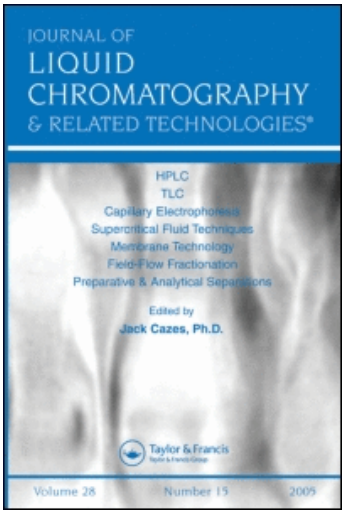

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SIMULTANEOUS HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF AMITRIPTYLINE HYDROCHLORIDE AND PERPHENAZINE IN TABLET FORMULATIONS

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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 perphenazine in two component tablet formulations. The related compounds of amitriptyline hydrochloride were separated, making the determination specific for amitriptyline hydrochloride and perphenazine. The method was used for the assay and content uniformity for three commercial products. The mobile phase was 0.02 M ammonium acetate in acetonitrile: methanol: water (45:15:40) solution and the pH was adjusted to 5.0 by acetic acid. The column was a supelcosil (5 μm) LC-8-DB (250 mm x 4.6 mm i.d). The method was tested for linearity, recovery, and specificity.

INTRODUCTION

The combination of amitriptyline hydrochloride (I) (antidepressent) and perphenazine (II)(a tranquilizing drug) is recommended

for the treatment of patients with agitation and/or anxiety (whether moderate or severe) and depressed mood.

The conventional methods of analysis, such as the pharmacopeial procedures for the individual drugs (1,2) are not suitable because of interferences from the other components. Traditional procedures, such as the spectrophotometric determination of the bromocresol green complex of amitriptyline hydrochloride are tedious, time-consuming, and lack specificity.

Numerous LC reports for the determination of I and II in drugs (3-6), and in body fluids (7-8) have been reported. However, none of the methods is applicable to the simultaneous determination of these compounds in tablet formulations.

This paper presents an HPLC method for the quantitative determination of both drugs in two-component tablet formulations. The analysis can be performed in a reasonable time and eliminates interference due to possible impurities of I: 10, 11-dihydro-5-{3-(dimethyl amino)propyl} -5H-dibenzo{a,d} cyclohepten-5-ol (III) and 10,11-dihydro-5H-dibenzo{a,d}cyclohepten-5-one (IV).

EXPERIMENTAL

Apparatus

The apparatus employed was a Varian 2010 pump (Varian Associates, Inc. Palo Alto, CA, U.S.A.) equipped with a 10-µL loop injector 7125 (Rheodyne, Cotati, CA , U.S.A.) connected to a Varian 2050 spectrophotometric detector and a Varian 4290 integrator. A reverse phase column (250 mm x 4.6 mm i.d.) Supelcosil LC-8-DB (5 µm) (Supelco, Inc. Pennsylvania, U.S.A.) was used at ambient temperature.

Materials

The reference standards I and II were obtained from the USP (Rockville, MD, U.S.A.). The related compounds, III and IV, were kindly supplied by F. Hoffmann-La Roche & Co. (Nutley, NJ, U.S.A.). The internal standard, Naphthalene (99%), from E. Merck (Darmstadt W.G.). Acetonitrile-HPLC, methanol-HPLC, and acetic acid (96%) were from May & Baker Ltd (Dagenham, U.K.), Gainland Chemical Company (Clwyd, U.K.), and Merck, respectively. Ammonium acetate, purum grade, was obtained from Fluka A.G. (Switzerland). Water was always distilled and deionized.

Excipients usually used in the tablet formulations:lactose, calcium dihydrogen phosphate, starch, gelatin , magnesium stearate, acacia , talc, calcium carbonate, magnesium carbonate, sucrose, titanium oxide, povidone,microcrystalline cellulose , methyl paraben, and coloring agents were supplied by Al-Hikma Pharmaceuticals, Amman-Jordan. Commercial tablets were purchased locally.

Chromatographic Conditions

The mobile phase consists of 2.0 mM ammonium acetate in acetonitrile: methanol: water (45:15:40) solution; the pH was adjusted to 5.0 with acetic acid. The mobile phase was always filtered using 0.45 μm-membrane filters (Supelco, Inc.) and degassed by vacuum prior to use. The flow rate was 1.5 mL/min. The wavelength was 254 nm and the sensitivity was set at 0.2 AUFS. The chart speed was 0.25 cm/min.

Study of The Interferences of Placebo Excipients - A mixture of the excipients was dissolved and treated in the same manner as the

sample solution. Ten-µL injections were made under the chromatographic conditions described.

Preparation of The Standard Solutions:

Internal Standard Solution - The internal standard solution was prepared by dissolving 100 mg of naphthalene in 1.0 L of methanol.

Standard Solutions For Linearity - Standard solutions of I in the internal standard solution containing 262.5, 210.0, 175.0, 140.0, 105.0, and 87.5 µg/mL and standard solutions of II in the internal standard solution containing 21.0, 17.5, 14.0, 11.2, 9.1, and 7.0 µg/mL were prepared.

Standard Solutions of I and II - Twenty five mg of I and 2.0 mg of II were dissolved in 25.0 mL of the internal standard solution. This was further diluted with the internal standard solution to obtain a final concentration of 175 and 14 µg/mL of I and II, respectively.

Preparation of The Sample Solution:

Twenty tablets (one tablet if content uniformity was to be determined) were weighed and powdered. Accurately weighed portions of the powder (equivalent to the weight of one tablet) were placed in 25 mL-volumetric flasks. Each sample was sonicated for three minutes with 20 mL of the internal standard solution, then completed to volume with the internal standard solution. Samples (Mutabon-D and Minitran tablets) were further diluted with the internal standard solution to obtain a concentration of 175 and 14 µg/mL of I and II, respectively. For Mutabon-A tablets, the final concentrations were 160 and 16 µg/mL of I and II. The solutions were filtered through 0.45 µm-membrane filters.

Percent Recovery Study - The study was performed by preparing synthetic mixtures identical to the pharmaceutical formulations and were spiked with known amounts of I (12.5, 15.0, 20.0, 25.0, 30.0 and 37.5 mg) and II (1.0, 1.3, 1.6, 2.0, 2.5, and 3.0 mg) spanning the range of 50-150% of the expected assay values. The resulting mixtures were assayed and the results obtained were compared with the expected ones.

Assay Method - Equal volumes (10-µL) and and approximately equal concentrations of the standard and sample solutions were injected into the HPLC and chromatographed under the conditions described above. The standard and the sample solutions contained the same concentration of the internal standard. The quantity of each component injected was always within the linearity range.

Calculations - The results were calculated using response ratios (RR) relative to internal standard based on peak areas:

$$\text{Percent of the label claim found} = \frac{C_s}{C_l} \times \frac{RR_x}{RR_s} \times 100$$

where RR_x = sample response ratio; RR_s = standard response ratio; C_s = standard concentration; C_l = label claim concentration.

RESULTS AND DISCUSSION

Under the chromatographic conditions chosen for this method, the chromatographic peaks of I, II, III, and IV were sharp and well resolved from the internal standard. Figure 1 is a representative chromatogram which illustrates the specificity of the method.

To determine the linearity of the detector response, a plot of peak area versus the amount injected was linear between 2.6 and 0.9 µg of I and 70.0 and 210.0 ng of II with a correlation coefficient of

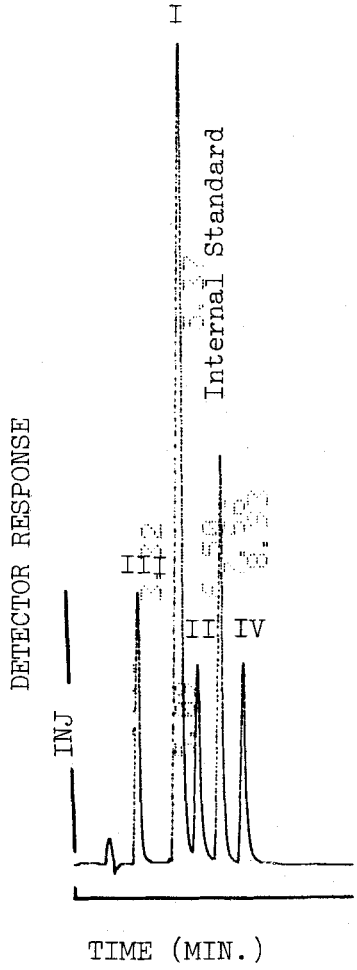

Figure 1. A typical chromatogram of a 10-μL injection of a synthetic mixture of III (t_R = 3.32 min.), I (t_R = 5.37 min.), II (t_R = 6.50 min), naphthalene (internal standard) (t_R = 7.66 min.), and IV (t_R = 8.93 min.).

0.999 for each compound. The working concentrations were 1.4 - 1.8 µg for I and 140.0 - 160.0 ng for II.

To determine the accuracy of the method, each standard was spiked with a placebo and subjected to HPLC analysis. In all cases, satifactory recoveries and reproducibility of peak areas were obtained. A linear regression of the data shows excellent correlation over the analysis range studied (Table I). No iterferences due to excipients were detected in the chromatograms produced, which validates the selectivity of the method. The detection limit based on signal-to-noise ratio of 2 was less than 1.0 ng for I and II as determined by diluting a standard solution with methanol and injecting 10-µL into the column.

The results of analysis of three commercial products (Table II, Figures 2-4) indicate that the proposed assay can be used for the qunatitation of I and II in commercial tablets. The accuracy of the method was supported by the closeness of the results to the label claim. The precision of the HPLC method is supported by the very small relative standard deviation (RSD) based on 3x6 readings.

The specificity of the method is further confirmed by the results of content uniformity (Table III) which show compliance to specifications of all dosage forms.

A stability study was performed on synthetic tablets mixture of I and II by placing samples in water-glycerin bath at 60°C for

TABLE I

Recovery Study Of Amitriptyline Hydrochloride And Perphenazine From Spiked Placebo Samples.

Amitriptyline HCl			Perphenazine		
mg Added/Tablet	mg Found/Tablet[a]	%Recovery[a]	mg Added/Tablet	mg Found/Tablet[a]	% Recovery[a]
12.50	12.58 ± 1.61	100.36 ± 1.61	1.00	1.00 ± 1.61	100.36 ± 1.61
15.00	14.99 ± 0.86	99.96 ± 0.86	1.30	1.31 ± 0.38	100.71 ± 0.37
20.00	20.34 ± 0.34	101.69 ± 0.35	1.60	1.61 ± 0.78	100.72 ± 0.78
25.00	25.21 ± 0.74	100.83 ± 0.74	2.00	2.04 ± 0.74	102.20 ± 0.74
30.00	30.20 ± 0.67	100.61 ± 0.67	2.50	2.50 ± 0.80	99.98 ± 0.80
37.50	37.24 ± 0.67	99.31 ± 0.67	3.00	2.99 ± 1.02	99.61 ± 1.03
R	0.9998			0.9999	
Slope	0.9912			0.9912	
Intercept	0.2983			0.0195	

[a]Mean ± RSD for 6 determinations

TABLE II

HPLC Assay Results of Three Commercial Products

Product	Label Claim (mg/Tablet)		Percent Label Claim Found ± RSD[a]	
	I	II	I	II
Mutabon-D[b]	25.00	2.00	100.69 ± 0.95	99.41 ± 1.17
			98.76 ± 1.19	98.30 ± 1.21
			97.98 ± 1.40	97.60 ± 1.12
Mutabon-A[c]	10.00	4.00	99.58 ± 0.82	100.00 ± 0.52
			100.38 ± 0.49	101.40 ± 0.28
			100.57 ± 1.12	99.38 ± 0.86
Minitran[d]	25.00	2.00	100.15 ± 0.81	100.19 ± 0.30
			100.40 ± 0.59	99.48 ± 0.39
			99.24 ± 0.78	101.67 ± 1.34

[a]Mean ± RSD for 6 determinations

[b]Lot CA 4 ANCG 2 (Schering Corporation, N.J., U.S.A.)

[c]Lot CA 4 ANBA 1 (Schering Corporation, N.J. ,U.S.A.)

[d]Lot 986 (Chromatourgia Athinon-Colocotronis Bros, Moschato, Athens).

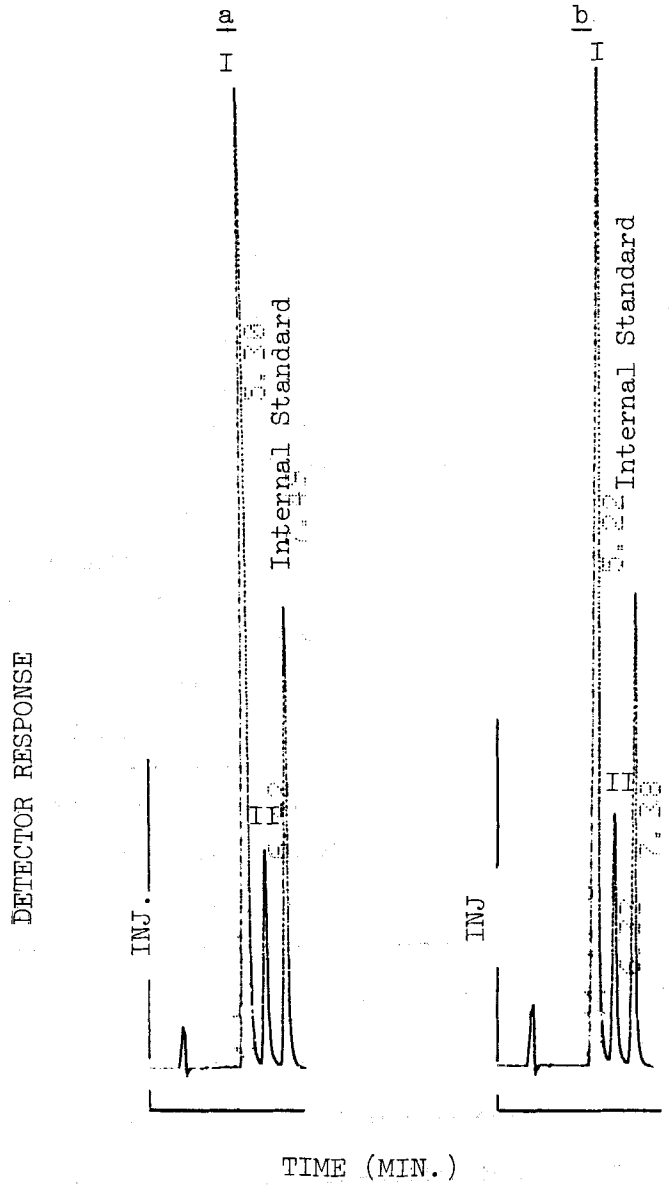

Figure 2. a. A typical chromatogram of a 10-µL injection of a standard solution containing 1.75 µg of I, 0.14 µg of II, and 1.0 µg of the internal standard.

b. A chromatogram for a 10-µL injection of a solution made equivalent to one tablet Mutabon-D (the assay method procedure) containing 1.75 µg of I, 0.14 µg of II, and 1.0 µg of naphthalene.

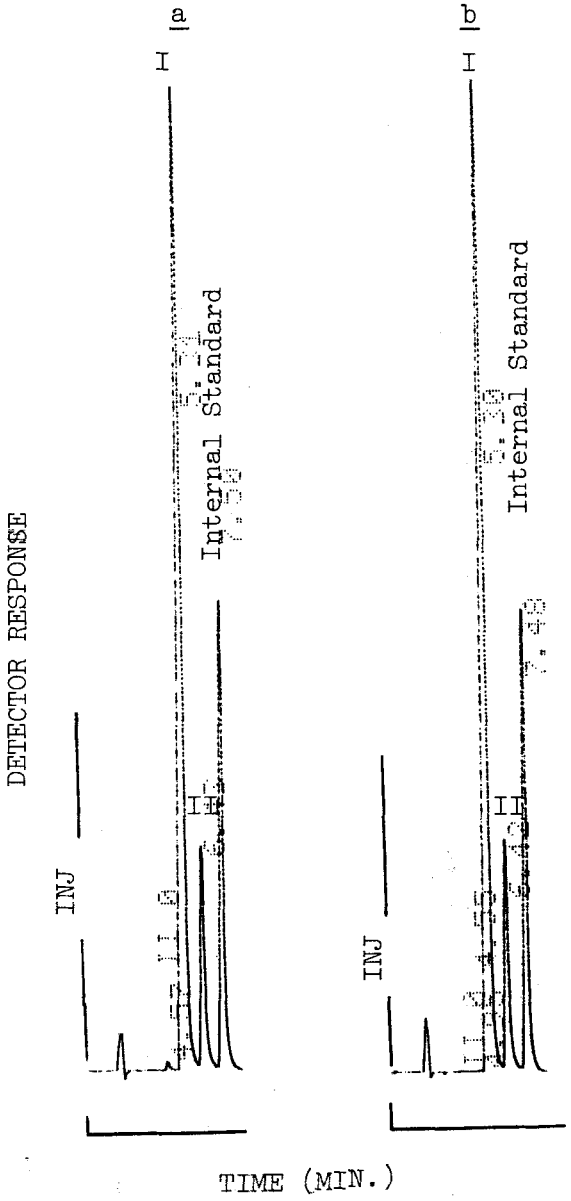

Figure 3. a. a typical chromatogram for a 10-μL injection of a standard solution containing 1.75 μg of I, 0.14 μg of II, and 1.0 μg of naphthalene.

b. A chromatogram for a 10-μL injection of a sample (Minitran) containing 1.75 μg of I, 0.14 μg of II, and 1.0 μg of naphthalene.

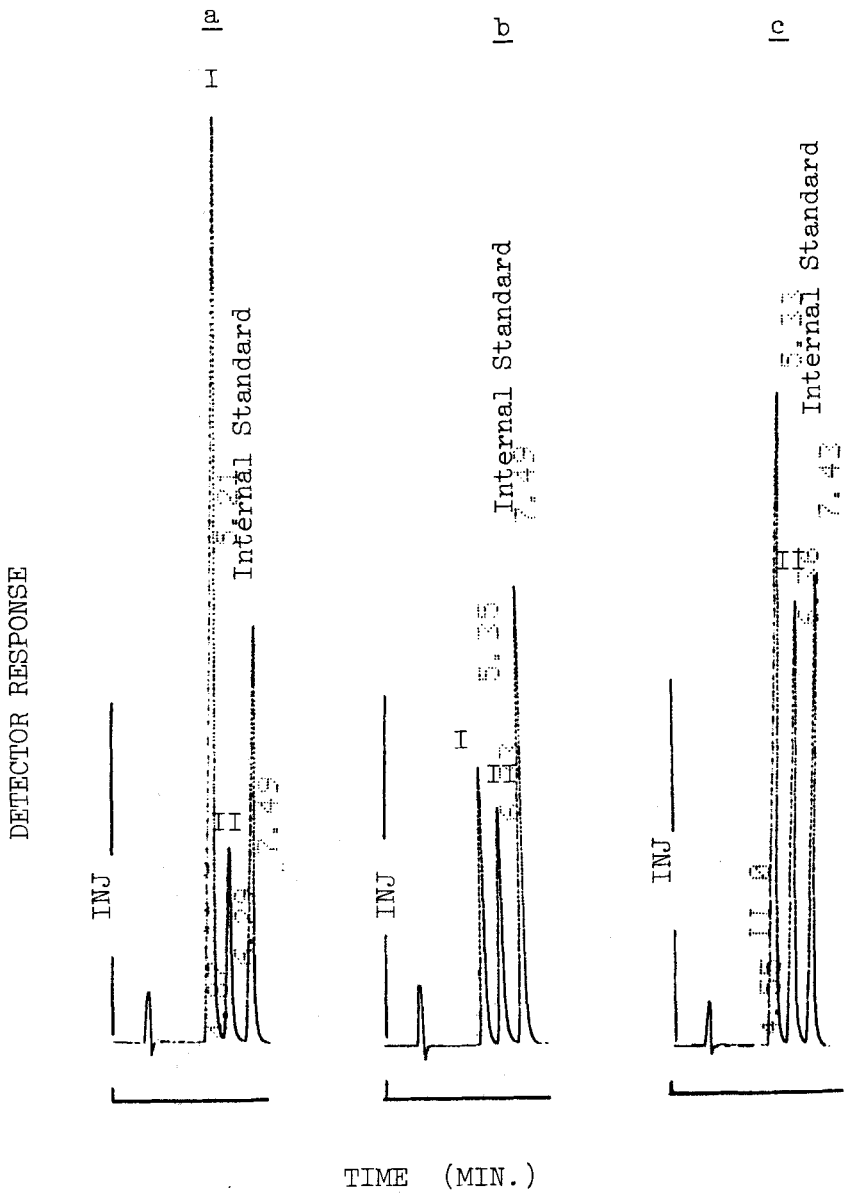

Figure 4. a. A typical chromatogram for a 10-µL injection of a standard solution containing 1.75 µg of I, 0.14 µg of II, and 1.0 µg of naphthalene.

b. A chromatogram for a 10-µL injection of a sample of Mutabon-A, for the analysis of II, containing 0.4 µg of I, 0.16 µg of II, and 1.0 µg Naphthalene.

c. A chromatogram for a 10-µL injection of a sample of Mutabon-A, for the analysis of I, containing 1.6 µg of I, 0.64 µg of II, and 1.0 µg naphthalene.

TABLE III

Content Uniformity For Amitriptyline Hydrochloride And Perphenazine In Commercial Tablets.

	Percent Label Claim Found					
	Mutabon-D		Mutabon-A		Minitran	
Tablet No.	I	II	I	II	I	II
1	104.70	101.11	103.21	101.15	99.22	92.30
2	101.70	103.56	102.35	103.20	102.38	103.96
3	100.47	102.07	98.87	98.15	103.67	105.16
4	98.06	96.98	100.32	98.14	100.05	100.56
5	102.46	101.19	98.78	97.77	99.14	101.43
6	100.60	96.75	104.75	102.04	104.16	105.79
7	99.34	96.19	101.84	101.12	100.87	102.38
8	99.41	98.89	102.87	100.74	102.46	103.33
9	99.83	102.11	103.28	103.68	103.92	102.54
10	98.21	97.70	106.98	105.65	103.33	101.59
Mean	100.48	99.66	102.34	101.16	101.92	101.90
RSD	2.02	2.67	2.49	2.58	1.91	3.69
High	104.70	103.56	106.98	105.65	104.16	105.79
Low	98.06	96.19	98.78	97.77	99.22	92.30

one month. In the chromatograms produced I and II were stable for the study period without any measurable degradation.

In conclusion, the HPLC assay described here has been shown to be of general applicability to commercially available products. The method is accurate, precise, rapid, and easy to perform. It can be easily applied for the determination of the related compounds of I.

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