

### Method A

**Columns**—Stainless steel columns (6 ft.  $\times$   $\frac{1}{8}$  in.) packed with 10% Carbowax 20 M on Diatoport S, 60-80 mesh (prepared by F & M Scientific Co.).

**Chromatography Conditions**—Column temperature, 170°; injection port temperature, 170°; detector temperature, 170°; amplifier gain  $5 \times 10^8$  ohms at an attenuation of 2; helium flow, 40 ml./min.; hydrogen 44 ml./min.; air, 360 ml./min.

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

**Procedure**—Transfer about 200  $\mu$ g. of terpin hydrate, accurately weighed, into a 50-ml. volumetric flask and dissolve in about 30 ml. of absolute alcohol. Add 10.0 ml. of internal standard solution, dilute to volume with absolute alcohol, and mix. A 0.5- $\mu$ l. portion of this solution was injected into the chromatograph. A typical chromatogram is shown in Fig. 1.

**Calculation**—The per cent of anhydrous terpin hydrate is calculated by the formula:

$$H \times \frac{C \times V}{W} \times F \times 100$$

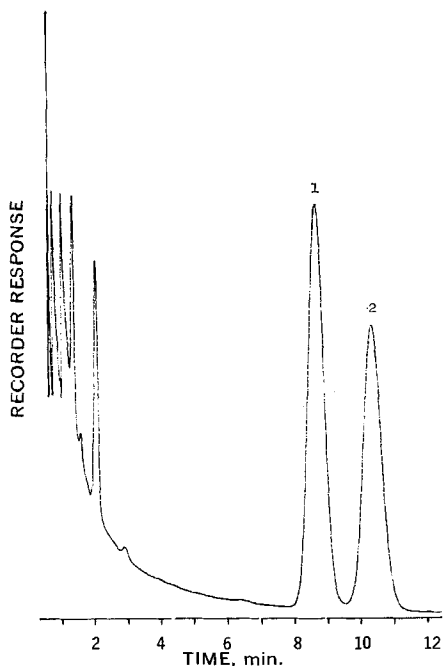


Fig. 2—Chromatogram of ether derivatives of terpin hydrate and dodecyl alcohol on a SE-30 column. Key: 1, terpin hydrate (TMS derivative); 2, dodecyl alcohol (TMS derivative).

in which  $H$  = peak ht. of terpin hydrate  
 peak ht. of internal std.  
 $C$  = concentration, in mg./ml., of internal standard preparation,  
 $V$  = volume, in ml., of internal standard added in procedure,  
 $W$  = weight, in mg., of terpin hydrate taken calculated on an anhydrous basis using a water content determined by Karl Fischer titrimetry,  
 $F$  = relative response of equal weights of dodecyl alcohol (DDA) and terpin hydrate (TH) standard as determined by peak height measurement (DDA/TH).

#### Method B

**Columns**—Stainless steel columns (6 ft.  $\times$   $\frac{1}{8}$  in.) packed with 10% SE-30 on Diatoport S, 60–80 mesh (prepared by F & M Scientific Co.)

**Chromatography Conditions**—Column temperature, 140°; injection port temperature, 145°; detector temperature, 150°; amplifier gain  $5 \times 10^8$  ohms at an attenuation of 1; helium flow, 65 ml./min.; hydrogen, 58 ml./min.; air, 370 ml./min.

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

**Procedure**—Transfer about 200 mg. of terpin hydrate, accurately weighed, into a 50-ml. volumetric flask and dissolve in about 25 ml. of pyridine. Add 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

TABLE I—RECOVERY OF TERPIN HYDRATE FOR COMMERCIAL SAMPLES BY METHOD A

Sample	Terpin Hydrate, mg. Added	Found	Recovery, % <sup>a</sup> (Anhydrous)
A	201.5	180.2	98.8
	203.5	181.9	98.7
B	198.6	177.8	98.9 <sup>b</sup>
	200.4	178.2	98.8
	210.4	187.7	98.2
C	202.4	181.1	98.5
	210.5	187.5	99.3
	207.4	186.2	99.1
D	201.8	182.0	99.6
	204.0	183.8	99.8

<sup>a</sup> Based on water content determined by Karl Fischer titrimetry: sample A, 9.54%; sample B, 9.45%; sample C, 9.87%; sample D, 9.63%. <sup>b</sup> Average of 12 determinations by three analysts with S.D.  $\pm$  0.27.

TABLE II—RECOVERY OF TERPIN HYDRATE FOR COMMERCIAL SAMPLES BY METHOD B

Sample	Terpin Hydrate, mg. Added	Found	Recovery, % <sup>a</sup> (Anhydrous)
A	201.5	181.3	99.5
	213.6	192.6	99.6
B	203.3	181.6	98.8
	206.2	184.2	99.1
	208.3	187.5	99.9
C	206.5	185.2	99.2
	210.6	188.3	99.2
	202.6	182.0	99.2
	202.7	182.5	99.4
	...	...	99.2 <sup>b</sup>

<sup>a</sup> Water contents used for calculation included in Table I. <sup>b</sup> Average of 5 determinations of S.D.  $\pm$  0.20.

to stand for 1 hr. and inject 0.4  $\mu$ l. of supernatant liquid into the chromatograph. A typical chromatogram is shown in Fig. 2.

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

## RESULTS AND DISCUSSION

Series of tests were conducted to determine the stability of terpin hydrate and its TMS derivative at various component temperatures. Results of the investigations showed that terpin hydrate could be chromatographed directly at a temperature not exceeding 175° and terpin hydrate bis-trimethylsilyl ether could be exposed to a temperature of about 300° before thermal decomposition was evidenced. Additional studies produced the instrument parameters established for methods A and B. Results obtained for commercial samples of terpin hydrate by each method are summarized in Tables I and II, respectively.

The method is specific for the determination of terpin hydrate in the presence of its dehydration products. A sample of terpin hydrate was boiled in dilute sulfuric acid, the mixture steam distilled, the water immiscible portion distilled, and an alcoholic solution of the distillate chromatographed

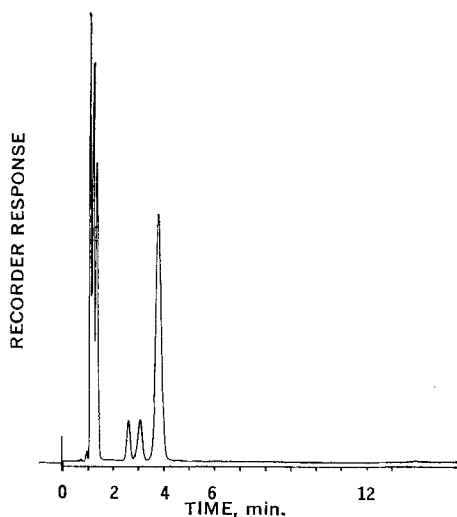


Fig. 3—Chromatogram of dehydroxylated terpin hydrate in alcohol solution on a Carbowax 20M column.

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*.

#### REFERENCES

- (1) Fernandez, O., and Luengo, N., *Anal. fis. quim.*, **18**, 158(1920); through *Chem. Abstr.*, **15**, 1869(1921).
- (2) Mesnard, P., and Bertucat, M., *Bull. Soc. Chim. France*, **1959**, 307; through *Chem. Abstr.*, **53**, 16919(1959).
- (3) Mesnard, P., and Bertucat, M., *Boll. Chim. Farm.*, **101**, 519(1962).
- (4) Mitchell, J., Jr., and Smith, D. M., "Aquametry," Interscience Publishers, Inc., New York, N. Y., 1948, pp. 267-282.
- (5) Domange, L., and Longueval, S., *Compt. Rend.*, **247**, 209(1958).
- (6) Porcaro, P. J., and Johnston, V. D., *Anal. Chem.*, **33**, 361(1961).

## Determination of Terpin Hydrate in Elixirs by Gas Chromatography

By LEON KURLANSIK, CAROLYN DAMON, HANNAH KLEIN, and EDWARD F. SALIM

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.

**T**ERPIN 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.

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.,

Received April 13, 1967, from the Drug Standards Laboratory, AMERICAN PHARMACEUTICAL ASSOCIATION FOUNDATION, Washington, DC 20037.

Accepted for publication May 25, 1967.