NMR Analysis of Pharmaceuticals XII: Determination of Amantadine Hydrochloride in Soft Gelatin Capsules and Syrups

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Abstract □ An NMR procedure is described by which amantadine hydrochloride is determined in soft gelatin capsules and syrup preparations. A standard deviation of 0.8% was obtained on eight synthetic mixtures. Analysis of capsule and syrup preparations by NMR and NF XIII procedures indicates a maximum difference of 1.6%, but generally a difference of less than 1.0% was obtained. The NMR spectrum provides a very specific means of identification of amantadine hydrochloride.

Keyphrases □ Amantadine hydrochloride—NMR analysis of soft gelatin capsules and syrups □ NMR spectroscopy—analysis, amantadine hydrochloride in soft gelatin capsules and syrups

Amantadine (1-adamantanamine) (I) and its hydrochloride salt (I · HCl), whose structure is shown in Fig. 1, are synthetic chemicals unrelated to other chemotherapeutic anti-infective drugs that represent a new approach to the prevention of respiratory infection caused by the influenza A_2 (Asian) virus strain as well as to the treatment of Parkinson's disease.

Some analytical methods for this drug have been reported. Indirect procedures carried out after derivative formation (1) include a GLC determination, which is based on the conversion of I into 1-(chloroacetamido)adamantate by reaction with chloroacetic anhydride, and a spectrophotometric method, which consists of measuring the UV absorption of 1-(*p*-nitrobenzylidineamino)adamantane obtained by the reaction of I with *p*-nitrobenzaldehyde. Other procedures are direct and are based on reaction with coulometrically generated bromine (2) in a buffered medium, titrimetry (3) in which I is determined by residual base titration of a distillate collected in 0.1 *N* HCl, and potentiometric titration (4) of I in a nonaqueous medium with perchloric acid. In addition to quantitative methods, a few qualitative procedures are described, including GLC (5) and NMR spectrometry (6), in the characterization of I and its derivatives. The official NF XIII procedure (7) for the determination of I in soft gelatin capsules and in syrup involves the solvent extraction of I with benzene and titration of the free base with perchloric acid.

The procedure for I · HCl proposed here uses NMR spectroscopy, a very specific measurement technique which avoids difficulties such as those inherent in titration of a basic center or complete conversion to a desired derivative. The isolation scheme developed in this work is based on the NF method (7) with the addition of one more liquid–liquid partitioning step, a necessary extension to avoid interferences. Briefly, the scheme involves the addition of an internal standard followed by the solvent extraction, NMR spectral scan, and integration. Good quantitative results can be obtained together with a positive identification of the active component in terms of the NMR spectrum.

EXPERIMENTAL[1]

Materials—The following were used: standard, amantadine hydrochloride[2]; internal standard, succinimide[3] (II); and reference standard, Tiers' salt (sodium 2,2-dimethyl-2-silapentane-5-sulfonate) (III).

Compound I capsules and syrup, used as the samples, were obtained from a commercial source.

Procedures—*Capsules*—Dissolve a number of soft gelatin capsules, equivalent to 200 mg of I · HCl, in 10 ml of water by warming on a steam bath with intermittent shaking. Cool, transfer the sample quantitatively with water into a 125-ml separator, make basic with 30% sodium hydroxide solution, add exactly 50 ml of benzene, stopper, and shake gently for a few minutes. Transfer a 25-ml aliquot to a second 60-ml separator; add approximately 200 mg of II, accurately weighed, 4 ml of deuterium oxide, and 4 drops of concentrated hydrochloric acid; stopper; and shake. Allow the layers to separate and transfer about 0.4 ml of the bottom layer to an analytical NMR tube. Place into an NMR spec-

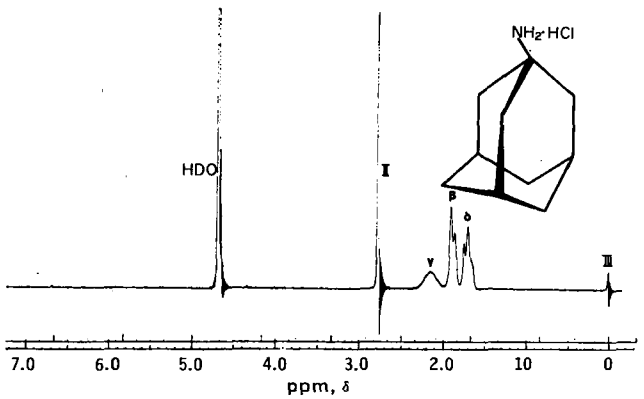

Figure 1—*NMR spectrum of amantadine hydrochloride in acidified deuterium oxide. Key: II, succinimide; and III, Tiers' salt.*

[1] 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°.

[2] DuPont, Lot QCD 1-156.

[3] Eastman Kodak Co., Rochester, NY 14650

trometer and obtain the spectrum, adjusting the spin rate to about 30 Hz. All peak field positions are referred to III at 0 ppm using the δ-scale. Integrate the peaks of interest at least five times and average the results.

Syrup—Pipet a portion of the sample solution, equivalent to 200 mg of I · HCl, into a 125-ml separator. Make basic with 30% sodium hydroxide solution and proceed as in the procedure for capsules, beginning with "... add exactly 50 ml of benzene, stopper, ..."

For the procedures given here, the amount of I · HCl may then be calculated as follows:

$$\frac{\text{mg I}}{\text{capsule}} = \frac{A_I}{A_{II}} \times \frac{EW_I}{EW_{II}} \times \frac{\text{mg II}}{C} \times \frac{50}{25} \qquad \text{(Eq. 1)}$$

$$\frac{\text{mg I}}{\text{ml of syrup}} = \frac{A_I}{A_{II}} \times \frac{EW_I}{EW_{II}} \times \frac{\text{mg II}}{V} \times \frac{50}{25} \qquad \text{(Eq. 2)}$$

where:

A_I = integral value of the signal representing I·HCl
A_{II} = integral value of the signal representing II
EW_I = formula weight of I·HCl/15 = 12.514
EW_{II} = formula weight of II/4 = 24.773
C = number of capsules taken initially
V = volume of syrup taken initially

Any variations introduced into the described procedure may introduce changes into Eqs. 1 and 2. The required changes may be simply applied.

RESULTS AND DISCUSSION

When the choice of suitable solvents for NMR is considered, the complex nature of dosage forms sometimes complicates the particular system to be used. Although water is an obvious and excellent solvent for the dissolution and analysis of I · HCl, this solvent cannot be used in a simple analytical scheme for I · HCl in various dosage forms because both the soft gelatin capsules and the syrups encountered in this work have some water-soluble excipients with proton resonance in the vicinity where the I protons resonate, thereby interfering with the integration of the peaks ascribable to I · HCl. As a result, a simple selective dissolution could not be used to isolate I · HCl in a suitable fashion.

To overcome this difficulty, a solvent distribution procedure was evolved. I · HCl was neutralized to yield I by pH adjustment (making the solution alkaline) followed by extraction into benzene and then a second extraction of the benzene with acidified deuterium oxide.

The second partitioning—*viz.*, from benzene into acidified deuterium oxide, is necessary to eliminate obvious benzene-soluble interferences that were in evidence in the NMR spectrum.

The choice of deuterium oxide eliminates the integration interference arising from the strong water resonance that would otherwise extend to the nearby region where the four methylene protons of II resonate. Although the slight amount of residual HDO present in deuterated water is further increased by the two exchangeable amino protons of I, the imide proton of II *(vide infra)*, and the small amount of hydrochloric acid used, the increase in the intensity of this resonance and its side bands is fortunately not great enough to interfere at any time (Fig. 1) with the integration and subsequent quantitation of I · HCl.

The choice of an internal standard requires the compound to be a pure chemical, preferably with a single, strong (large number of equivalent protons) signal at an appropriate field position. Compound II satisfies these requirements and, advantageously, is soluble in water but not benzene. This solubility behavior is important since II is introduced at a point where partitioning between deuterium oxide and benzene is taking place. The results indicate that II does, in fact, behave well in the described scheme.

A 60-MHz NMR spectrum of I obtained in the analysis is exhibited in Fig. 1. The resonance signals used in the actual calculations are the multiplet between 1.5 and 2.4 ppm due to the six β and the six δ methylene protons and the three γ methine protons of I · HCl and the singlet at about 2.75 ppm ascribable to the four methylene protons of II (internal standard).

The unambiguous assignment of chemical shifts for substituted adamantane hydrogens is not a straightforward task. No problem is presented in the case of the three γ hydrogens since the peak ascribable to them at 2.16 ppm is distinct from other spectral features and gives rise to three relative area units. The difficulty, however, arises in the differentiation of the peaks ascribable to the β and δ hydrogens. Fort and Schleyer (6) pointed out that the δ proton multiplet is broader than the β multiplet because the two methylene protons in the δ position are not equivalent. Although there is some reservation about the magnitude of the effect of a charge transmitted from the protonated amine (in the α position) to the β hydrogens in a rigid constrained diamondoid system such as adamantane, there is no question that this effect should be greater than the effect on the δ hydrogens. Thus, on this basis, the multiplet at 1.9 ppm is assigned to the β hydrogens whereas the δ hydrogens give rise to the somewhat broader multiplet at 1.7 ppm. This assignment is consistent with that made previously (8) in work with the 1-adamantyl carbonium ion.

In addition to the I peaks in Fig. 1, other spectral components are observed, some of which can prove troublesome in the analytical scheme. The singlet at about 4.7 ppm is due to the HDO present as a solvent impurity as well as to exchangeable protons from the solutes, whereas the singlet at 0 ppm arises from the nine methyl protons of Tiers' salt (III). Although the resonance signals ascribable to the six methylene protons α, β, and γ to the sulfonyl group in the III reference standard are not visually detectable in the spectrum, they are present in the analytical region and contribute to the integration, thereby interfering with measurement of I. Consequently, the use of III was restricted to the assignment of the chemical shifts seen in Fig. 1; in the actual analysis, no reference standard (III) is used or needed to carry out satisfactory measurements. Furthermore, since Fig. 1 is a spectrum that includes the reference standard (III) for the sake of completeness, no integration of the peaks of interest is made; the spectrum is not one that would be used for actual quantitative analysis.

Table I—Determination of Amantadine Hydrochloride in Standard Mixtures by NMR

Standard Mixture	Succinimide Internal Standard, mg	Amantadine Hydrochloride Added, mg	Found, mg	Recovery, %
1	155.7	80.4	80.8	100.5
2	102.2	101.8	101.2	99.4
3	200.0	101.0	100.5	99.5
4	100.3	101.1	100.2	99.1
5	300.2	99.8	100.9	101.1
6	200.8	102.4	101.3	98.9
7	201.5	99.9	99.3	99.4
8	200.1	102.9	103.4	100.5
			Average =	99.8
			SD =	0.8
			NF procedure =	99.3

Table II—Determination of Amantadine Hydrochloride in Capsules and Syrups by NMR

Sample	Declared Dosage, mg/unit[a]	By NMR Procedure mg/unit[a]	By NMR Procedure %	By Official Procedure mg/unit[a]	By Official Procedure %
Capsules					
1	100	99.7	99.7	98.9	98.9
2	100	98.7	98.7	99.2	99.2
3	100	101.5	101.5	99.9	99.9
4	100	99.3	99.3	100.1	100.1
5	100	99.0	99.0	99.4	99.4
Syrups					
1	50	49.95	99.9	50.5	101.0
2	50	49.8	99.5	49.9	99.7
3	50	49.8	99.6	50.3	100.5
4	50	50.5	101.0	50.4	100.8
5	50	49.9	99.8	50.3	100.6

[a] For syrup (mg/5 ml).

The accuracy and precision of the NMR procedure were studied by analyzing solutions of pure I · HCl in the range from about 80 to 100 mg in 3–4 ml of solution together with varying amounts of II internal standard. Five integrations were run on each of eight samples prepared by using the double-extraction procedure described (Table I). Since the sample taken was a pure chemical, the average value is close to 100% as expected. The standard deviation obtained for the eight known mixtures was 0.8%. The agreement between the NMR and NF procedures is excellent; both results show the measurements to be accurate. 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.

The suitability of this procedure for the analysis of actual samples was established since 15 trials involving commercial I · HCl soft gelatin capsules and syrups were analyzed by NMR with no evidence of interference from excipients present. For comparison, 10 of these samples were also analyzed by the NF XIII procedure. The results (Table II) indicate good agreement between the NMR and official procedures, the average values being within at most 0.5% of each other. The reproducibility of the NMR measurement is 0.9% *SD*.

As previously demonstrated, the use of NMR for quantitative analysis offers a number of advantages, experienced in this case as well. Thus far, monograph specifications for the identification and purity of pharmaceutical substances drawn up by books of standards do not use NMR spectroscopy. There can be little doubt, however, that NMR has an important role to play in the area of good drug standards. This situation arises probably because many people are unaware of the full potential of the NMR technique and hesitate before allowing a relatively new, and apparently expensive, method to take its rightful place alongside the established methods such as IR, UV, and visible spectroscopy.

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Quantitative Analytical Method for Determination of Drugs Dispersed in Polymers Using Differential Scanning Calorimetry

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Abstract □ A differential scanning calorimeter was used to determine quantitatively the concentration of dispersed progesterone and cholesterol in a silicone rubber matrix. With this technique, the heat of mixing and the solubility of the drug at the drug melting point also were obtained. In the progesterone study, four polymorphic forms in silicone rubber were observed.

Keyphrases □ Polymers, silicone rubber matrix—determination of dispersed progesterone and cholesterol, differential scanning calorimetry □ Progesterone—determination of concentration dispersed in silicone rubber matrix, four dispersed polymorphic forms observed, heat of mixing and solubility at drug melting point determined, differential scanning calorimetry □ Cholesterol—determination of concentration dispersed in silicone rubber matrix, differential scanning calorimetry □ Silicone rubber—determination of dispersed progesterone and cholesterol, differential scanning calorimetry □ Polydimethylsiloxane—determination of dispersed progesterone and cholesterol, differential scanning calorimetry □ Differential scanning calorimetry—analysis, progesterone and cholesterol dispersed in silicone rubber matrix

Differential scanning calorimetry has been employed for semiempirical determinations of purities of drug substances (1, 2) and for kinetic studies of polymorphic phase transitions (3). In the present study, the technique was used to determine quantitatively the concentration of solid drugs dispersed in a polymeric matrix. Polydimethylsiloxane (silicone rubber) was selected as the matrix material since it has been widely used as a rate-controlling membrane in sustained-release drug delivery devices (4–12). With these devices, it is important to know in which physical state the drug is present; in quantitative work, determination of the total amount of drug remaining in the matrix is a frequent analytical problem. Conventionally, radiolabeled drug can be used or the matrix can be extracted and the drug determined spectrophotometrically or by GLC. These techniques are often time consuming and yield no in-