rin, a more polar molecule, showed no residue on autopsy, indicating complete absorption from the olive oil suspension.

It would be expected that compounds containing more polar groups, such as the dicinnamoyl lysines, would more clearly reveal any relationship between the alkylene and cycloalkylene diamides and the natural product, cynarin. The work is currently under study.

The lack of any cholesterol-lowering effect of cynarin in the present test system suggests that this group of compounds may only show their effectiveness in previously hypercholesterolized animals, as originally reported by Preziosi and Loscalzo (1).

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DRUG STANDARDS

Determination of Chloral Hydrate in Soft Gelatin Capsules by NMR

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Abstract □ An accurate and specific procedure is described by which chloral hydrate is determined. Analysis of six synthetic mixtures showed that this method is accurate to ±1% with a standard deviation of 0.6. The analysis of commercial chloral hydrate capsules provides results that are within 1–2% by the NMR method, whereas the USP XVIII assay yields values that are, on the average, 6% higher than the labeled amount. It is felt that the high results obtained with the official procedure may be ascribed to the nonspecificity of the acid–base titration.

Keyphrases □ Chloral hydrate soft gelatin capsules—NMR analysis, compared to compendial method □ Capsules, chloral hydrate—NMR analysis, compared to compendial method □ NMR spectroscopy—analysis, chloral hydrate soft gelatin capsules

Published procedures for the analysis of chloral hydrate (2,2,2-trichloro-1,1-ethanediol) abound. Some are based on the Fujiwara color-producing reaction with alkaline pyridine (1–6). This reaction is nonspecific, since the red color may be produced by many polyhalogenated aliphatic compounds. Other procedures, such as the one by Stehwien and Kuhmstedt (7) which reacts chloral hydrate with 2,6-diaminopyridine, seem specific but critical conditions are required. The one developed by Archer and Haugas (8), based upon the reaction of chloral hydrate with quinaldine ethyl iodide to produce a stable blue cyanine dye, shows no interference from polyhalogenated compounds.

In addition to the colorimetric methods, other approaches have included GC, polarography, and titra-

Table I—Determination of Chloral Hydrate in Standard Mixtures by NMR[a]

Standard Mixture	Maleic Acid Internal Standard Added, mg.	Chloral Hydrate Added, mg.	Chloral Hydrate Found, mg.	Chloral Hydrate Recovery, %
1	126.0	505.1	508.6	100.7
2	176.9	507.9	510.0	100.4
3	228.1	505.9	505.9	100.0
4	182.6	524.4	524.9	100.1
5	180.5	519.8	514.6	99.0
6	177.6	504.1	501.1	99.4

[a] $SD = 0.6$.

Table II—Determination of Chloral Hydrate in Commercial Capsules by NMR

Commercial Capsules	Maleic Acid Internal Standard Added, mg.	Chloral Hydrate					
		Declared, mg./Capsule	Found (NMR) mg./Capsule	Found (NMR) %	Found (USP XVIII) mg./Capsule	Found (USP XVIII) %	Difference[a], %
1	180.3	500	500.5	100.1	536.0	107.2	7.1
2	182.1	250	251.8	100.7	260.3	104.1	3.4
3	179.4	500	491.5	98.3	528.5	105.7	7.4
4	175.2	500	495.0	99.0	524.0	104.8	5.8
5	174.9	500	498.5	99.7	535.0	107.0	7.3
6	174.8	250	245.8	98.3	265.0	106.0	7.7
7	171.4	500	496.5	99.3	525.0	105.0	5.7
8	174.1	250	252.3	100.9	261.3	104.5	3.6
9	177.5	500	498.5	99.7	530.0	106.0	6.3
			Average	99.6		105.6	6.0

[a] Difference between NMR and USP XVIII procedures.

tion techniques. GLC methods for chloral hydrate were developed by Anderson *et al.* (9) and Garrett and Lambert (10), using an electron-capture detector. Elving and Bennett (11), in a review of methods for chloral hydrate published prior to 1954, described a polarographic method usable in the presence of dichloro- and chloroacetaldehyde. Other published procedures for chloral hydrate are potentiometric titration (12), iodometric titration (13, 14), and polarography after reaction with hydroxylamine sulfate (15).

The official USP procedure (16) for the determination of chloral hydrate in capsules involves its alkaline decomposition into chloroform and sodium formate, followed by a titration of the residual alkali with sulfuric acid. The procedure, while probably adequate for the assay of chloral hydrate crystals, is subject to many interferences and is unsuitable for many chloral hydrate formulations.

The alternative method proposed here uses NMR spectroscopy. In this laboratory, many high dosage pharmaceuticals have been successfully analyzed by adding an internal standard and then extracting with a suitable solvent. In most cases the recorded NMR spectrum provides an identification of the active ingredient, which contributes to the specificity of the procedure. Chloral hydrate was determined by this technique using deuterated water as the solvent and maleic acid as the internal standard. The procedure was used to analyze known mixtures and commercial capsules, and it is rapid and specific.

EXPERIMENTAL

Materials—An NMR spectrometer[1] equipped with a variable temperature probe (V-6031) having a six-turn insert was used. All spectra were scanned at a probe temperature of 42°.

The standard used was chloral hydrate[2], and the internal standard was maleic acid[3]. The samples, chloral hydrate capsules, 0.25 and 0.50 g./capsule, were obtained from various commercial sources. The solvent used was deuterium oxide, 99.75 atom %[4].

Procedure—Place capsules, equivalent to 500 mg. of chloral hydrate, into a glass-stoppered test tube and add about 175 mg. of maleic acid followed by 2 ml. of deuterated water (D_2O). Stopper the test tube and heat on the steam bath with frequent shaking until the capsules are dissolved. After the mixture has cooled, shake and transfer approximately 0.4 ml. of the solution to an analytical NMR tube. Place in an NMR spectrometer and obtain the spectrum, adjusting the spin rate so that no spinning side bands occur between 5.16–5.50 and 6.27–6.60 p.p.m., using the δ scale. The peaks of interest should be integrated at least five times. The amount of chloral hydrate per capsule may then be calculated as follows:

$$\frac{\text{mg. of chloral hydrate}}{\text{capsule}} = \frac{A_c}{A_m} \times \frac{\text{E.W.}_c}{\text{E.W.}_m} \times \frac{\text{mg. maleic acid}}{\text{number of capsules}} \qquad \text{(Eq. 1)}$$

where:

A_c = integral value of the signal representing chloral hydrate
A_m = integral value of the signal representing maleic acid
E.W._c = formula weight of chloral hydrate/1 = 165.42
E.W._m = formula weight of maleic acid/2 = 58.04

RESULTS AND DISCUSSION

Water was a logical choice as a solvent for chloral hydrate in soft gelatin capsules, since with it the method is rapid and simple and requires no further manipulations. Deuterated water (D_2O) was chosen to eliminate the integration interferences due to strong water resonance that otherwise overwhelms the near region where the methine proton of chloral hydrate resonates. Although the slight amount of residual HDO present in deuterated water is further increased by the two exchangeable OH protons of chloral hydrate, the increase in intensity of the resonance and its side bands do not interfere at any time with the integration and subsequent quantitation.

The amount of chloral hydrate is determined from the integration of the singlet due to the methine proton of chloral hydrate at approximately 5.3 p.p.m. and of the singlet due to two ethylene protons of maleic acid at approximately 6.6 p.p.m. with respect to tetramethylsilane.

Table I is a summary of the analysis of a group of standard chloral hydrate mixtures by NMR. As noted, the method is accurate and precise, with a standard deviation of 0.6. The proportions of maleic acid to chloral hydrate show no significant bearing on the accuracy of the determination for the range of proportions shown in Table I.

With this method, approximately 25 commercial preparations of soft gelatin capsules were analyzed by NMR. Nine of these samples were also analyzed by the official USP procedure (16) (Table II). The data in Table II indicate that the results by the official procedure ranged from 3.4 to 7.7% higher than those obtained by the described procedure. The differences between the methods are ascribable to a number of reasons, one of which is the nonspecificity of the official USP method. Harrington *et al.* (17) showed that some hydrolysis of the chloroform occurs at room temperature in the excess alkali present, contributing to high results. Other reasons are the residual acidity found in soft gelatin capsules and the coloring present in the capsules which can interfere with the visual endpoint.

Such compounds as trichloroethanol, trichloroacetic acid, trichloroacetaldehyde, chloroform, or formate, which are sometimes

[1] Varian A-60.
[2] Fisher Chemical Co.
[3] Eastman White Label.
[4] Prochem Ltd., Carolyn House, Croydon, England.

found as impurities with or decomposition products of chloral hydrate, will not interfere with its analysis.

The speed, accuracy, and specificity of the NMR method make it a useful procedure which can provide a rapid assay with an accuracy of about 1–2%. This method can be very useful for stability studies of chloral hydrate alone or in the capsule dosage form. It could also be utilized for individual capsule analysis.

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Modified Method for Determining Tetracycline, 4-Epitetracycline, and Anhydrotetracyclines in Tetracycline Base or Hydrochloride

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Abstract □ A partition column chromatographic procedure was developed for the assay of tetracycline samples for tetracycline (I), 4-epitetracycline (II), anhydrotetracycline (III), and 4-epianhydrotetracycline (IV). Two separate portions of a sample are used, one for the assay of I and II and the other, after a concentration step, for the assay of III and IV. Both portions are assayed with the same chromatographic system. The compounds are eluted from a column of diatomaceous earth coated with a pH 7.0 buffer containing disodium ethylenediaminetetraacetate, glycerin, and polyethylene glycol 400. The individual eluates are assayed by absorption spectroscopy. The precision of the assay for I, $SD \pm 0.5\%$, suggests that a chemical method such as this should be considered as the official assay method for tetracycline samples.

Keyphrases □ Tetracycline base or hydrochloride—column chromatographic analysis for tetracycline and impurities, evaluated as potential compendial method □ 4-Epitetracycline—column chromatographic analysis, tetracycline base or hydrochloride □ Anhydrotetracyclines—column chromatographic analysis, tetracycline base or hydrochloride □ 4-Epianhydrotetracycline—column chromatographic analysis, tetracycline base or hydrochloride □ Column chromatography, partition—analysis, tetracycline base or hydrochloride, evaluated as potential compendial method

The analysis of tetracycline samples for tetracycline (I) and three associated impurities, 4-epitetracycline (II), anhydrotetracycline (III), and 4-epianhydrotetracycline (IV), has been carried out by various chromatographic procedures (1–8). Only two of these procedures (7, 8) include methods for the assay of all four compounds. A TLC procedure (7) gave recoveries of I lower than those desirable for accurate quantitation. The second method (8) extended earlier work (6) to include the determination of III and IV.

The chromatographic system of Ascione *et al.* (6) has been used in this laboratory for the analysis of tetracycline raw materials and for stability assays of syrups and tablets. The method, as extended (8), does not give accurate assay values for the amounts of impurities in actual samples. The synthetic mixtures analyzed (8) contained about 10% each of III and IV. Work in this laboratory and in that of others (9) has shown that samples of tetracycline hydrochloride or base usually contain a few tenths of a percent or less of each impurity. These low amounts require a concentration step prior to the assay of III and IV; this, in turn, requires two samples for a complete analysis. The method given here is applicable to actual samples of tetracycline base and hydrochloride and has been so used (Table I). The method should be equally applicable to tetracycline phosphate.

EXPERIMENTAL

Materials and Reagents—Benzene, chloroform, *n*-butanol, methanol, acetic acid, ammonium hydroxide, and disodium ethylenediaminetetraacetate (V) were reagent grade.

For the 20% polyethylene glycol 400 in glycerin solution, add polyethylene glycol 400 to 80 ml. of glycerin USP to make 100 ml.

For the glycol buffer solution, adjust a 0.1 *M* solution of V to pH 7.0 with concentrated ammonium hydroxide. To 95 ml. of this solu-