Determination of Terpin Hydrate in Elixirs Containing Codeine or Dextromethorphan Hydrobromide by Gas–Liquid Chromatography

By F. P. MAHN, V. VISWANATHAN, and B. Z. SENKOWSKI

A gas–liquid chromatographic procedure for the quantitative analysis of terpin hydrate was investigated. A Pyrex column packed with 10 percent Carbowax 20M terephthalic acid on Chromosorb W, hexamethyldisilane (HMDS) treated, was found to give satisfactory elution characteristics. Pharmaceutical preparations containing terpin hydrate, such as elixirs with dextromethorphan hydrobromide or codeine, were analyzed by the proposed procedure with recoveries of 100 ± 3 percent. The internal standard used was biphenyl. The effect of temperature on retention volume is reported.

THE CHEMICAL STRUCTURE of terpin hydrate indicated that the compound could lend itself to rapid and precise gas chromatography. A survey of the literature failed to disclose any previous studies utilizing this technique.[1] Procedures were reported by Platt and James (1) for the quantitative analysis of terpin hydrate using colorimetry. These authors treated phosphomolybdic acid with terpin hydrate under controlled conditions and measured the resulting color. Milos (2) reported on collaborative results which supported the adoption of the colorimetric determination. Vadodaria, Parikh, and Mukheyi (3) modified the colorimetric method of Platt and James and reported results with a precision of ±3%. These methods are time-consuming and involve the isolation of terpin hydrate in comparatively pure form by solvent extraction prior to actual analysis. Microscopic methods (4, 5) and refractometric methods (6) also were reported for the analysis or identification of terpin hydrate. However, the use of these techniques was limited primarily to qualitative identification.

Vapor-phase chromatography was investigated, and it was found that elution of terpin hydrate under isothermal conditions resulted in a symmetrical peak. The column selected for best results was 10% Carbowax 20M terephthalic acid on Chromosorb W, HMDS treated. Since analysis could be carried out directly on alcoholic dilutions of terpin hydrate preparations, errors due to excessive handling were minimized. The precision of the method therefore is dependent primarily upon the instrumental conditions and response. In order to compensate for column characteristics, instrumental variations, and sample introduction technique, an internal standard, biphenyl, was employed. Biphenyl is well suited for the experimental conditions in terms of detector linearity and retention time. Analyses were carried out on elixir terpin hydrate and elixir terpin hydrate with codeine or dextromethorphan hydrobromide NF.

Linearity of detector response with concentration, detection levels, interferences, and recovery data are presented.

EXPERIMENTAL

Operational Parameters—The instrument used for this work was a Varian Aerograph model 600 gas chromatograph equipped with a hydrogen flame ionization detector. The column used was a Pyrex coil, 5 ft. long and 3 mm. i.d., packed with 10% Carbowax 20M terephthalic acid on Chromosorb W, HMDS treated, 60/80 mesh. The temperatures were: column, 160°; injector port, 250° with a Pyrex insert; and detector, 160°. The flow rates were: carrier gas, nitrogen, 40 ml./min.; detector gas, hydrogen, 20 ml./min.; and air, 300 ml./min. All injections were made using a 10-μl. Hamilton syringe with the injection volume being approximately 2 μl. The instrument was operated at a range of 10 and attenuation of 64×, or equivalent, to result in at least a 50% response of the recorder scale. The recorder used was a 0–1 mv. Texas Instrument with a pen response of 0.4 sec. and a chart speed of 0.75 in./min. All peak areas were measured using a disk integrator. Under the conditions stated the relative retention time of terpin hydrate was 1.38 with respect to the internal standard, biphenyl, which has a specific retention time of approximately 4 min. (Fig. 1).

Standard Preparation—*Internal Standard*—Accurately weigh 50 mg. of biphenyl into a 25-ml. volumetric flask. Dissolve and dilute to volume with absolute ethanol. Mix well.

Reference Standard—Accurately weigh 85 mg. of terpin hydrate into a 25-ml. volumetric flask. Dissolve and dilute to volume with absolute ethanol. Mix well.

Working Standard (Prepare in Duplicate)—Into a small glass-stoppered flask, pipet 1 ml. of the bi-

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[1] Subsequent to the submission of this paper, a similar method was published: Kurlansik, L., Damon, D., Klein, H., and Salim, E. F., *J. Pharm. Sci.*, **56**, 1160(1967).

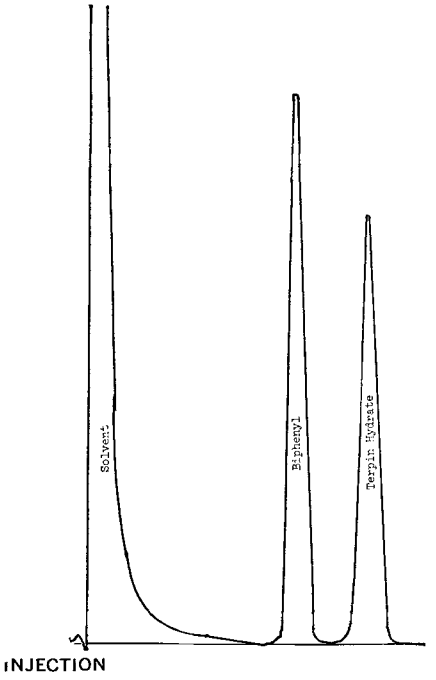

Fig. 1—Standard chromatogram.

phenyl standard solution and 1 ml. of the terpin hydrate standard solution. Stopper and mix thoroughly. Chromatograms were run and all peak areas were measured from the integrator pen trace.

Sample Preparation—Accurately pipet a 5-ml. aliquot of elixir terpin hydrate sample, equivalent to 85 mg. of terpin hydrate, into a 25-ml. volumetric flask. Dilute to volume with absolute ethanol and mix thoroughly.

Into a small glass-stoppered flask, pipet 1 ml. of the above prepared sample solution and 1 ml. of the biphenyl solution (internal standard solution). Stopper and mix the solutions thoroughly (working sample solution).

Chromatograph this mixture in duplicate as described in the above procedure. After the elution of the terpin hydrate, the chromatogram is allowed to run for an additional 10 min. to allow for the elution of glycerol, if present, which has an approximate retention time of 12 min. When the glycerol peak has been completely eluted, the column is ready for another run.

Calculations—Assay for terpin hydrate in the elixir.

mg. of terpin hydrate per 5 ml. of elixir =

$$\frac{Ths}{Bps} \times \frac{Bp}{Th} \times 85 \text{ mg.}$$

Ths = peak area of terpin hydrate in sample,
Bps = peak area of biphenyl in sample,
Bp = peak area of biphenyl in standard,
Th = peak area of terpin hydrate in standard,
85 = mg. of terpin hydrate.

RESULTS AND DISCUSSION

Although daily conditioning of the column was found to be unnecessary, it is not advisable to use the first run for quantitative information. This initial run should be used to determine instrument sensitivity and retention data.

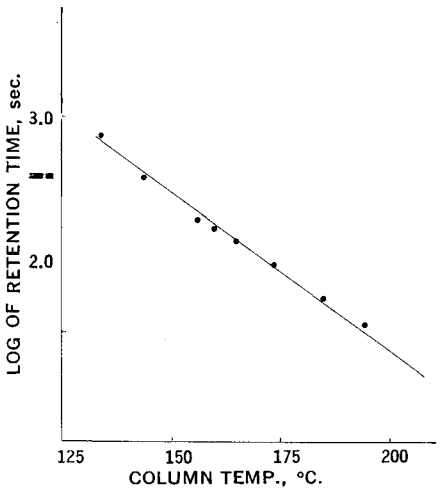

Fig. 2—Column temperature versus *retention time for terpin hydrate.*

As is well known, insufficiently loaded columns may cause tailing due to adsorptive effects which result from incomplete coverage of the support by the liquid phase. If adsorption occurs, quantitative work is considerably hampered. The column used in this study contained a high ratio of liquid phase to inert support in order to minimize any adsorptive effects.

An attempt was made to utilize standard copper tubing for the column, however, the erratic results obtained were indicative of possible decomposition due to catalytic effects. A Pyrex column therefore was used in all experiments.

The detector response for approximately 3.4 mcg. of terpin hydrate and 2.2 mcg. of biphenyl with the change in hydrogen flow rate, at constant air and carrier gas was obtained. The optimum flow ratio of hydrogen to carrier gas was 1:2.

TABLE I—ANALYSIS OF PRODUCTS CONTAINING TERPIN HYDRATE AND RECOVERY DATA

	Terpin Hydrate, mg./5 ml.			
	Theoret.	Added	Found	
Sample 1				
Elixir terpin hydrate placebo	...	...	Av. of 2 detn.	91.2
	...	90.7		101%
Sample 2				
Elixir terpin hydrate	85.0	...	Av. of 2 detn.	82.4
				97%
	85.0	18.8	Av. of 2 detn.	103.3
				100%
Sample 3				
Elixir terpin hydrate with dextromethorphan hydrobromide	85.0	...	Av. of 2 detn.	85.3
				100%
	85.0	15.8	Av. of 2 detn.	98.2
				97%
Sample 4				
Elixir terpin hydrate with codeine	85.0	...	Av. of 4 detn.	82.0
				97%
	85.0	13.5	Av. of 10 detn.	97.7
				99%
Sample 5				
Elixir terpin hydrate with dextromethorphan hydrobromide	50.0	...	Av. of 6 detn.	50.0
				100%
	50.0	11.2	Av. of 4 detn.	59.6
				97%

The detector response with varying concentrations of terpin hydrate and biphenyl followed a linear relationship and the minimum measurable quantity was 5 nanograms.

The effect of column temperature on retention time was investigated and a linear relationship obtained (Fig. 2). The equation calculated by the method of least squares is $\log t_r = -0.014\ T_c + 4.7$, where t_r = retention time in seconds and T_c is the column temperature in °C. A plot of the log of the retention volume *versus* $1/T$, where T = absolute temperature, also yielded a linear relationship. The apparent ΔH_s was calculated from the equation $\log V_R = -\Delta H_s/2.3RT + C$ and found to be −12.4 Kcal./mole, where the slope of the resulting straight line was determined by the method of least squares.

A number of products containing terpin hydrate was assayed. The results and recovery data are presented in Table I. The precision is ±3%.

Under the conditions described, no interference in the analysis of the samples was encountered. Apparently dextromethorphan hydrobromide and codeine were not eluted, or were in too low a concentration to result in a measurable detector response. However, it should be pointed out that measurable interference due to glycerol will occur unless complete elution of this compound from the column is carried out prior to subsequent injections. The retention time of glycerol is approximately 12 min. Continued use of the column will result in the build-up of sugar which tends to skew the elution peaks and decrease the precision of the method. When this occurs, the column should be replaced.

SUMMARY

A gas–liquid chromatographic method for the determination of terpin hydrate in elixirs has been developed. The procedure involves diluting the sample with ethanol, adding an internal standard, biphenyl, and chromatography.

The precision of the developed method was ±3% and duplicate analyses can be completed within 1 hr.

Detection levels, interferences, and recovery data are presented.

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Keyphrases

Terpin hydrate elixirs
Codeine terpin hydrate elixir—analysis
Dextromethorphan terpin hydrate hydrobromide elixir—analysis
GLC analysis

Technical Articles

Unit Tablet Assay of 17α-Ethynylestradiol-3-methyl Ether (Mestranol) by a Direct Colorimetric and an Automated Fluorometric Method

By J. P. COMER, P. HARTSAW, and C. E. STEVENSON

Unit tablet assays of 17α-ethynylestradiol-3-methyl ether (mestranol) were obtained with optimum precision and accuracy by direct dissolution of sample tablets and standardized reference tablets in sulfuric acid–methanol (70:30) reagent. An automated method which utilized basic automatic analyzer (AutoAnalyzer) components and a Turner fluorometer was more rapid but less precise than the direct method.

THE ASSAY of low dosage combination steroid tablets has been a challenge to several analysts. Gänshirt and Polderman (1) separated ethinyl estradiol from tablet excipients and decomposition products by thin-layer chromatography (TLC), and measured the color formed with sulfuric acid–water (80:20) with a relative standard deviation (RSD) of ±3.2%. Comer (2) mentioned the utility of the sulfuric acid–methanol (70:30) reagent for the determination

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