NMR Analysis of Pharmaceuticals VIII: Determination of Trimethadione in Various Dosage Forms

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Abstract □ An NMR procedure is described for the determination of trimethadione in its various pharmaceutical dosage forms. The method is both accurate and precise, with a standard deviation of ±0.3%. The NMR results of synthetic mixtures and commercial preparations were compared to those obtained by USP XVIII and NF XIII procedures. The NMR technique offers the advantages of speed, simplicity, and specificity.

Keyphrases □ Trimethadione dosage forms—NMR analysis, compared to compendial methods □ NMR spectroscopy—analysis, trimethadione dosage forms

Trimethadione (3,5,5-trimethyl-2,4-oxazolidinedione) (I) is an anticonvulsant drug which is used primarily in the treatment of petit mal epilepsies.

Approaches to the quantitative determination of I vary. Gallelli and Kostenbauder (*1*) reported an IR assay method in which I is determined in the presence of its hydrolysis products. A manometric procedure based upon the decomposition of I by alkali and the subsequent measurement of the carbon dioxide formed was described by Taylor and Bertcher (2). Booker and Darcey (3) developed a GC method for the quantitation of I and its metabolite dimethadione in blood serum. Other techniques used for the assay of I in the pure form and in combination with other drugs include spectrophotometry (4), volumetric analysis (5), and the measurement of alkali consumed during the hydrolysis of I in strong base (6).

The method adopted by USP XVIII (7) for the analysis of I in the capsule and solution dosage forms, and by NF XIII (8) for the analysis of I in tablets, is based upon the hydrolysis of I with sodium hydroxide. The reaction products formed in the hydrolysis are *N*-methylcarbamylo-α-hydroxyisobutyric acid and *N*-methyl-α-hydroxyisobutyramide. The volatile amines produced during the total degradation of the amide (9) are refluxed and distilled into a boric acid solution and are subsequently measured by direct titration with standard sulfuric acid.

By virtue of its relatively high unit dosage (150 and 300 mg.) and characteristic resonance pattern, a quantitative NMR procedure was investigated for the determination of I. With the addition of an internal standard followed by extraction with a suitable solvent, a rapid and accurate quantitation can be accomplished.

An NMR procedure is described here for the analysis of I in its official pharmaceutical dosage forms. *tert*-Butyl alcohol was employed as the internal standard and water as the solvent. Besides providing quantitative results, the recorded NMR spectrum furnishes an identification of the active ingredient, thereby contributing to the specificity of the technique. The method was used to analyze both known mixtures and commercial preparations.

EXPERIMENTAL

Apparatus—An NMR spectrometer[1] equipped with a variable temperature probe[2], having a six-turn insert, was used.

Standard—Trimethadione reference standard was obtained from a commercial source[3].

Internal Standard—*tert*-Butyl alcohol[4], 99+ mol. %, was used.

Samples—Trimethadione capsules, solutions, and tablets were used.

Procedure—*Tablet Preparation*—Weigh and finely powder not less than 20 tablets.

Capsule Preparation—Weigh and mix thoroughly the contents of not less than 20 capsules.

Weigh accurately a portion of the powder equivalent to about 150 mg. of I into a glass-stoppered centrifuge tube. In the case of I solution, pipet an equivalent portion into the tube. Add about 50 mg., accurately weighed, of the *tert*-butyl alcohol internal standard and fill the tube to approximately the 4-ml. mark with water. Stopper the tube and shake thoroughly for about 2 min. The tablet and capsule sample should be centrifuged to separate the solution from the insoluble excipients.

Transfer about 0.4 ml. of the sample solution to an analytical NMR tube. Place in an NMR spectrometer and obtain the spectrum. Care should be taken to adjust the spin rate so that no spinning side band occurs in the region of interest between 1.0 and 1.8 p.p.m., using the delta scale to express magnetic field strength. All peak field positions are referenced to sodium 2,2-dimethyl-2-silapentanesulfonate (III) at 0 p.p.m. Integrate the peaks of interest at least five times.

The amount of I may be calculated as follows:

$$\frac{\text{mg. I}}{\text{tablet}} = \frac{A_{spl}}{A_{std}} \times \frac{\text{E.W.}_{spl}}{\text{E.W.}_{std}} \times \frac{\text{mg. } tert\text{-butyl alcohol}}{\text{mg. sample}} \times \text{average tablet weight} \quad \text{(Eq. 1}a\text{)}$$

$$\frac{\text{mg. I}}{\text{capsule}} = \frac{A_{spl}}{A_{std}} \times \frac{\text{E.W.}_{spl}}{\text{E.W.}_{std}} \times \frac{\text{mg. } tert\text{-butyl alcohol}}{\text{mg. sample}} \times \text{average capsule contents} \quad \text{(Eq. 1}b\text{)}$$

$$\frac{\text{mg. I}}{\text{ml. solution}} = \frac{A_{spl}}{A_{std}} \times \frac{\text{E.W.}_{spl}}{\text{E.W.}_{std}} \times \frac{\text{mg. } tert\text{-butyl alcohol}}{\text{ml. solution used}} \quad \text{(Eq. 1}c\text{)}$$

where:

A_{spl} = integral value of the signal representing I
A_{std} = integral value of the signal representing *tert*-butyl alcohol
E.W._{spl} = formula weight of I/6 = 23.86
E.W._{std} = formula weight of *tert*-butyl alcohol/9 = 8.236

Although no evidence of the instability of I was encountered in the dosage forms studied, the use of the described NMR procedure in those cases where decomposition products are present is discussed later.

[1] Varian A-60.
[2] V-6031.
[3] Abbott Laboratories, North Chicago, IL 60064
[4] Matheson, Coleman and Bell, East Rutherford, N. J.

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

Standard Mixture	*tert*-Butyl Alcohol Internal Standard Added, mg.	Trimethadione Added, mg.	Trimethadione Found, mg.	Recovery, %
1	54.2	158.9	159.7	100.5
2	58.1	154.0	155.2	100.8
3	51.2	150.7	151.6	100.6
4	52.4	157.2	157.2	100.0
5	78.4	155.5	156.0	100.3
6	29.1	148.3	148.4	100.1
7	51.9	154.1	154.9	100.5

[a] *SD* ±0.3%.

RESULTS AND DISCUSSION

The selection of water as the solvent was a relatively straightforward choice. Compound I is soluble in water to about 5%. Furthermore, I solution USP contains about 40 mg./ml. in water. Utilizing this solvent obviates the necessity for a required extraction procedure and thus provides for a more simplified analysis. Equally important is the fact that the broad resonance signal of the solvent is sufficiently downfield from the I resonance pattern to allow for interference-free quantitation and distinct identification of the active ingredient.

tert-Butyl alcohol proved to be a suitable internal standard because it is very soluble in water and provides a single strong spectral signal at a useful upfield position.

Figure 1 represents a spectrum of trimethadione under the prescribed analytical conditions. The amount of I is calculated from the area integration of the singlet at about 1.55 p.p.m. due to the six methyl protons on the oxazolidine ring at position 5 on the ring, and the singlet at 1.25 p.p.m. is attributed to the nine methyl protons of the *tert*-butyl alcohol internal standard. The additional resonance peaks present in the spectrum are due to the methyl group of I at position 3 on the ring at about 3.02 p.p.m. and the broad solvent signal at approximately 4.6 p.p.m. In the actual analysis, it is preferred not to add the III reference standard since the methylene group beta to the sulfonyl group of III exhibits a multiplet that would overlap the sample signal in the analytical region of interest between 1.3 and 2.2 p.p.m. Even though the concentration of III in Fig. 1 is reduced to the point where no observable integration error would occur, the absence of III in the described analytical procedure provides for truer results and a more simplified analysis.

A group of known standard I mixtures was analyzed by this NMR technique, and the results are summarized in Table I. The method was both accurate and precise, with a standard deviation of ±0.3%. The relative proportions of I to *tert*-butyl alcohol had no significant bearing on the accuracy of the determination.

Approximately 10 commercial preparations of I in its various dosage forms were assayed by the described NMR procedure. Six of the results were also compared to results obtained using the official USP and NF procedure, and the findings are listed in Table II. Although the results are in good agreement, the NMR procedure has several distinct advantages over the official method. Banes (9) pointed out that the quantitative analysis of I as performed in the official procedure would be more conclusive if the drug constituents

Table II—Determination of Trimethadione in Commercial Preparations by NMR

Dosage Form	Declared, mg./Unit[a]	Found (NMR) mg./Unit[a]	Found (NMR) %	Found (Official Method) mg./Unit[a]	Found (Official Method) %
Tablet	150	141.9	94.6	141.3	94.2
Tablet	150	146.3	97.5	144.6	96.4
Capsule	300	305.1	101.7	298.5	99.5
Capsule	300	294.0	98.0	296.7	98.9
Solution[b]	1200	1175	97.9	1165	97.1
Solution	1200	1150	95.8	1156	96.3

[a] Single dosage form analysis. [b] For solution (mg./oz.).

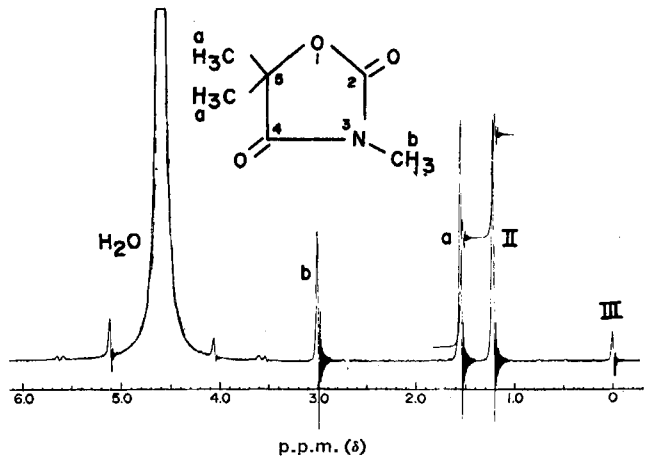

Figure 1—*NMR spectrum of trimethadione in water. Key: II, tert-butyl alcohol; and III, sodium 2,2-dimethyl-2-silapentanesulfonate.*

were first separated to ensure identity of the compound being hydrolyzed. The specificity drawback is not applicable to the NMR procedure as a result of the distinguishing resonance pattern ascribable to I. Speed and simplicity are additional characteristic traits of the NMR technique when applied to the quantitative analysis of pharmaceuticals. A complete assay can be performed in approximately 30 min. in the case of I, thus avoiding the tedious and time-consuming refluxing and distillation called for by the official method.

No interferences from any of the capsule, solution, or tablet excipients were observed in any of the analyses.

The ability of an analytical method to determine a drug in the presence of its decomposition products is a desirable attribute. Since I is stable under the analytical conditions described, no decomposition will take place as a result of the NMR procedure. However, the history of the drug could include conditions leading to decomposition. The kinetics study of Gallelli and Kostenbauder (1) demonstrated that I was hydrolyzed primarily in alkaline solution. As a result, the possibility of using this procedure in those instances where decomposition products are present was investigated, even though no evidence of decomposition was evident from the dosage forms analyzed.

The spectrum of the partially hydrolyzed drug provides an opportunity to analyze the extent of decomposition as well as the intact drug. The presence of the hydrolysis products can be detected by the appearance of two singlets at about 7 and 13 cycles upfield from the singlet due to the two methyl groups at position 5 on the oxazolidine ring and the appearance of two singlets at about 17 and 22 cycles upfield from the singlet due to the methyl group at position 3 on the ring. The total drug may be calculated by comparing the area of the three-peak system ascribable to the methyl groups on position 5 of the drug and the corresponding groups of the decomposed compounds with the area of the internal standard signal. The extent of decomposition may be measured by first comparing the area of the methyl group singlet for the intact drug at 3.02 p.p.m. (noting the correct equivalent weight) with the internal standard area and then dividing this weight by the weight of the total drug found.

The choice of the latter procedure is dictated by the fact that the differential integration is possible without difficulty since the peaks are well separated. On the other hand, although decomposition is manifest in the first three-peak system mentioned, the chemical shifts within the system do not allow for satisfactory integration and subsequent calculation of decomposition. It is possible to determine the extent of decomposition to about 2%.

In conclusion, the NMR technique, when applied to the analysis of trimethadione in its various pharmaceutical dosage forms, can provide an assay with an accuracy of about 1–2% while offering the benefits of speed, simplicity, and specificity.

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PHARMACEUTICAL TECHNOLOGY

Analog Computer Program for Simulating Variable Dosing Regimens

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Abstract □ Based on a single-compartment model, an analog computer program was developed which utilizes both analog and logic components of a general-purpose hybrid computer. The program permits the simulation of the blood and urine levels that would be obtained following the administration of an initial dose followed by a series of additional doses. The magnitude of the doses, as well as the time period between doses, may be varied independently. The program can be used to aid the formulator in establishing the amount of drug that should be released during selected time periods from a timed-release dosage form to provide uniform blood levels.

Keyphrases □ Computer program, analog—simulation of variable dosing regimens □ Simulation of variable dosing regimens—analog computer program □ Timed-release dosage formulation—use of computer simulated blood and urine levels □ Dosing regimens, variable—simulation of blood and urine levels

The use of the analog computer for simulating drug distribution in the body has been widely accepted (1–5). Many of the reported analog computer programs have been limited to simulating drug levels after single-dose administration (6–14). Several authors have reported simulations based on multiple-dose administrations that were achieved by either digital computer techniques (15–17) or manual manipulation of analog computer components (18–21). At best, the manual methods are cumbersome and there is a possible loss of accuracy due to time-dependent manual manipulation. In addition, digital computer simulation does not permit the instantaneous observation of the effect of parameter modification on the drug level in a particular compartment.

This paper discusses how digital logic components (22), which have recently been made available as an integral part of general-purpose analog computers, can be used in the simulation of variable dose sequencing regimens. Such components permit manipulation, in microseconds, of operations that were previously performed manually. With the aid of the logic components, programs can be prepared that change parameter values automatically and make decisions based on either timing considerations or other arbitrary conditions inherent in the problem to be simulated.

The flexible analog computer program described here permits the simulation of a variety of dosing regimens and has many applications in pharmacokinetic research. It has the feature of simulating the administration of an initial dose followed by a sequence of doses of variable size where the time periods between the administration of doses may be varied independently.

EXPERIMENTAL

The program is based on the single-compartment model:

$$G \xrightarrow{k_a} B \xrightarrow{k_{el}} U \qquad \text{(Eq. 1)}$$

where G = amount of drug in the gut, B = amount of drug in the blood, U = cumulative urinary excretion level, k_a = first-order rate constant of absorption, and k_{el} = first-order rate constant of excretion.

The model is described by the following differential equations:

$$\frac{dG}{dt} = -k_a G \qquad \text{(Eq. 2a)}$$

$$\frac{dB}{dt} = k_a G - k_{el} B \qquad \text{(Eq. 2b)}$$

$$\frac{dU}{dt} = k_{el} B \qquad \text{(Eq. 2c)}$$

Although the single-compartment model was chosen for demonstration purposes, the program can be easily modified to represent almost any pharmacokinetic model.