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Stability of Amitriptyline Hydrochloride in a Commercial Aqueous Solution

R. ROMAN ˣ, E. M. COHEN, M. E. CHRISTY, and W. B. HAGERMAN

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Abstract □ A commercial amitriptyline hydrochloride solution was stored at 80° for up to 3 months. High-performance liquid chromatography showed no evidence of amitriptyline hydrochloride degradation. The method also indicated that two reported degradates, 3-(propa-1,3-dienyl)-1,2:4,5-dibenzocyclohepta-1,4-diene and dibenzosuberone, were present at levels less than 0.1% (the detection limit of the method) under the storage conditions. The stability of the commercial solutions is attributed to their relatively low ratio of headspace oxygen to amitriptyline hydrochloride.

Keyphrases □ Amitriptyline hydrochloride—stability, commercial aqueous solutions, high-performance liquid chromatography □ Antidepressants—amitriptyline hydrochloride, stability, commercial aqueous solutions, high-performance liquid chromatography □ High-performance liquid chromatography—analysis, amitriptyline hydrochloride in commercial aqueous solutions, stability

Amitriptyline hydrochloride decomposition products in aqueous solution were identified by Enever *et al.* (1). More recently, the same investigators reported a number of factors that influence the drug's decomposition rate (2). The latter study indicated that aqueous amitriptyline hydrochloride solutions could undergo appreciable decomposition after even a few days of storage at 80°.

Neither study reported data for amitriptyline hydrochloride stability in marketed parenteral solutions. The purpose of this investigation was to assess the stability of an aqueous amitriptyline hydrochloride solution in one such formulation[1].

EXPERIMENTAL

Materials—Amitriptyline hydrochloride[2], dibenzosuberone[3], and methanesulfonic acid[4] were used without further purification. All other chemicals were reagent grade.

High-Performance Liquid Chromatography (HPLC)—*Apparatus*—The liquid chromatograph[5] was equipped with a fixed-wavelength detector (254 nm), an oven, and an integrator.

Column—A 30 × 0.39-cm (i.d.) column containing a nitrile-bonded phase packing[6] was used at 30° with a mobile phase flow of 2 ml/min.

Mobile Phase—For amitriptyline hydrochloride analysis, acetonitrile–0.02 *M* ammonium acetate plus 0.01 *M* methanesulfonic acid in distilled water (90:10) was used. For degradate detection, the ratio was 50:50.

Sample Preparation—The sample was prepared by diluting a 2.0-ml aliquot to 25.0 ml with distilled water. The sample was filtered prior to analysis, and 10 μl was injected onto the column (attenuation 0.0256 absorbance unit/cm). Samples containing degradates were prepared in the same manner and injected at an attenuation of 0.0032 absorbance unit/cm.

Quantitation—Quantitation was achieved using the ratio of the sample peak area to that of an amitriptyline hydrochloride reference standard[2].

Storage of Amitriptyline Hydrochloride Solutions—Multiple-dose vials[1] (10 ml) were stored in a forced-air oven[7] maintained at 80°. Vials were withdrawn at predetermined times and stored at 5° prior to analysis at the completion of the study (3 months).

Synthesis of 3-(Propa-1,3-dienyl)-1,2:4,5-dibenzocyclohepta-1,4-diene (I)—An authentic sample of I was prepared from amitriptyline *N*-oxide dihydrate (3) by Cope elimination (125°/2 hr). The product was isolated by ether extraction and purified by column chromatography on silica gel with carbon tetrachloride elution. The purified sample was characterized by TLC and spectra (UV, NMR, and mass) and was stored at 5°.

Table I—Amitriptyline Hydrochloride in Aqueous Solution Stored at 80°

Days	Found, mg/ml	Percent of Initial
Initial	10.21	
4	10.32	101
8	10.25	100.4
11	10.24	100.3
16	10.20	99.9
20	10.10	98.9
24	10.15	99.4
28	10.16	99.5
35	10.19	99.8
90	10.08	98.7

RESULTS AND DISCUSSION

The results (Table I) showed no detectable amitriptyline hydrochloride loss in aqueous solutions stored at 80° for up to 90 days. The results are unusual only in that the solutions studied by Enever *et al.* (2) showed amitriptyline hydrochloride losses from 5 to 90% after storage at 80° for 30 days. The solutions used in the earlier study contained 2 mg of amitriptyline hydrochloride/ml buffered at pH 3.0 or 5.0 and were sealed in ampuls with a 4:1 ratio of headspace to solution (2). The commercial formulation contained 10 mg of amitriptyline hydrochloride/ml in an unbuffered solution that also included 44 mg of dextrose/ml, 1.5 mg of

[1] Elavil, 10 mg/ml in a 10-ml multidose vial, Merck Sharp & Dohme, West Point, Pa.
[2] Merck Sharp & Dohme, West Point, Pa.
[3] Aldrich Chemical Co., Milwaukee, Wis.
[4] Eastman Kodak Co., Rochester, N.Y.
[5] Hewlett-Packard model 1084, Avondale, Pa.
[6] μBondapak CN, Waters Associates, Milford, Mass.
[7] Model OV-490A-2, Blue M Electric Co., Blue Island, Ill.

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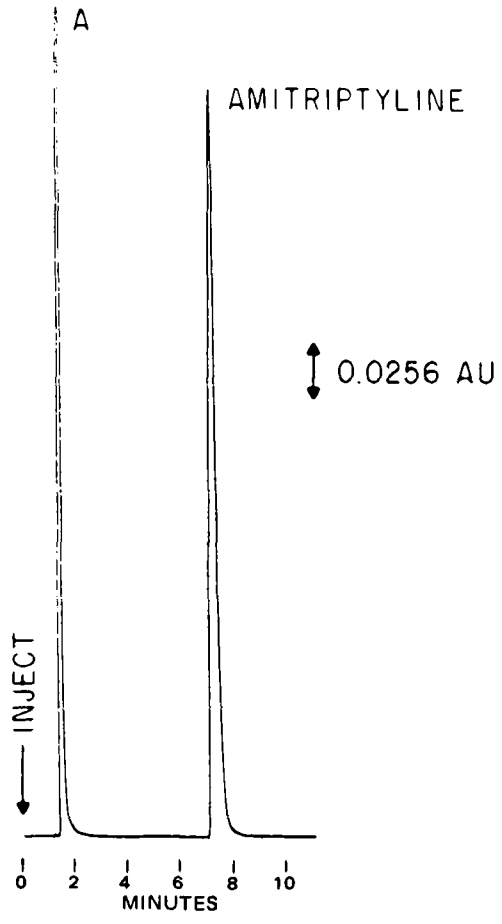

Figure 1—*Chromatogram of diluted amitriptyline hydrochloride formulation. Peak A represents methylparaben, propylparaben, and I and II, if present. The mobile phase was acetonitrile–0.02* M *ammonium acetate and 0.01* M *methanesulfonic acid (90:10).*

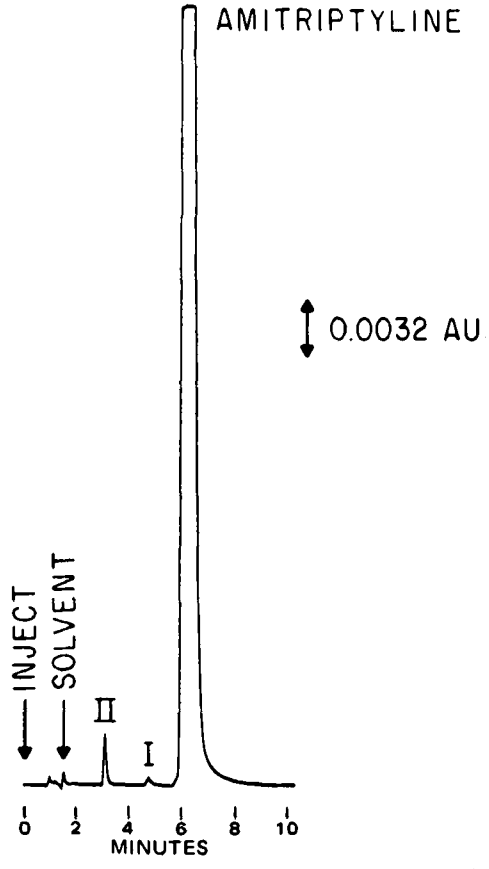

Figure 2—*Chromatogram of amitriptyline hydrochloride standard (0.64 mg/ml) spiked with I (0.00065 mg/ml) and II (0.0016 mg/ml). The mobile phase was acetonitrile–0.02* M *ammonium acetate and 0.01* M *methanesulfonic acid (50:50).*

methylparaben/ml, and 0.2 mg of propylparaben/ml. The ratio of headspace to liquid in the multiple-dose vial was 1:6, and the pH was 4.0–6.0 (4).

Enever *et al.* (2) demonstrated the involvement of oxygen in amitriptyline hydrochloride degradation with an experiment showing no decomposition of drug sealed in ampuls under nitrogen. An accurate prediction of the headspace oxygen effect on amitriptyline hydrochloride decomposition would require detailed knowledge of the reaction kinetics. However, the fact that the headspace to amitriptyline ratio in the marketed formulation was less than 1/100th of that for the solutions studied previously (2) is a likely explanation for the different extents of degradation.

Assay—Enever *et al.* (2) used ether extraction followed by GLC to assay the intact amitriptyline. In the present study, an HPLC procedure using a dilute solution of the formulation was more convenient. In this method, which was modified from one reported for amitriptyline hydrochloride–perphenazine tablets (5), the neutral compounds, methyl- and propylparaben, as well as the degradates, 3-(propa-1,3-dienyl)-1,2:4,5-dibenzocyclohepta-1,4-diene (I) and dibenzosuberone (II), were unretained and eluted well before the basic amitriptyline (Fig. 1). A third degradate reported by Enever *et al.* (1), 3-(2-oxoethylidene)-1,2:4,5-dibenzocyclohepta-1,4-diene (III), was not available for testing. Since III is a neutral compound, it should exhibit polarity similar to II and should almost certainly be separated from amitriptyline under the conditions used.

Detection of Degradates—The k' values for I and II could be increased by decreasing the acetonitrile percentage in the mobile phase while maintaining a relatively constant k' value for amitriptyline by increasing the methanesulfonic acid level. Thus, it was possible to separate and detect I, II, and amitriptyline in the same chromatogram (Fig. 2). This chromatogram of amitriptyline spiked with I and II illustrates potential degradate detection at levels as low as 0.1%. Even under these conditions, it was not possible to detect either I or II in amitriptyline hydrochloride solutions at 80° for up to 3 months. To test whether storage for longer periods at lower temperatures might induce degradation, three lots of commercial material that had been stored at room temperature for longer than 5 years (the expiration date for the product) were analyzed, but neither I nor II could be detected.

Although the commercial formulation is clearly more stable than the aqueous amitriptyline hydrochloride solutions studied by Enever *et al.* (2), the absence of detectable degradates in the former solutions even after 3 months of storage at 80° is surprising. One explanation for the absence of I and II might be the formation of other transformation products. Compound I polymerized readily. Both the formulation and solutions containing all ingredients except amitriptyline hydrochloride discolored during storage at 80° and eventually formed a brown oil, presumably due to dextrose decomposition, thus obscuring the visual detection of insoluble amitriptyline degradates.

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