^{1}H-NMR: δ 2.60 (s, 3), 2.9 (d, br, 4), 4.0 (d, br, 4), 4.10 (s, 3), 5.60 (s, 1), and 7.6–8.2 ppm (m, 6).

Anal.—Calc. for $C_{17}H_{21}IN_2S_2$: C, 45.94; H, 4.76; N, 6.30. Found: C, 45.59; H, 4.79; N, 6.32.

1-Methyl-2 - [2-methylthio-2-(1-piperidino)vinyl] - quinolinium Iodide—To a suspension of II (R = H) (2.72 g, 0.007 mole) in 25 ml of dimethyl sulfoxide was added freshly distilled piperidine (1.0 g, 0.012 mole), and the mixture was stirred at 30° for 5 days. It was treated as in the previous procedure, and the crude precipitate was dissolved in 1-propanol (300 ml), treated with charcoal, and filtered while hot. This procedure was repeated three times, and the combined filtrates were evaporated to dryness under reduced pressure. The residue was recrystallized from 2-propanol to give 2.70 g (90%) of orange crystals, m.p. 195–196°. ^{1}H-NMR: δ 1.75 (s, 6), 2.63 (s, br, 3), 3.81 (s, br, 4), 4.10 (s, 3), 5.55 (s, 1), and 7.6–8.2 ppm (m, 6).

Anal.—Calc. for $C_{18}H_{23}IN_2S$: C, 50.70; H, 5.43; N, 6.57. Found: C, 50.93; H, 5.23; N, 6.38.

1-Methyl-2-[2-methylthio-2-(4-methyl-1-piperazino)vinyl]-quinolinium Iodide—Following the previous procedure, II (R = H) (1.20 g, 0.0031 mole) in dimethyl sulfoxide (25 ml) was treated with 1-methylpiperazine (0.35 g, 0.0035 mole). The product was recrystallized from acetic acid and again from absolute ethanol to give 0.63 g (48%) of orange crystals, m.p. 188–191°. ^{1}H-NMR: δ 2.58 (s, 3), 2.95 (s, 3), 3.40 (br, 4), 3.83 (br, 4), 4.10 (s, 3), 5.80 (s, 1), and 7.6–8.2 ppm (m, 6).

Anal.—Calc. for $C_{18}H_{24}IN_3S$: C, 48.98; H, 5.48; N, 9.52; S, 7.26. Found: C, 49.00; H, 5.49; N, 9.29; S, 7.30.

1-Methyl-2-[2-methylthio-2-(3-methyl-1-piperazino)vinyl]-quinolinium Iodide—Following the previous procedure, II (R = H) (2.72 g, 0.007 mole) in dimethyl sulfoxide (25 ml) was treated with 2-methylpiperazine (0.70 g, 0.007 mole). The thick, oily product crystallized on long storage in the refrigerator. It was recrystallized from absolute ethanol and ether to give 0.41 g (14%) of orange-brown crystals,m.p. 188–190°. ^{1}H-NMR: δ 2.13 (s, 3), 2.58 (s, 3), 3.40–3.70 (br, 7), 4.10 (s, 3), 5.58 (s, 1), and 7.6–8.2 ppm (m, 6).

Anal.—Calc. for $C_{19}H_{24}IN_3S$: C, 48.98; H, 5.48; N, 9.52; S, 7.26. Found: C, 48.83; H, 5.58; N, 9.11; S, 7.08.

REFERENCES

(1) W. O. Foye, Y. J. Lee, K. A. Shah, and J. M. Kauffman, *J. Pharm. Sci.*, **67**, 962 (1978).

(2) W. O. Foye and J. M. Kauffman, *J. Pharm. Sci.*, **68**, 336 (1979).

(3) W. O. Foye and J. M. Kauffman, *J. Pharm. Sci.*, **69**, 477 (1980).

(4) W. O. Foye, J. M. Kauffman, and R. Ganapathi, "Current Chemotherapy and Infectious Disease," Vol. II, Proc. 11th Int. Congress of Chemotherapy and 19th Intersc. Conf. on Antimicrobial Agents and Chemotherapy (1979), pp. 1629–1631, 1980.

(5) W. O. Foye, O. Vajragupta, and S.K. Sengupta, *J. Pharm. Sci.*, **71**, 253 (1982).

(6) R. Gompper, B. Wetzel, and W. Elser, *Tetrahedron Lett.*, **53**, 5519 (1968).

(7) K. Mizuyama, Y. Tominaga, Y. Matsuda, and G. Kobayashi, *Yakugaku Zasshi*, **94**, 702 (1974).

(8) R. M. Silverstein, G. C. Bassler, and T. C. Morrill, "Spectrometric Identification of Organic Compounds," 3rd ed., Wiley, New York, N.Y., 1974, p. 223.

(9) K. Mizuyama, Y. Tominaga, Y. Matsuda, and G. Kobayashi, *Yakugaku Zasshi*, **95**, 290 (1975).

(10) R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep.*, Part 3, **3**, 1 (1972).

ACKNOWLEDGMENTS

Supported by funds from the John R. and Marie K. Sawyer Memorial Fund, Massachusetts College of Pharmacy and Allied Health Sciences.

Quantitative Analysis of Ethchlorvynol in a Capsule Dosage Form by NMR Spectroscopy

KAREN B. FEKETY ˣ **and THOMAS MEDWICK**

Received July 19, 1982, from the *College of Pharmacy, Rutgers University, Piscataway, NJ 08854.* Accepted for publication October 14, 1982.

Abstract □ A quantitative ^{1}H-NMR procedure is described for measuring ethchlorvynol in capsules. Deuterochloroform is used as the solvent, and hexamethylenetetramine as the internal standard; the analysis is based on the comparison of the area of the AB peak system of ethchlorvynol with the area of the hexamethylenetetramine singlet. The ^{1}H-NMR method yields results that are precise to within 1% and agree well with results of the more cumbersome and less specific USP titrimetric procedure.

Keyphrases □ Ethchlorvynol—NMR quantitative analysis, capsule dosage form □ NMR quantitative analysis—ethchlorvynol, capsule dosage form

Ethchlorvynol (I), a nonbarbiturate hypnotic, is a tertiary acetylenic carbinol (1-chloro-3-ethyl-1-penten-4-yn-3-ol). The official USP XX procedure for the analytical determination of this drug substance, both alone and in its pharmaceutical dosage form, is based on the reaction of I with excess silver nitrate, producing the silver acetylide and nitric acid (1, 2). The resultant acid is immediately titrated with ~0.05 *N* NaOH; however, end point determination with the methyl red–methylene blue indicator is hampered by the precipitation of the silver acetylide. According to the official procedure, capsules must be weighed, carefully opened, emptied, and reweighed, with the difference taken as the capsule contents. The difficult end point and sample manipulations often lead to poor results.

Although there are several procedures published for the determination of I in biological fluids (3–8), there are only two other published methods for its determination in the pharmaceutical dosage form. Davidson (9), proposed a GLC analysis, accepted by the AOAC (10), and that uses 1,3-dichloro-2-propanol as an internal standard; a standard deviation range of 1.3–2.9% on assays of known solutions and the 500-mg capsule dosage form is reported. Drawbacks inherent in this procedure include the need for column preparation and overnight conditioning and the necessity of injecting a I standard (purified by vacuum distillation and quantified by the USP XX titrimetric procedure) along with the unknown solutions. Rizk and associates (11) proposed a colorimetric method for the determination of certain monosubstituted acetylenic hypnotic drugs, including I. In this procedure, silver acetylide

0022-3549/83/1100-1358$01.00/0

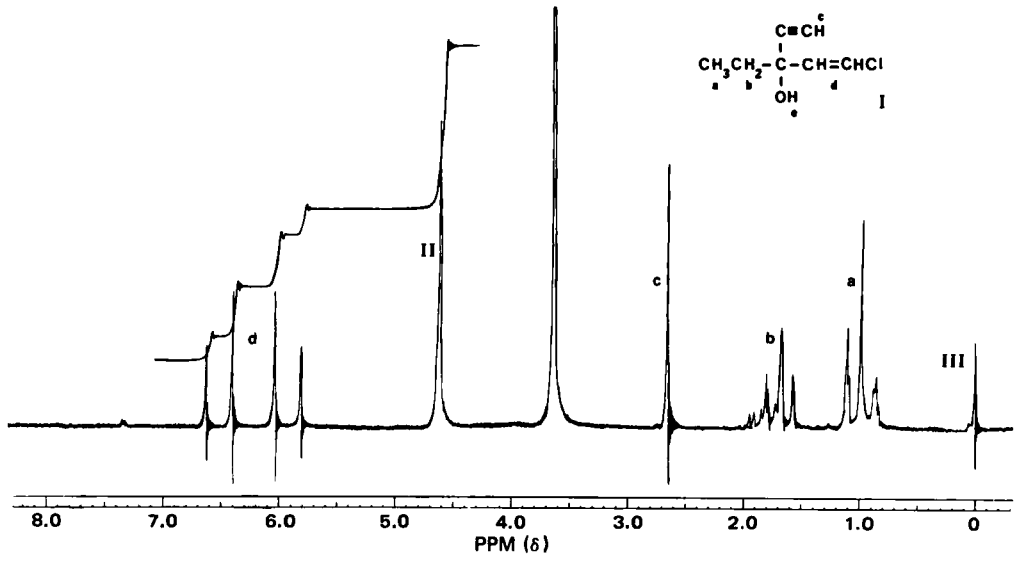

Figure 1—*^{1}H-NMR spectrum of ethchlorvynol in deuterochloroform. Key: (II) hexamethylenetetramine (internal standard); (III) tetramethylsilane.*

is formed and extracted into 4-methyl-2-pentanone, and after acidification, an equivalent amount of silver ions is liberated and assayed colorimetrically as silver dithizonate (λ_{max} = 472 nm). Although the method claims a standard deviation of 1.5–2.9%, it is complicated by the need for a calibration curve for silver nitrate and an involved extraction procedure.

The procedure proposed in this study utilizes quantitative ^{1}H-NMR. Rucker and Natarajan (12) used NMR spectroscopy to quantitatively determine nine sedatives (including I) in mixtures using calibration curves obtained from the responses of standard solutions. However, this study describes the use of an internal standard, which results in a simple, rapid, specific, precise, and accurate analytical method.

EXPERIMENTAL

Materials—The following were used: internal standard, hexamethylenetetramine (methenamine USP), 100.05%, 30 mesh (II)[1]; reference standard, tetramethylsilane (III)[2]; solvent, deuterochloroform, 99.8% D[3] (IV). Ethchlorvynol capsules, 500 mg and 750 mg, were obtained from commercial sources.

Procedure—An individual capsule of I was placed into a suitable glass-stoppered vessel for cutting (*e.g.*, a weighing bottle). With a dissecting scalpel, the capsule was cut cleanly in half, and the blade was rinsed with a few drops of IV to ensure recovery of the entire capsule contents. The appropriate amount of accurately weighed II (~80 mg for a 500-mg capsule and 120 mg for a 750-mg capsule) was added to the container, followed by ~3 ml of IV. The container was stoppered and shaken to ensure complete dissolution of II. The solution was transferred to a glass syringe fitted with a filter apparatus[4], and ~0.5 ml was filtered directly into a standard 5-mm analytical NMR tube. A drop of III was added as needed to reference the peak field positions to 0 ppm on the δ scale. The tube was placed into an NMR spectrometer[5], and the spectrum was obtained adjusting the spin rate so that no spinning side bands interfered with the peaks of interest. The peaks were integrated at 5.81, 6.05, 6.40, and 6.62 ppm and the singlet at 4.65 ppm, not fewer than five times, taking care to avoid saturation.

[1] Merck and Co., Rahway, N.J.
[2] Aldrich Chemical Co., Milwaukee, Wis.
[3] Sci-Graphics, Wayne, N.J.
[4] A Millipore HA filter type 0.45 μm and a 2-ml B-D Yale Luerlok glass syringe.
[5] 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°.

The amount of I per capsule was calculated as follows:

$$\text{mg of I/capsule} = \text{mg of II} \times (\text{Au/As}) \times (\text{Eu/Es})$$

where Au is the integral value representing I (5.81, 6.05, 6.40, and 6.62 ppm), As is the integral representing II (4.65 ppm), Eu is the proton equivalent weight of I (FW/2 = 72.305), and Es is the proton equivalent weight of II (FW/12 = 11.683).

RESULTS AND DISCUSSION

The solubility of ethchlorvynol (I) and hexamethylenetetramine (II) in deuterochloroform (IV) and the insolubility of the capsule shell makes this solvent a good choice for this ^{1}H-NMR procedure. Although the actual formulation of the dosage form is unknown to the authors, the only peak not ascribable to I occurs at 3.60 ppm and does not interfere with the spectrum of I. Filtration of the solution removes undissolved material, since the unfiltered samples leave difficult-to-remove beadlets in the NMR tube. Filtering the sample does not affect the analytical results, as evidenced by studies of samples before and after filtration.

The ^{1}H-NMR spectrum for I with the addition of II is seen in Fig. 1. The assignments made in this work are in agreement with the limited assignments made by Rucker and Natarajan (12) for the ^{1}H-NMR spectrum of I. For I, the resonances, characteristic of an AB system (d) at δ 5.81, 6.06, 6.40, and 6.62 ppm, correspond to the olefinic protons in the molecule. The methylene protons (b) are not chemically equivalent because of the adjacent asymmetric carbon atom, and therefore are split into 2 quartets (band center δ 1.67 ppm) by the methyl protons; the methyl protons (a) are seen as a triplet centered at δ 1.00 ppm. The acetylenic hydrogen (c) resonates as a singlet at δ 2.62 ppm. The broad based peak at δ 3.60 ppm appears to be a consequence of the formulation and may be a polar material that exchanges with the hydroxyl proton (e) of I. Compound II exhibits only one resonance signal at δ 4.65 ppm, since all 12 of its protons are equivalent. Finally, a very small peak from an impurity in IV is seen at δ 7.32 ppm. All chemical shifts are measured with respect to III at 0 ppm.

The quantitative analysis of I is based on the integration of the area of the AB system quartet which is compared with the area arising from the singlet for II. Summaries of the analyses of a series of both the 500- and 750-mg commercial capsules appear in Tables I and II, respectively. As the results indicate, the ^{1}H-NMR method is both precise, with a relative standard deviation of ~1%, and accurate, as evidenced by the good agreement of the ^{1}H-NMR results in comparison with samples analyzed by the official USP procedure.

The potential value of the proposed ^{1}H-NMR procedure in the analysis of dosage forms of I is established by these measurements, with no evidence of interference from other capsule components or the capsule shell. Blank systems including only the solvent and the capsule shell, with and without the internal standard, showed that no signals are derived from the capsule shell ingredients. This analytical measurement by quantitative ^{1}H-NMR circumvents the lengthy manipulations inherent in the

Table I—Analysis of 500-mg Ethchlorvynol Capsules

Sample	^{1}H-NMR			USP	
	Internal Standard Added, mg	Ethchlorvynol, mg	Percent of Label Claim	Ethchlorvynol, mg	Percent of Label Claim
1	86.86	480.50	96.1	492.33	98.5
2	77.19	473.05	94.6	494.00	98.8
3	81.17	487.33	97.4	485.40	97.1
4	80.74	485.74	97.1	484.33	96.9
5	83.57	482.37	96.5	489.31	97.9
6	79.03	487.01	97.4	484.00	96.8
7	86.72	486.56	97.3	485.61	97.1
8	89.43	487.02	97.4	479.77	96.0
9	81.53	480.53	96.1	491.95	98.4
10	82.92	475.73	95.1	488.74	97.8
Mean		482.58	96.6	487.54	97.5
SD		± 5.10	± 1.02	± 4.47	± 0.89

Table II—Analysis of 750-mg Ethchlorvynol Capsules

Sample	^{1}H-NMR			USP	
	Internal Standard Added, mg	Ethchlorvynol, mg	Percent of Label Claim	Ethchlorvynol, mg	Percent of Label Claim
1	116.71	724.00	96.5	719.05	95.9
2	116.89	738.97	98.5	725.27	96.7
3	116.46	712.64	95.0	714.47	95.2
4	120.73	726.72	96.9	720.82	96.1
5	117.53	722.34	96.3	724.43	96.6
6	119.77	731.19	97.4	717.44	95.7
7	119.35	724.57	96.6	720.28	96.1
8	116.33	722.19	96.3	724.24	96.6
9	118.54	724.07	96.5	728.65	97.2
10	118.83	723.38	96.5	719.65	96.0
Mean		725.01	96.7	721.43	96.2
SD		± 6.73	± 0.89	± 4.20	± 0.57

USP procedure, and avoids the necessity of a reference standard of I, as in the procedure of Rucker and Natarajan (12). In brief, the method is more specific and appears superior to the existing analyses.

REFERENCES

(1) "The United States Pharmacopeia," 20th rev., U.S. Pharmacopeial Convention, Rockville, Md., 1980, p. 305.

(2) N. D. Cheronis and T. S. Ma, "Organic Functional Group Analysis," Wiley-Interscience, New York, N.Y., 1964, p. 385.

(3) D. W. Robinson, *J. Pharm. Sci.*, **57**, 185 (1968).

(4) P. F. Gibson and N. Wright, *J. Pharm. Sci.*, **61**, 169 (1972).

(5) E. J. Algeri, G. G. Katsas, and M. A. Luongo, *Am. J. Clin. Pathol.*, **38**, 125 (1962).

(6) C. S. Frings and P. S. Cohen, *Am. J. Clin. Pathol.*, **54**, 833 (1970).

(7) J. E. Wallace, W. J. Wilson, and E. V. Cahoe, *J. Forensic Sci.*, **9**, 342 (1964).

(8) J. E. Wallace, H. E. Hamilton, J. Ariloff, and K. Blum, *Clin. Chem.*, **20**, 159 (1974).

(9) A. W. Davidson, *J. Assoc. Off. Anal. Chem.*, **53**, 834 (1970).

(10) "Official Methods of Analysis," 13th ed., AOAC, Washington, D.C., 1980, sec. 37.100–37.104.

(11) M. S. Rizk, M. I. Walash, and A. Elbrashy, *J. Assoc. Off. Anal. Chem.*, **63**, 88 (1980).

(12) G. Rucker and P. N. Natarajan, *Arch. Pharm.*, **300**, 276 (1967).

ACKNOWLEDGMENTS

Taken in part from the thesis submitted by Karen B. Fekety to the Graduate School, Rutgers University in partial fulfillment of the requirements for the degree of Master of Science.

Gratitude is extended to the New Jersey Pharmaceutical Quality Control Association for granting to Karen B. Fekety their Summer Fellowship for 1982.