High-Performance Liquid Chromatographic Determination of Perphenazine 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 perphenazine and amitriptyline hydrochloride in two-component tablet formulations. An aliquot of a methanolic extract of the tablet, containing trifluoperazine hydrochloride as an internal standard, is chromatographed on a nitrile bonded phase microparticulate column using a 0.005 *M* ammonium acetate–methanol (20:80) mobile phase. Quantitation is by peak area. The relative standard deviations for the procedure are 0.34 and 0.54% for the simultaneous determination of perphenazine and amitriptyline, respectively. Eight commercial tablet formulations were analyzed and found to contain 96.5–101.5 and 96.5–103.3% of the labeled amounts of perphenazine and amitriptyline hydrochloride, respectively.

Keyphrases □ Perphenazine—high-performance liquid chromatographic analysis, pharmaceutical formulations containing amitriptyline hydrochloride □ Amitriptyline hydrochloride—high-performance liquid chromatographic analysis, pharmaceutical formulations containing perphenazine □ High-performance liquid chromatography—analyses, perphenazine and amitriptyline hydrochloride, two-component pharmaceutical formulations □ Tranquilizers—perphenazine, high-performance liquid chromatographic analysis, pharmaceutical formulations containing amitriptyline hydrochloride □ Antidepressants—amitriptyline hydrochloride, high-performance liquid chromatographic analysis, pharmaceutical formulations containing perphenazine

Perphenazine–amitriptyline hydrochloride two-component tablet formulations are produced by several manufacturers (1). To monitor the quality of these preparations, a rapid, accurate procedure, applicable to all formulations, was required for the simultaneous determination of both drugs. Conventional methods of analysis (2–6), such as the pharmacopeial procedures for the individual drugs (5, 6) are not suitable because of interference from the other components.

High-performance liquid chromatography (HPLC), however, is ideally suited to the analysis of mixtures (7–9) and has been employed extensively for the analysis of both phenothiazine (10–13) and dibenzcycloheptadiene (14, 15) drugs, including the quantitative determination of amitriptyline hydrochloride (15). This report describes an HPLC procedure for the simultaneous determination of perphenazine and amitriptyline hydrochloride in two-component tablet formulations.

EXPERIMENTAL

Materials—Amitriptyline hydrochloride USP[1] and perphenazine NF[2] were used as received. Both drug substances were compared chromatographically (HPLC) for retention time, impurities, and response factor to appropriate reference standards (NF and USP) and were assayed by nonaqueous titration procedures (5, 6). Both drug substances were essentially identical to their respective reference standards.

Trifluoperazine hydrochloride[3] was used as received. Solvents and reagents were commercial analytical reagent grade. Water was double distilled in an all-glass still and filtered through a 0.2-μm filter[4] with the aid of suction.

Apparatus—A liquid chromatograph[5], fitted with a septumless injection port[5], a fixed wavelength UV detector[5] (254 nm), and a computing integrator[6], was used. The detector was attenuated to 0.08 absorbance unit full scale (aufs), and the integrator output was attenuated ×4 throughout.

Column—A 25 × 0.216-cm i.d. column containing a nitrile bonded phase packing[7] was used at ambient temperature and at a mobile phase flow rate of 80 ml/hr (150 bar).

Mobile Phase—Methanol–0.005 *M* ammonium acetate in double-distilled water (80:20) was prepared as required, degassed (refluxed for 5 min), and then stored in the solvent reservoir of the instrument.

Internal Standard Solution—A solution of trifluoperazine hydrochloride in methanol (0.25 mg/ml) was used.

Standard Curves—Methanolic stock solutions of perphenazine (1.0 mg/ml), amitriptyline hydrochloride (5.0 mg/ml), and trifluoperazine hydrochloride (1.25 mg/ml) were prepared. Standard solutions were prepared by pipetting 5.0 ml of trifluoperazine stock solution into each of seven 25-ml volumetric flasks along with 10.0, 8.0, 6.0, 5.0, 4.0, 2.0, or 1.0 ml of both perphenazine and amitriptyline stock solutions. Each solution was diluted to volume with methanol, if necessary, and duplicate 5-μl aliquots of each standard solution were chromatographed.

Calibration Standard Solutions—Approximately 3 mg of perphenazine and 15 mg of amitriptyline hydrochloride, accurately weighed, were transferred to a 20 × 150-mm screw-capped culture tube[8] and dissolved in 25.0 ml of internal standard solution. Duplicate 5-μl aliquots of the resulting solution were chromatographed periodically to check the slopes of the calibration curves.

Analysis of Pharmaceuticals—*Single-Tablet Assay*—A tablet was placed in a 20 × 150-mm screw-capped culture tube and crushed to a fine powder with a glass rod. Internal standard solution, 25.0 ml, was added to the tube, which was then closed, tumbled on a rotator[9] at 30 rpm for 15 min, and centrifuged[10] at 2000 rpm for 5 min. Duplicate 5-μl aliquots of the supernate were chromatographed.

Tablet Composite Assay—A tablet composite was prepared by grinding 20 tablets in a mechanical mill[11]. A quantity of powdered tablet material equivalent to one tablet was transferred to a 20 × 150-mm screw-capped culture tube. The sample was treated as described previously.

Quantitation—Quantitation was by peak area ratio of the sample to the internal standard.

RESULTS AND DISCUSSION

Evaluation of Chromatographic Systems—Table I lists retention times (R_T), column efficiency (N), capacity factors (k'), selectivity (α), and resolution (R) data for perphenazine and amitriptyline hydrochloride on three bonded phase columns. Perphenazine eluted behind amitriptyline on the octadecylsilane bonded phase column[12] and ahead on the fluoroether bonded phase column[13]. The early elution of perphenazine is desirable, since it is the minor component in perphenazine–amitriptyline formulations. The fluoroether column was not as efficient and did not provide as high resolution as the nitrile bonded phase

[1] Merck Sharp and Dohme, Kirkland, Quebec, Canada.
[2] Schering, Pointe-Claire, Quebec, Canada.
[3] Smith Kline and French, Montreal, Canada.
[4] FGLP04700, Millipore Ltd., Mississauga, Ontario, Canada.
[5] Model 4100, Varian Aerograph, Palo Alto, Calif.
[6] Autolab System I, Spectra-Physics, Santa Clara, Calif.
[7] Micropak-CN, Varian Aerograph, Palo Alto, Calif.
[8] Canadian Laboratory Supplies, Montreal, Canada.
[9] Multi-Purpose rotator, Scientific Industries, Springfield, Mass.
[10] Model K, International Equipment Co., Needham Heights, Mass.
[11] Micro Mill, Chemical Rubber Co., Cleveland, Ohio.
[12] Prepared by bonding octadecyltrichlorosilane (Aldrich) to dried LiChrosorb SI-60 (Merck) under anhydrous conditions.
[13] FE Sil-X-I, Perkin-Elmer Corp., Norwalk, Conn.

Table I—Comparison of Chromatographic Parameters for Perphenazine and Amitriptyline Hydrochloride on Three Columns

Column	Retention Time, R_T, min	Capacity Factor, k'	Number of Theoretical Plates, N	Selectivity, α	Resolution, R	Compound
A[a]	4.2	3.2	560	—	—	Perphenazine
	3.0	2.0	800	1.6	2.2	Amitriptyline
B[b]	3.5	3.7	175	—	—	Perphenazine
	4.9	5.5	340	1.5	1.5	Amitriptyline
C[c]	1.3	1.5	490	—	—	Perphenazine
	4.2	8.0	820	5.3	6.0	Amitriptyline

[a] Octadecyltrichlorosilane reacted with dry 10-μm silica under anhydrous conditions. The mobile phase was 2 drops of acetic acid–30% 0.1 *M* ammonium acetate–70% acetonitrile. The column was 250 × 2.2 mm i.d., and the flow rate was 60 ml/hr. [b] Commercial fluoroether bonded phase. The column was 250 × 2.2 mm i.d., and the flow rate was 60 ml/hr. The mobile phase was the same as for Column A, except that 50% acetonitrile was used. [c] Commercial nitrile bonded phase. The column was 250 × 2.2 mm i.d., and the flow rate was 80 ml/hr. The mobile phase was 20% 0.005 *M* ammonium acetate–80% methanol.

column[7], which also allowed perphenazine to elute first; accordingly, the nitrile column was chosen.

While searching for a suitable internal standard, various related phenothiazines, dibenzcycloheptadienes, and several dibenzazepines were chromatographed on the nitrile column (Table II). Trifluoperazine hydrochloride eluted between the two compounds of interest and was chosen.

Figure 1 shows a chromatogram obtained when a representative tablet formulation was analyzed by the described procedure. The internal standard and the two compounds of interest were well resolved in less than 6 min.

Linearity and Standard Curves—A plot of peak area *versus* the amount of trifluoperazine hydrochloride injected was linear below about 3 μg/injection (0.6 mg/ml). A level of 0.25 mg/ml was used in the analytical procedure.

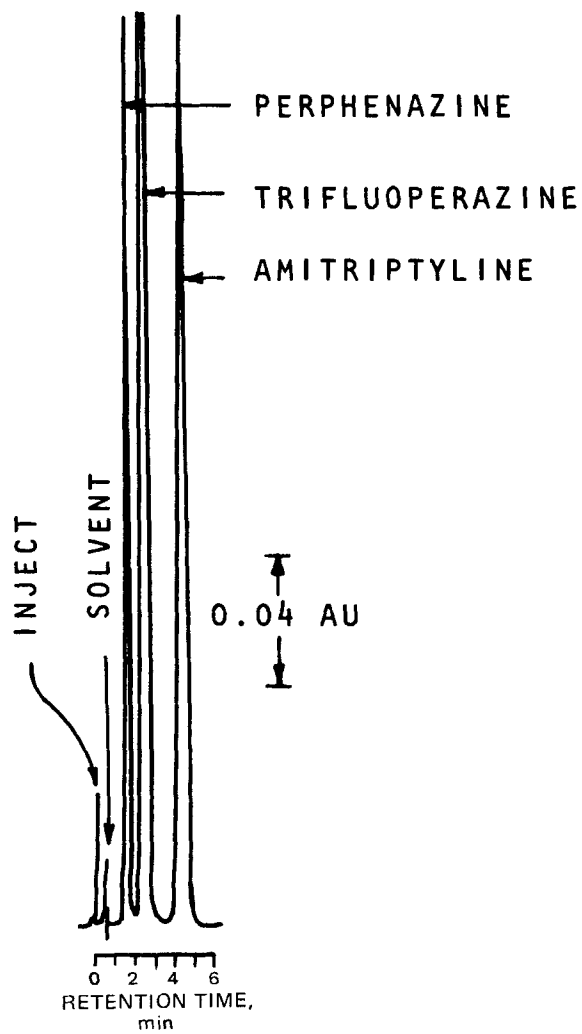

Figure 1—*High-performance liquid chromatogram of an extract of a perphenazine–amitriptyline hydrochloride tablet (4 and 25 mg, respectively).*

Standard curves for perphenazine and amitriptyline hydrochloride were constructed by plotting sample to internal standard peak area ratios *versus* sample to internal standard weight ratios.

The standard curve for perphenazine was linear with a negligible intercept to >1.25 μg/injection when examined over the range of 0.2–2.0 μg/injection (40–400 μg/ml). The mean slope of the linear portion of the line was 1.37 with a relative standard deviation of 1.4%. Similarly, the standard curve for amitriptyline hydrochloride was linear with a negligible intercept to >8 μg/injection when examined over the range of 1.0–10.0 μg/injection (0.2–2.0 mg/ml). The mean slope of the linear portion of the line was 0.42 with a relative standard deviation of 1.8%. Therefore, the standard curves are applicable to tablets containing from 1 to 6 mg of perphenazine and from 5 to 40 mg of amitriptyline hydrochloride using the described procedure.

Duplicate injections of replicate calibration standard solutions were chromatographed periodically to determine the slopes of the standard curves. When using a single calibration standard, stored at 4° between uses, the relative standard deviations for the slopes of the perphenazine and amitriptyline hydrochloride standard curves over 4 weeks were ±1.1 and ±0.7%, respectively.

Sample Preparation—Various extraction solvents and conditions were tried. When water, water–methanol, or water–acetonitrile was used as the extraction solvent, all tablets disintegrated rapidly, but a fine suspension did not completely settle when centrifuged for 10 min at 2000 rpm. The use of methanol as the extraction solvent did not result in a suspension but also would not disintegrate coated tablets. Crushing the tablets before extraction allowed rapid extraction of both drugs with methanol.

The time for constant extraction of perphenazine and amitriptyline hydrochloride from the tablet mass was determined by tumbling crushed

Table II—Retention Times of Various Phenothiazine, Dibenzcycloheptadiene, and Dibenzazepine Drugs

Compound	Retention Time[a], min
Phenothiazines	
Perphenazine	1.4
Trifluoperazine hydrochloride	2.2
Phenothiazine	0.5[b]
2-(Trifluoromethyl)phenothiazine	0.5[b]
10-(3-Chloropropyl)-2-(trifluoromethyl)-phenothiazine	0.5[b]
Isopromethazine hydrochloride	2.6
Promethazine hydrochloride	3.2
Promazine hydrochloride	5.4
Chlorpromazine hydrochloride	4.0
Trifluopromazine hydrochloride	3.3
Methotrimeprazine hydrochloride	3.7
Thioproperazine	3.0
Prochlorperazine maleate	2.9
Dibenzcycloheptadienes	
Amitriptyline hydrochloride	4.0
Nortriptyline hydrochloride	4.1
Protriptyline hydrochloride	4.3
Dibenzocycloheptanone	0.5[b]
Dibenzazepines	
Trimipramine maleate	3.7
Imipramine hydrochloride	5.0
Desipramine hydrochloride	4.6

[a] The column was 250 × 2.2-mm i.d. Micropak-CN, the flow rate was 80 ml/hr, and the mobile phase was 0.005 *M* ammonium acetate–methanol (20:80). [b] Solvent front (t_0).

Table III—Analysis of Synthetic Solutions

Sample	Amitriptyline Hydrochloride			Perphenazine		
	Calculated, mg/ml	Found, mg/ml	Recovery, %	Calculated, mg/ml	Found, mg/ml	Recovery, %
1	1.013	1.033	102.0	0.0962	0.0966	100.4
2	0.648	0.655	101.0	0.144	0.143	99.3
3	0.432	0.430	99.4	0.192	0.190	99.0
4	1.048	1.035	98.8	0.195	0.195	100.0
5	0.311	0.311	100.0	0.0962	0.0956	99.4
Mean recovery, %			100.2			99.6
RSD, %			1.2			0.6

single tablets of a representative formulation for 5, 10, and 15 min in 25 ml of the internal standard solution. In each case, the sample was centrifuged briefly after each 5-min period and a 5-μl aliquot was chromatographed. The peak area ratios were identical at all three times, indicating constant extraction of both compounds in less than 5 min. Extraction was approximately complete based on response factor data and the label claims for the formulation used; however, a dilution procedure was used to prove this finding. When tablet composite samples equivalent to the weights of one-half, one, and one and one-half tablets were analyzed by the described procedure, using a 15-min tumbling time, the concentrations of perphenazine and amitriptyline hydrochloride were in the ratio 1:2:3. These data showed that constant and complete extraction of the compounds of interest was effected in less than 15 min under the conditions described.

Quantitation—Quantitation was by peak area. The reproducibility of the chromatographic system was shown by chromatographing 12 5-μl aliquots of a calibration standard solution containing 1.04 mg of amitriptyline hydrochloride/ml, 0.096 mg of perphenazine/ml, and 0.55 mg of trifluoperazine hydrochloride/ml. The relative standard deviations of the peak area ratios were ±0.29 and ±0.74% for perphenazine and amitriptyline hydrochloride, respectively.

Five solutions containing perphenazine and amitriptyline hydrochloride, prepared in the same manner as the calibration standard solutions, were analyzed using the described procedure. Table III shows that the mean recoveries for the two compounds were 99.6 and 100.2%, respectively.

Table IV—Analysis of Perphenazine–Amitriptyline Hydrochloride Tablet Formulations [a] by HPLC

Formulation	Perphenazine		Amitriptyline Hydrochloride	
	Label, mg	Found[b], %	Label, mg	Found, %
A	3	97.9	15	96.9
B	4	96.8	25	99.5
C	2	96.5	25	98.9
D	2	101.5	25	103.3
E	4	96.8	25	96.5
F	2	99.9	25	102.0
G	4	100.0	10	102.2
H	2	98.9	25	100.4

[a] Tablet composite assay. [b] Percent of label claim.

Seven replicate samples of a formulation composite, prepared from tablets labeled to contain 4 mg of perphenazine and 25 mg of amitriptyline hydrochloride, were also assayed. The relative standard deviations of the peak ratios were 0.34% for perphenazine and 0.54% for amitriptyline hydrochloride.

Eight commercial formulations of perphenazine–amitriptyline hydrochloride tablets were analyzed in duplicate. Table IV shows that the levels of both drugs varied between 96.5 and 103.3% of the label claim amount.

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