Simultaneous High-Performance Liquid Chromatographic Determination of Chlordiazepoxide and Amitriptyline Hydrochloride in Two-Component Tablet Formulations

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Abstract ☐ A rapid, precise, and accurate high-performance liquid chromatographic procedure is presented for the simultaneous determination of amitriptyline hydrochloride and chlordiazepoxide in two-component tablet formulations. The impurities and decomposition products of both components were separated, making the determination specific for amitriptyline hydrochloride and chlordiazepoxide. The method was used for the assay, content uniformity, and dissolution testing of dosage forms containing 5–30 mg of chlordiazepoxide and 12.5–75 mg of amitriptyline.

Keyphrases ☐ Chlordiazepoxide—simultaneous determination with amitriptyline hydrochloride, high-performance liquid chromatography ☐ Amitriptyline hydrochloride—simultaneous determination with chlordiazepoxide, high-performance liquid chromatography ☐ High-performance liquid chromatography—simultaneous determination of chlordiazepoxide and amitriptyline hydrochloride

Numerous high-performance liquid chromatographic (HPLC) methods for the determination of amitriptyline hydrochloride (V), chlordiazepoxide (III), and related compounds have been reported (1–4). However, none of the methods is applicable to the simultaneous determination of these compounds in two-component tablet formulations.

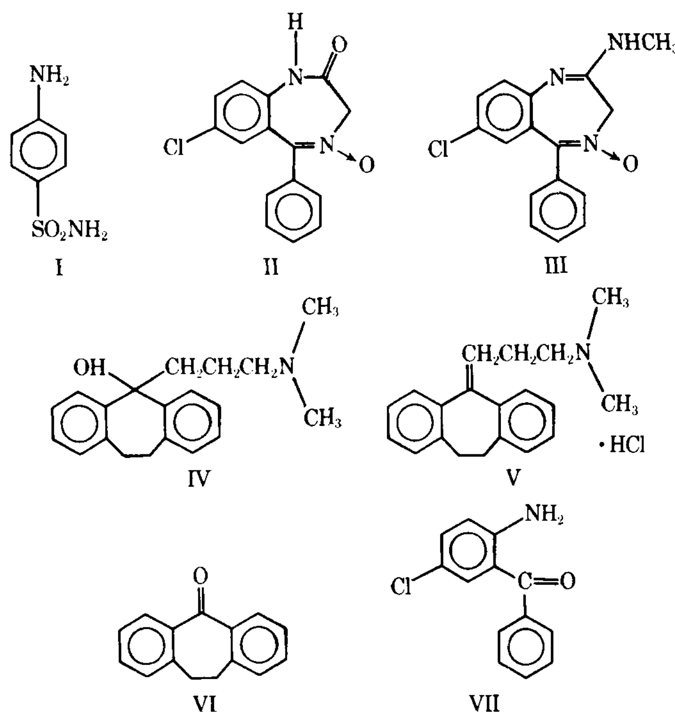
Conventional analyses, such as pharmacopeial procedures for the individual drugs (5), also are not suited for simultaneous determination. Traditional procedures, such as the spectrophotometric determination of the bromcresol green complex of amitriptyline hydrochloride and of the diazotization and coupling product of chlordiazepoxide, are tedious and time consuming, especially for content uniformity and dissolution testing.

This report presents an HPLC method for the quantitative determination of both substances in two-component tablet formulations. The analysis can be performed in a reasonable time and eliminates interference due to possible impurities: 10,11-dihydro-5-[3-(dimethylamino)propyl]-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ol (IV) and 10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-one (VI) in amitriptyline hydrochloride and 7-chloro-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one-4-oxide (II) and 2-amino-5-chlorobenzophenone (VII) in chlordiazepoxide.

EXPERIMENTAL

Apparatus—The liquid chromatograph consisted of a pump[1] with a flow capacity of 16–160 ml/hr and a fixed-wavelength UV detector[2] (254 nm). Solutions were injected with a valve loop[3] with a capacity of 20 or 50 μl. A 30 × 0.39-cm i.d. column containing an octadecyl bonded-phase packing[4] was used.

Chromatographic Conditions—The mobile phase consisted of 0.01



M sodium lauryl sulfate in tetrahydrofuran–methanol–pH 2.5 Britton–Robinson buffer (120:30:150). A flow rate of ~1 ml/min was established.

Reagents and Samples—The solvents were commercial analytical grade, and the water was double distilled. The Britton–Robinson buffer was prepared by adding 10.5 ml of 0.2 *N* NaOH to 100 ml of stock solution consisting of 0.04 *M* acetic acid, 0.04 *M* phosphoric acid, and 0.04 *M* boric acid to obtain pH 2.5 (6). The sample solvent was water–tetrahydrofuran–methanol (150:120:30). Sulfanilamide[5] (I) solution in the sample solvent at ~1 mg/ml was the internal standard. The standard solution contained ~0.3 mg of amitriptyline hydrochloride[6]/ml, 0.1 mg of chlordiazepoxide[7]/ml, and 0.1 mg of sulfanilamide/ml.

All standard and sample solutions were prepared and stored in low-actinic glassware.

Assay and Calculation—For the content uniformity test and tablet composite assay, one tablet or the equivalent tablet mass was triturated with the sample solvent and transferred quantitatively to a volumetric flask. The internal standard was added, and the flask contents were diluted to volume with the sample solvent to obtain ~0.3 mg of amitriptyline hydrochloride/ml, 0.1 mg of chlordiazepoxide/ml, and 0.1 mg of sulfanilamide/ml. An aliquot was filtered, and 20 μl was injected.

For analysis of the tablets after dissolution, a portion of the sample solution was withdrawn after an appropriate time and filtered, and an aliquot was transferred to a volumetric flask. The pH was adjusted to 2.5, and the aliquot was diluted to volume with the sample solvent to obtain 0.011 mg of amitriptyline hydrochloride/ml and 0.004 mg of chlordiazepoxide/ml. A solution of reference standards of the same amitriptyline hydrochloride and chlordiazepoxide concentrations was prepared similarly, and 50 μl of each solution was injected.

[1] Model 396/2396, Laboratory Data Control, Riviera Beach, Fla.
[2] Model 1285, Laboratory Data Control, Riviera Beach, Fla.
[3] Model 7010, Rheodyne, Inc., Berkeley, Calif.
[4] μBondapak C_{18}, Waters Associates, Milford, Mass.

[5] Eastman Organic Chemicals, Rochester, N.Y.
[6] USP reference standard.
[7] Chlordiazepoxide (99.3% pure), Hoffmann-La Roche Inc., Nutley, N.J.

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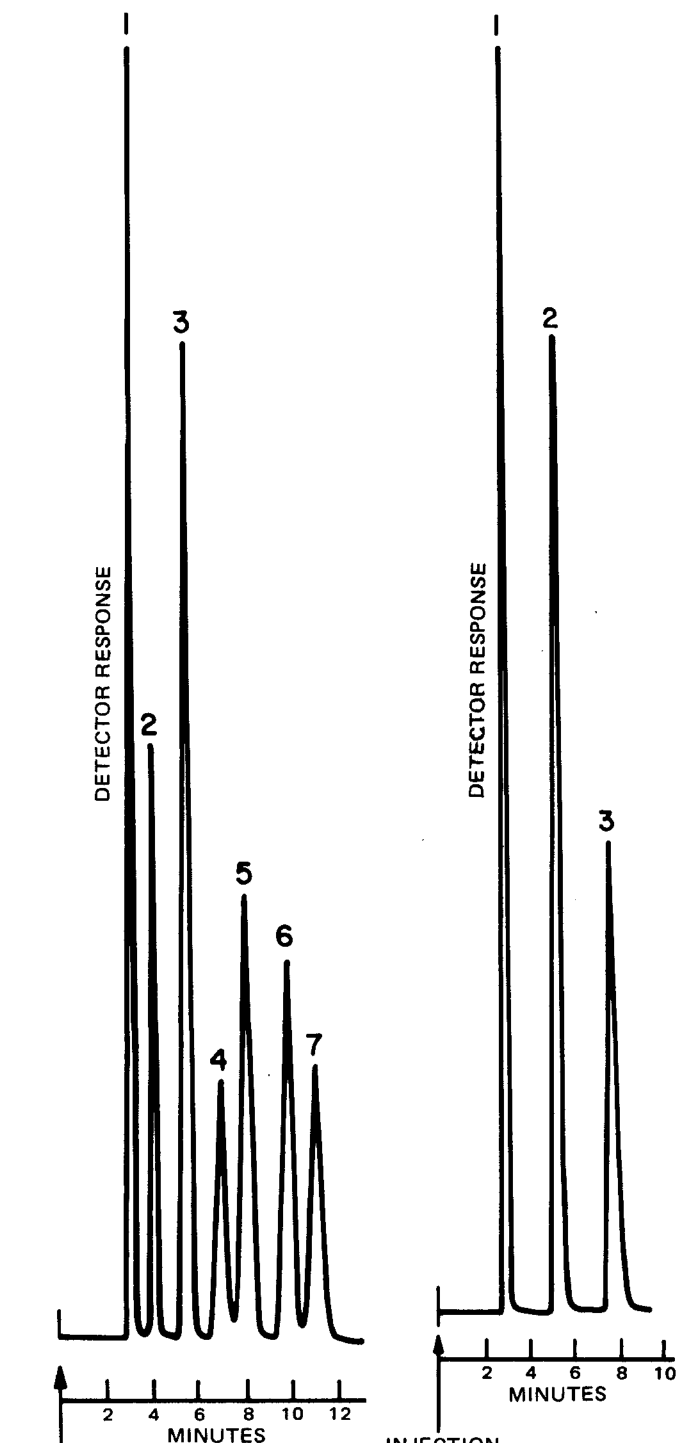

Figure 1—*Chromatogram of a mixture of I (1), II (2), III (3), IV (4), V (5), VI (6), and VII (7).*

Figure 2—*Chromatogram of a tablet containing 5 mg of III (2) and 12.5 mg of V (3) with I (1) (internal standard) added.*

Quantitation was by the peak height ratio of the reference standards and sample to the internal standard for the content uniformity test and tablet composite assay and by comparison of the peak height of the sample to that of the external standard for dissolution calculations.

RESULTS AND DISCUSSION

This investigation was initiated for use in control laboratories where rapid and simple methods are required. Simple and rapid sample preparation was needed, particularly for content uniformity and dissolution tests. The aqueous sample solution in the latter test can be used as is or with minimal sample treatment for reversed-phase chromatography.

To determine the appropriate HPLC parameters, some preliminary TLC was performed using silica gel plates coated with silicone oil. A mobile phase containing tetrahydrofuran and pH 3 Britton–Robinson buffer was selective in the migration of the six compounds chromatographed. The use of a μBondapak C_{18} column with the buffered mobile phase failed to separate V, II, and IV from each other. Variation of the mobile phase composition was unsuccessful. Complete separation was achieved by the addition to a mobile phase of sodium lauryl sulfate and methanol and by adjustment of the pH to 2.5. The paired-ion species were eluted within 12 min.

Table I—Chromatographic Characteristics of Amitriptyline Hydrochloride, Chlordiazepoxide, Their Impurities, and the Internal Standard

Compound[a]	Retention Time, min	Number of Theoretical Plates, N^b	Capacity Factor, k^c	Selectivity, α
I	3.0	580	0.6	
				1.9
II	4.5	660	1.1	
				1.6
III	5.5	780	1.8	
				1.4
IV	7.5	730	2.6	
				1.2
V	8.0	960	3.1	
				1.3
VI	10.0	1440	4.1	
				1.2
VII	11.0	1870	4.8	

[a] The calculations were made from the chromatogram in Fig. 1. [b] The number of plates was calculated from $N = 16\,(t_r/t_w)^2$. [c] The column void volume was 2.0 ml.

Table II—Comparison of HPLC and Spectrophotometric Tablet Assays

Sample	Amitriptyline Found, % HPLC	Amitriptyline Found, % Spectrophotometric	Chlordiazepoxide Found, % HPLC	Chlordiazepoxide Found, % Spectrophotometric
1	101.9	104.9	104.9	104.1
2	101.4	103.9	104.7	104.4
3	99.9	104.2	103.9	103.6
4	102.5	102.8	104.8	101.8
5	102.3	103.8	104.9	103.2
6	102.3	102.6	104.6	101.0
Average	101.7	103.7	104.6	103.0
RSD, %	0.96	0.84	0.36	1.3

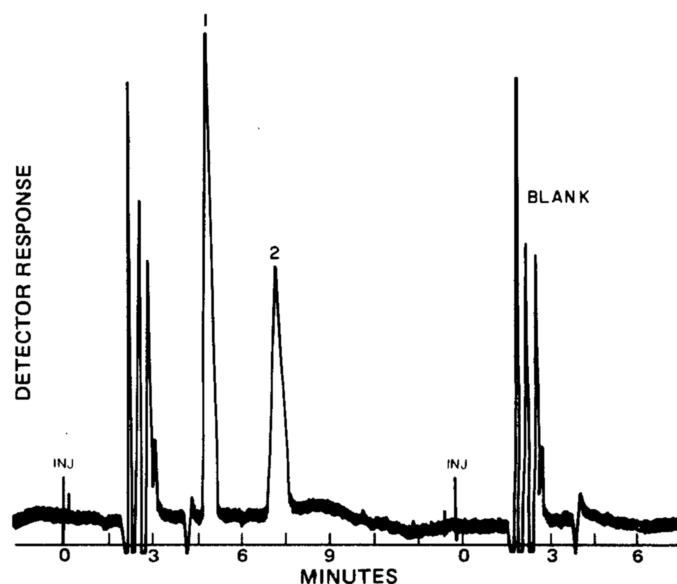

Figure 3—*Chromatogram of a tablet containing III (1) and V (2) after dissolution in simulated gastric fluid.*

Several tricyclic compounds and benzodiazepine derivatives were chromatographed in the selection of the internal standard necessary for the quantitation of amitriptyline hydrochloride and chlordiazepoxide, but a stable material with a suitable retention time was not found. Sulfanilamide eluted before amitriptyline hydrochloride and chlordiazepoxide and was well separated from their respective impurities, so it was chosen as the internal standard. Separation of the compounds including sulfanilamide is shown in Fig. 1, and the calculated chromatographic characteristics are listed in Table I.

Linearity—A plot of peak height *versus* the amount of the two components injected was linear between 0.3 and 11 μg of amitriptyline hydrochloride and 0.13 and 4 μg of chlordiazepoxide. The working concentration was ~5.7 μg of amitriptyline hydrochloride and 2 μg of chlordiazepoxide for the content uniformity test and assay. For dissolution measurements, the working concentration was 0.6 μg of amitriptyline hydrochloride and 0.2 μg of chlordiazepoxide.

Precision—Chromatographic system reproducibility was determined by injecting six 20-μl aliquots of a sample solution containing 0.1 mg of internal standard/ml, 0.3 mg of amitriptyline hydrochloride/ml, and 0.1 mg of chlordiazepoxide/ml as well as corresponding amounts of the reference standards. The relative standard deviation was ±0.16 and ±0.18% for amitriptyline hydrochloride and chlordiazepoxide, respectively. Six replicate samples of composite tablet mass also were assayed. The relative standard deviations were 0.96% for amitriptyline hydrochloride and 0.36% for chlordiazepoxide.

Recovery—A known amount of both active components was added to an assayed sample, and 97.7% of the amitriptyline hydrochloride and 100.2% of the chlordiazepoxide were recovered.

Figure 2 shows a typical chromatogram of the tablet assay. No interference due to a placebo was found. The completeness of the extraction of the tablet mass during sample preparation was checked; no active components remained in the tablet mass after filtering. Good agreement was obtained between the HPLC method and the spectrophotometric procedures, *i.e.*, measurements of the bromcresol green complex of amitriptyline hydrochloride and of the diazotization and coupling product of chlordiazepoxide (Table II).

For the dissolution test, external rather than internal standards were used since, at low concentrations, the sensitivity had to be increased and the blank interfered in the region of the internal standard. A typical chromatogram is shown in Fig. 3.

The described procedure subsequently was used for experimental tablet and capsule formulations containing only amitriptyline hydrochloride. The assay and content uniformity results correlated well with the data obtained using the pharmacopeial (5) procedures.

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Validation of Washing Procedures for Maintaining a Microbiologically Clean Gel Filtration Column

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Abstract □ Chromatographic gel filtration matrixes used in various separation techniques are subject to microbial contamination. The need for a microbe-free column is critical when preparing materials that require a low or zero microbial count. This report proposes two alternative washing systems: 0.02 *N* HCl containing 0.81% NaCl, and 0.1 *M* tromethamine–hydrochloride buffer (pH 7.0) containing 0.81% NaCl and 0.02% thimerosal. These washing systems were validated using a 100 × 2.6-cm column packed with a modified dextran gel slurry previously inoculated with known counts of USP test organisms. After each wash, the column separation characteristics were verified further with appropriate test proteins.

Keyphrases □ Chromatography—gel filtration, washing procedures for columns contaminated with microorganisms □ Contamination—microorganisms in gel filtration columns, washing procedures for column disinfection

Chromatographic column packing materials such as gel filtration and ion-exchange matrixes used in separation and the buffer systems usually employed with such matrixes are subject to microbial contamination and proliferation. Excessive microbial growth obstructs the flow, affects the chromatographic properties of these columns, and contaminates material that is affected adversely by microorganisms.

Gel filtration matrixes can be sterilized in the wet or dry state by autoclaving (1, 2), but such techniques are not feasible for a working column. Gaseous sterilization with formaldehyde or ethylene oxide is ineffective and unsafe due to the limited sterilant penetration and the residual toxic effects (2, 3). Organic solvents such as chloroform and toluene are incompatible with the matrixes and some column components. Several antimicrobial agents are compatible with the column packing matrixes. Thimerosal is one of the most commonly used preservatives because of its highly biostatic effect (2, 4). Its routine use as an applicable and effective antimicrobial agent in chromatographic columns has to be evaluated.

The present investigation was undertaken to develop effective methods to minimize or eliminate microbial contamination in a gel filtration column and to validate the antimicrobial effectiveness and applicability of these methods.

EXPERIMENTAL

Microbial Inoculum Suspension Preparation—The bacterial cultures, *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC

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