Determination of Pentylenetetrazol in Tablets and Injectables by NMR

JOHN W. TURCZAN▲ and BRUCE A. GOLDWITZ

Abstract □ A rapid and accurate NMR procedure is described for the analysis of pentylenetetrazol in tablet and injection dosage forms. A standard deviation of 0.7% was obtained on 10 synthetic mixtures. The NMR spectrum, in addition, provides a very specific means of identification for pentylenetetrazol.

Keyphrases □ Pentylenetetrazol—NMR analysis in tablets and injectables □ NMR spectroscopy—analysis, pentylenetetrazol tablets and injectables

Numerous procedures have been published for the determination of pentylenetetrazol (6,7,8,9-tetrahydro-5*H*-tetrazoloazepine), hereafter designated as I. Methods include the complexation of I with the chloride salts of cadmium (1, 2), copper (3), and mercury (4, 5). This is followed by either a gravimetric determinative step or a titration of the excess metal ion (or the complex itself). A technique developed by Throop (6) involves the precipitation of a phosphotungstate salt of a silver–I complex and subsequent direct titration with hydrochloric acid. Spectrophotometric approaches were investigated by Kolusheva and Nin'o (7) and by Daoust (8). Beyrich and Schlaak (9) and Popov and Marshall (10) quantitatively analyzed I using nonaqueous titration procedures. Other approaches to the assay of I include refractometry (11, 12), indirect polarography (13), and GC (14–16).

The method officially adopted by the National Formulary (17) for I injections involves the elution of the drug with water-saturated chloroform from an acid–base siliceous earth chromatographic column. The absorbance of the eluate is then determined using a suitable IR spectrophotometer. However, as was reported[1] and experienced by this laboratory, the official cleanup procedure is not adequate for commercial tablet preparations.

The use of NMR is very attractive for the quantitative analysis of high dosage pharmaceuticals. Addition of an internal standard followed by a suitable extraction and NMR scan is generally all that is required. Good quantitative results can be obtained, and the NMR spectrum provides an identification of the active ingredient, thereby contributing to the specificity of the method.

This report describes an NMR procedure for quantitative analysis of I as the pure drug and in the injection and tablet dosage forms. Although the applicability of the NMR technique to the analysis of I in ampuls was mentioned previously (18), the choice of solvent and reference protons of I for quantitative analysis is felt to be superior for this case. The results of this study show that the NMR procedure is simple, specific, accurate, and precise when used in the determination of the pure drug and dosage forms.

EXPERIMENTAL[2]

Standard—Pentylenetetrazol was used[3].

Internal Standard—The internal standard was hexamethylcyclotrisiloxane[3] II.

Solvent—Carbon tetrachloride[4] was used.

Samples—Compound I tablets and Compound I injectables were obtained from various commercial sources.

PROCEDURE

Tablet Preparation—Weigh and finely powder not less than 20 tablets. Weigh accurately a portion of the powder, equivalent to about 100 mg. of I, into a glass-stoppered centrifuge tube. Add about 60 mg. II, accurately weighed, and 3 ml. carbon tetrachloride. Stopper and shake for 2 min. and then centrifuge.

Injectable Preparation—Pipet a portion of the sample solution, equivalent to 100 mg. of I, into a 25-ml. glass-stoppered conical flask. Evaporate to dryness in a vacuum oven at 60°. Cool to room

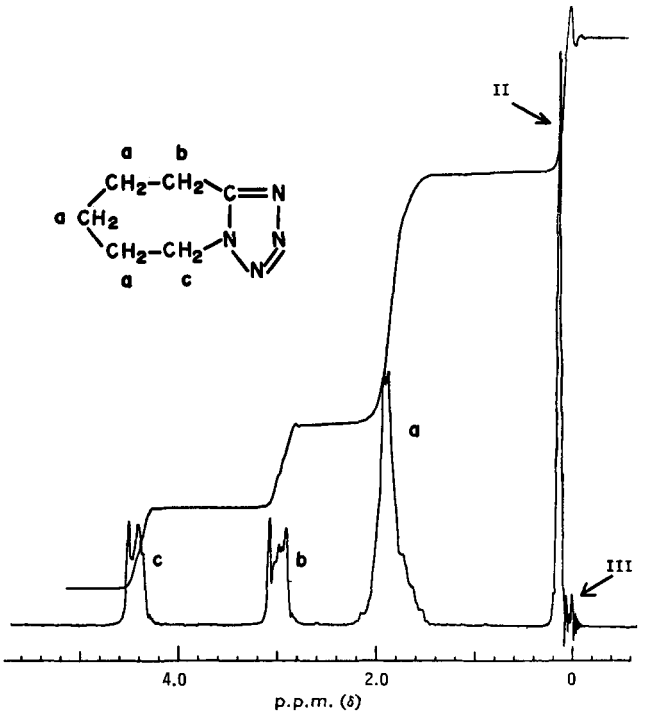

Figure 1—*NMR spectrum of pentylenetetrazol (I) in carbon tetrachloride. Key: II, hexamethylcyclotrisiloxane (internal standard); and III, tetramethylsilane.*

[1] J. C. Prinzo, private communication, Food and Drug Administration.

[2] A Varian A-60 NMR spectrometer, equipped with a V-6031 variable temperature probe having a six-turn insert, was used. All spectra were scanned at a probe temperature of 42°.

[3] K & K Laboratories, Plainview, N. Y.

[4] Fisher Scientific Co., Fair Lawn, N. J.

Table I—Determination of Pentylenetetrazol in Standard Mixtures by NMR

Standard Mixture	II Internal Standard Added, mg.	Pentylenetetrazol Added, mg.	Pentylenetetrazol Found, mg.	Recovery[a], %
1	60.1	104.6	104.1	99.5
2	59.7	104.0	104.5	100.5
3	62.2	102.2	100.6	98.4
4	60.7	52.0	52.0	100.0
5	64.5	155.5	154.9	99.6
6[b,c]	60.8	103.1	102.9	99.8
7[b,c]	61.5	105.2	106.0	100.8
8[b,c]	63.6	100.7	100.3	99.6
9[b,d]	61.2	107.1	106.2	99.2
10[b,e]	61.7	103.3	102.9	99.6

[a] The mean and standard deviation for all 10 results are 99.7 and 0.7%, respectively. [b] Standard dissolved in 1 ml. water and evaporated to dryness in a vacuum oven at 60°. [c] Dried for 1 hr. [d] Dried for 2 hr. [e] Dried for 3 hr.

Table II—Determination of Pentylenetetrazol in Commercial Preparations by NMR

Sample	Type	Tablet, mg./Tablet Injectable, mg./ml. Declared	Percent of Declared[a]
1	Tablet	100	100.4
2	Tablet	100	98.8
3	Tablet	100	100.9
4	Tablet	100	102.3
5	Tablet	100	99.9
6	Tablet	100	100.3
7	Injectable	100	99.7
8	Injectable	100	98.1
9	Injectable	100	99.6
10	Injectable	100	100.5

[a] Since the quantity of drug in the individual dosage unit is 100 mg., the quantity found in milligrams per unit and percent of declared values are identical.

temperature, add 60 mg. II, accurately weighed, and add 3 ml. carbon tetrachloride. Stopper the flask and mix thoroughly.

Transfer about 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 −0.4-0.6 p.p.m. and 1.2-2.5 p.p.m. using the delta scale. All peak field positions are referred to tetramethylsilane (III) at 0 p.p.m. Integrate the peaks of interest at least five times.

The amount of I may then be calculated as follows:

$$\frac{\text{mg. I}}{\text{tablet}} = \frac{A_p}{A_h} \times \frac{\text{E.W.}_p}{\text{E.W.}_h} \times \frac{\text{mg. II}}{\text{mg. sample}} \times \text{average tablet wt.} \quad \text{(Eq. 1)}$$

$$\frac{\text{mg. I}}{\text{ml. injectable}} = \frac{A_p}{A_h} \times \frac{\text{E.W.}_p}{\text{E.W.}_h} \times \frac{\text{mg. II}}{\text{ml. injectable}} \quad \text{(Eq. 2)}$$

where A_p = integral value of the signal representing I, A_h = integral value of the signal representing II, E.W._p = formula weight of I/6 = 23.03, and E.W._h = formula weight of II/18 = 12.35.

RESULTS AND DISCUSSION

The selection of a solvent and an internal standard is important in any NMR procedure. In this case, since both the drug, I, and the internal standard, II, are freely soluble in carbon tetrachloride, it becomes the solvent of choice. This solvent offers the obvious advantage of adding no proton signals to the spectrum. This fact, together with the convenient upfield position for the II protons, presents an uncomplicated spectrum composed of the proton resonance from the structural features of I alone. This is an ideal situation for the unambiguous identification and interference-free quantitative analysis of the drug. It is possible that II may be lost from the sample by sublimation. However, Barcza (19) reported that if II is the last component to be weighed and added to the tube which is then stoppered, no loss is incurred. The results from this laboratory corroborate these findings.

The spectrum for I is shown in Fig. 1. Three sets of peaks are noted (a, b, and c) in addition to the singlet at about 0.13 p.p.m., which is ascribable to the 18 methyl protons of the II standard. The multiplet at about 4.5 p.p.m. is assigned to the methylene protons, designated c in Fig. 1, whereas the one at about 3.0 p.p.m. is due to the methylene protons labeled b. Logically, the choice for quantitative analysis is the multiplet centered at about 1.9 p.p.m. and assigned to the six methylene hydrogens labeled a in Fig. 1. Integration of this broad pattern gives the largest single region for measurement.

The analysis of a group of standard I mixtures by NMR is summarized in Table I. To simulate the injectables, standards 6–10 were dissolved in 1 ml. of water and dried in a vacuum oven at 60° for the times specified in the table. The preparations were then carried through the described procedure. The method is both accurate and precise, with a mean of 99.7 ± 0.7%. The relative proportions of I to II, as noted in Table I, have no significant bearing on the accuracy of the determination for the range of proportions shown.

By applying this procedure to actual samples, approximately 15 commercial I tablet and injectable preparations were determined by NMR. The results are in good agreement with the declared dosages (Table II).

Some formulations encountered contained I in combination with either nicotinic acid or a mixture of methyl- and propylparabens. No interferences from either of these additives or from the excipients present were observed. In addition, NMR spectra were obtained and examined of such binders as starch, sucrose, lactose, sodium alginate, methylcellulose, and ethylcellulose and of some antisticking agents and lubricants in carbon tetrachloride. They were found not to interfere with the NMR analysis of I.

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▲ To whom inquiries should be directed.