SUMMARY

The silicone fluids appear to have certain physical, chemical, and biological properties which would make them useful as nonaqueous solvents for the administration of medicinal agents.

Based upon data from accelerated thermal studies, it was found that the dimethyl silicone fluid was superior to corn oil in preventing the thermal degradation of menadione.

The photodegradation studies indicated that, although corn oil was somewhat superior to the dimethyl silicone fluid in preventing the degradation of menadione, photodegradation was rapid in both vehicles. Since photodegradation can be controlled during normal storage by protective packaging, its practical significance is less than the relatively uncontrollable thermal storage conditions.

In consideration of these findings, it may be concluded that the dimethyl silicone fluid is superior to corn oil as a vehicle for menadione.

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Keyphrases

Menadione stability
Dimethylpolysiloxane fluid, corn oil—vehicles
Stability, menadione—nonaqueous vehicles
Photodegradation, menadione—vehicle effect
Thermal stability, menadione—vehicle effect
Colorimetric analysis—spectrophotometer

Drug Standards

Assay of Terpin Hydrate and Codeine Elixir by Gas Chromatography

By HAROLD J. WESSELMAN

Terpin hydrate and codeine after extraction from the elixir are separated and determined with the aid of two internal standards and the use of temperature-programmed gas chromatography. Using the same method, elixir of terpin hydrate can also be determined. A precision and accuracy study for both elixirs is included.

THE QUANTITATIVE determination of terpin hydrate, *cis-p*-menthane-1,8-diol hydrate, has always presented problems. Indeed the National Formulary XII (1) does not include an assay for terpin hydrate or codeine in either the elixir of terpin hydrate or the elixir of terpin hydrate and codeine. In 1921 Murray (2) used a gravimetric method for terpin hydrate. Harrison (3), Carol (4, 5), and Lund and Ameiss (6) improved this method. Later, in 1932, Perelmann (7) proposed a colorimetric method which was modified by Platt and James (8) and Vadodaria, Parikh, and Mukherji (9).

Dembeck (10) described a titrimetric assay for codeine while Stoicheva (11) determined terpin hydrate and codeine by subjecting them to microreactions and examining the results microscopically. Milos (12, 13) developed a spectrophotometric determination for these compounds. Using ion-exchange chromatography and nonaqueous titrimetry, Blake and Carlstedt (14) determined codeine in the elixir, while Mont-

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gomery, Jennings, and Weinswig (15) applied ion-exchange chromatography followed by ultraviolet absorption analysis.

Domange and Longuevalle (16) suggested that terpin hydrate could be determined by gas chromatography but provided no tangible evidence. Recently Kurlansik, Damon, and Salim (17, 18) and Mahn, Viswanathan, and Senkowski (19) devised gas chromatographic methods for terpin hydrate in elixir of terpin hydrate and elixir of terpin hydrate and codeine.

The gas chromatography of codeine was investigated qualitatively by Parker, Fontan, and Kirk (20) and Massingill and Hodgkins (21) and made quantitative by Mule (22) and Schmerzler *et al.* (23).

The literature does not record the use of gas chromatography for the simultaneous determination of terpin hydrate and codeine. These compounds are difficult to separate by isothermal gas chromatography because of their widely spaced boiling points. Another difficulty in the case of the elixir of terpin hydrate and codeine is that terpin hydrate being the more volatile of the two is present in the greatest amount, 17.0 mg./ml., while the higher boiling codeine has only a concentration of 2.0 mg./ml.

The present work overcomes these problems by using a combination of isothermal and temperature-programmed gas chromatography when both compounds are present. By using isothermal gas chromatography, terpin hydrate in elixir of terpin hydrate can be determined easily.

EXPERIMENTAL

Equipment—A linear programmed-temperature gas chromatograph, F & M Scientific Corp., model 402, equipped with a flame-ionization detector, was used for the experimental work. The detector signal was fed to a Honeywell Electronic 16 1-mv. recorder with a chart speed of 15 in./hr. and a 1-sec. full-scale response. Samples were injected with a 10-μl. Hamilton, No. 701, syringe.

Materials—Helium was used as a carrier gas, while electrolytic hydrogen and oxygen were used in the detector. The stationary phase was 3.8% Linde W-98 silicone gum applied by the solution technique of Horning *et al.* (24) to Diatoport S (80–100 mesh) and packed in dual borosilicate glass columns 0.91 m. × 0.64 cm. (3 ft. × 1/4 in. o.d.). A mixture of chloroform and ethanol (1:1) was used to dissolve the analytical reagent grade benzoic acid, terpin hydrate, codeine, and cholestane.

Operating Conditions—For the elixir of terpin hydrate and codeine, the columns were operated isothermally at 140° for 4 min. and then programmed to 275° at a heating rate of 10° per min. At the end of each run, the oven was cooled for exactly 10 min. and then equilibrated at 140° for exactly 10 min. before injecting the next sample. The electrometer range was 1,000 with an attenuation of 64 for terpin hydrate and benzoic acid and 4 for codeine and cholestane. In the case of the elixir of terpin hydrate, the columns were operated isothermally at 135° with an attenuation of 32 on range 1,000.

The helium flow rate was 55 ml./min. with an inlet pressure of 40 psig. Oxygen and hydrogen flow rates were 250 and 35 ml./min., respectively. The sample injection port was maintained at 240° and the detector block at 215°. One-microliter injections of all samples were used throughout.

Quantitative Analysis—This method uses two internal standards—benzoic acid for terpin hydrate and cholestane for codeine.

Elixir of Terpin Hydrate and Codeine—Prepare a standard solution by adding exactly 85.0 mg. benzoic acid, 85.0 mg. terpin hydrate, 10.0 mg. codeine alkaloid, and 10.0 mg. cholestane to a 5-ml. volumetric flask and diluting to volume with a 1:1 mixture of chloroform and ethanol.

Prepare a sample solution by transferring exactly 10.0 ml. of the elixir to a 125-ml. separator and adding 3 ml. sodium hydroxide solution (1:10 distilled water). Extract with four 20-ml. portions of chloroform, collecting the chloroform extracts in a 150-ml. beaker. Carefully evaporate the chloroform, using a stream of dry air and a water bath, to a volume of about 2 ml. Transfer the concentrated extract to a 10-ml. volumetric flask with the aid of chloroform. Carefully evaporate the chloroform to dryness. Dilute to volume with the internal standard solution which is prepared by diluting 850 mg. benzoic acid and 100 mg. cholestane to 50 ml. with a 1:1 mixture of chloroform and ethanol. Chromatograph the standard and the extracted sample solutions and measure the peak height of each component.

Elixir of Terpin Hydrate—Prepare a standard solution by adding exactly 85.0 mg. benzoic acid and 85.0 mg. terpin hydrate to a 5-ml. volumetric flask and diluting to volume with a 1:1 mixture of chloroform and ethanol.

A 10-ml. sample of the elixir is extracted as discussed above. The final dilution is made with an internal standard solution prepared by adding exactly 850.0 mg. benzoic acid to a 50-ml. volumetric flask and diluting to volume with a 1:1 mixture of chloroform and alcohol. Chromatograph the standard and the extracted sample solutions and measure the peak height of each component.

Calculations.

$$\frac{\text{peak height standard benzoic acid}}{\text{peak height standard terpin hydrate}} = A$$

$$\frac{\text{peak height sample benzoic acid}}{\text{peak height sample terpin hydrate}} = B$$

$$A/B \times 17 = \text{mg. terpin hydrate/ml. elixir.}$$

$$\frac{\text{peak height standard cholestane}}{\text{peak height standard codeine}} = C$$

$$\frac{\text{peak height sample cholestane}}{\text{peak height sample codeine}} = D$$

$$C/D \times 2 = \text{mg. codeine/ml. elixir.}$$

Precision and Accuracy—To determine the precision and accuracy of the methods, freshly prepared solutions of terpin hydrate and codeine and terpin hydrate in distilled water were used as standard solutions. The sample solutions were prepared

TABLE I—PRECISION AND ACCURACY STUDY FOR ELIXIR TERPIN HYDRATE AND CODEINE

	Terpin Hydrate	Codeine
Standard solution, mg./ml.	17.00	2.00
Sample solution, mg./ml.	16.47	2.00
	16.53	2.03
	16.88	2.01
	17.29	2.01
	17.32	1.99
$\sum x$	84.49	10.04
$\bar{x}$	16.90	2.01
$\sum x^2$	1428.3627	20.1612
s	0.2500	0.0132
RSD	±1.48%	±0.66%
Mean error	−0.10	+0.01
Relative error	−0.59%	+0.50%

TABLE II—PRECISION AND ACCURACY STUDY FOR ELIXIR TERPIN HYDRATE

	Terpin Hydrate
Standard solution, mg./ml.	17.00
Sample solution, mg./ml.	16.83
	16.81
	16.68
	16.66
	16.81
$\sum x$	83.79
$\bar{x}$	16.76
$\sum x^2$	1404.18
s	0.24855
RSD	±1.48%
Mean error	−0.24
Relative error	−1.41%

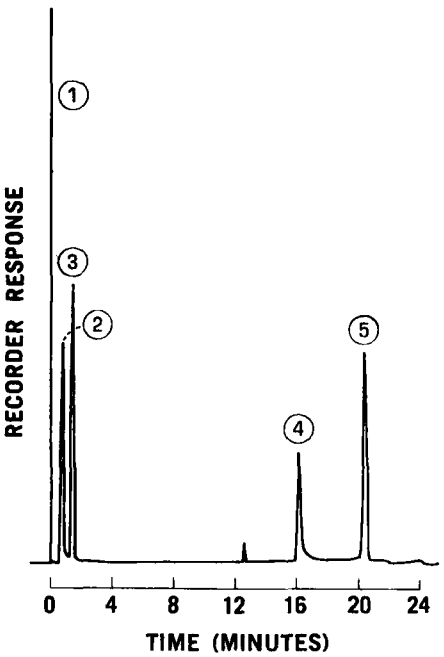

Fig. 1—Typical chromatogram of chloroform-ethanol (1), benzoic acid (2), terpin hydrate (3), codeine (4), and cholestane (5).

by dissolving the same components in freshly made elixir blanks. These studies were carried out in accordance with the suggestions of the Advisory Board of Analytical Chemistry (25) and the recommended nomenclature is used. Tables I and II show the results of these studies.

RESULTS AND DISCUSSION

A typical chromatogram of a mixture of terpin hydrate, benzoic acid, codeine, and cholestane is shown in Fig. 1. Table III gives the results of the assays of three production lots of elixir of terpin hydrate and codeine. Table IV shows the results obtained from three production lots of elixir of terpin hydrate. Triplicate assays in each case show good agreement.

TABLE III—RESULTS OF ASSAY OF ELIXIR TERPIN HYDRATE AND CODEINE

	Terpin Hydrate	Codeine
Theory, mg./ml.	17.00	2.00
Lot A	16.34	2.00
	16.04	2.00
	16.19	1.99
Lot B	17.32	2.00
	17.13	2.02
	17.29	2.01
Lot C	16.83	2.03
	16.84	2.03
	16.88	2.01

TABLE IV—RESULTS OF ASSAY OF ELIXIR TERPIN HYDRATE

	Terpin Hydrate
Theory, mg./ml.	17.00
Lot D	16.39
	16.30
	16.41
Lot E	17.62
	17.50
	17.45
Lot F	17.36
	17.12
	17.45

CONCLUSION

A new method for determining terpin hydrate and codeine simultaneously using a combination of isothermal and programmed-temperature gas chromatography has been developed. The use of two internal standards permits a high degree of precision and accuracy. The relative standard deviations for terpin hydrate and codeine are ±1.48% and ±0.66%, respectively. The procedure is fast and accurate and easily carried out. The overall time for determining the standard and the sample is approximately 1 hr.

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Keyphrases

Terpin hydrate-codeine elixir—analysis
GLC—analysis
Benzoic acid—internal standard, terpin hydrate
Cholestane—internal standard, codeine
Isothermal, programmed temperature—GLC

Technical Articles

Automated Colorimetric Analysis of Ethinyl Estradiol and Mestranol in Pharmaceutical Tablets

By WILLIAM F. BEYER

An automated colorimetric procedure is described for the determination of ethinyl estradiol and ethinyl estradiol-3-methyl ether in pharmaceutical tablets. Chloroform solutions of the estrogen are automatically extracted with alcohol–sulfuric acid and analyzed at a rate of 20 samples/hr. The relative standard deviation of repetitive sampling of tablet extracts was less than 1 percent, and Beer's law was obeyed over the range of 0.40–8.00 mcg./ml. of sample solution. The procedure is applicable to unit dosage assays and to assays requiring composite samples of pulverized tablets or numerous whole tablets.

NUMEROUS METHODS have been reported for the determination of ethinyl estradiol (EE) or its 3-methyl ether derivative (EE-3ME) from tablets (1–5). The majority of these procedures depends upon the spectrophotometric analysis of sulfuric acid-induced color, and involve somewhat lengthy separative techniques.

A recent publication of Khoury and Cali (6) describes an automated assay for EE and EE-3ME based on the fluorescence exhibited by the estrogens in 90% sulfuric acid. The necessity of assaying large numbers of tablets for EE in combination with nonestrogenic steroids also prompted the investigation of automation in these laboratories. A relatively simple, automated method has been developed with instruments normally found in laboratories employing automatic analysis—sampler, proportioning pump, heating bath, spectrophotometer, and recorder. The procedure is based on a manual method developed in these laboratories (7), a modification and quantitation of the USP XVII sulfuric acid identification test for EE (1). The red color formed when sulfuric acid is added to a chloroform solution of EE is extractable with sulfuric acid and exhibits a maximum absorption at 518 mμ. The color develops rapidly and is

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