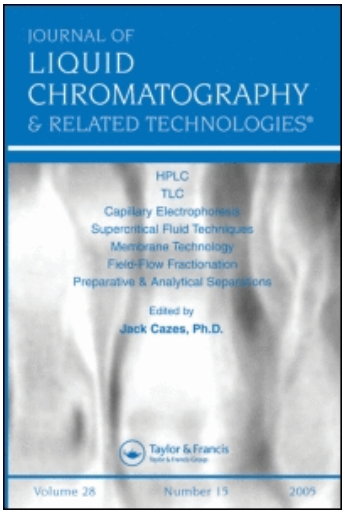

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A SIMULTANEOUS ANALYSIS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF BAMIPINE COMBINED WITH TRICYCLIC ANTIDEPRESSANTS AND/OR ANTIPSYCHOTICS IN DOSAGE FORMS

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ABSTRACT

A reversed phase high-performance liquid chromatographic method is described for the simultaneous determination of antihistamines, tricyclic antidepressants and antipsychotics in pharmaceutical formulations and in spiked placebos. The separation was performed on an octadecyl-silica column using acetonitrile: tetrahydrofuran : 0.015 M aqueous ammonium acetate (53:42 : 5) as mobile phase. The presence of ammonium acetate both shortens the elution time and improves the symmetry of the chromatographic peaks. Measurements were made at 251 nm.

INTRODUCTION

Antihistamines are one of the most widely used group of drugs. They are formulated as single entities or combined with other drugs with different pharmacological action. Bamipine is an antihistamine and also possesses mild sedative properties and is mostly

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administered in tablet form. It can also be combined with tricyclic antidepressants or antipsycotics since it is claimed that this produces better therapeutic results in the treatment of depression.[1]

Although a considerable number of papers refer to the assay of bamipine, [2-4], tricyclic antidepressants [5-11] and antipsycotics [12-14] by using HPLC, no method for the simultaneous quantitation of the above drugs appears to be available. In the proposed HPLC method more than three different drugs can be determined simultaneously.

EXPERIMENTAL

Apparatus: A Perkin Elmer Series 3B high performance liquid chromatograph equipped with two reciprocating pumps controlled by a microcomputer, a Reodyne 7010 20 μl loop injector valve and a LC 75 UV spectrophotometric detector with a single-beam variable wavelenhth system was used. The spectrophotometer was operated at 0.04 absorbance units full scale(a.u.f.s.)(1cm path length).The use of a higher sensitivity was unnecessary for these determinations. The spectrophotometer was insensitive to flow noise and to changes in the refractive index of the solvents. The chromatographic peaks were recorded by employing a LKB 2210 Bromma potentiometetric recorder connected to the spectrophotometer, with an operating voltage of 10mV and chart speed of 2 mm/min. A 250 X 2.1 mm I.D. stainless-steel column containing Bondapak, C_{18}, 10 μ, was used. A flow rate of 1ml/min eluted the analysed compounds in the range from 3.10′ to 9.35′ as illustrated in Table 1. The wavelength was set at 251 nm.

Materials: Analytical grade (Ferak Berlin, F.R.G.) acetonitrile and tetrahydrofuran were used. Ammonium acetate was zur Analyse, Merck. The water was purified by a Millipore filtration unit (deionized $< 10\ \mu\Omega$).

The following reference standards were purchased from Sigma Chemical Company: chlorprothixene, haloperidol, trimipramine maleate, trimeprazine tartrate, thioridazine hydrochloride, trifluoperazine dihydrochloride, promethazine hydrochloride, prochlorperazine edisylate, imipramine hydrochloride. Bamipine hydrochloride was kindly donated by Knoll (Ludwigshafen, W. Germany).

Mobile Phase: The mobile phase consisted of acetonitrile: tetrahydrofuran:0.015 M aqueous ammonium acetate 53:42:5. It was degassed by vacuum filtration through a 0.2-μm Sartorius S 11807 polytetrafluoroethylene membrance filter while the container (flask) was in an ultrasonic bath.

System Suitability: The column was equilibrated with mobile phase at a flow rate of 1ml/min. After a stable line was achieved, the standard and the sample solutions were injected into the column. The peaks appeared over the increased retention time. The

TABLE 1

High Performance Liquid Chromatographic characteristics of the Drug Separation

Compound	Retention Time/min (t_R)	Capacity Factor (k′)	Resolution (R_s)
Bamipine Hydrochloride	5.50′	1.80	–
Promethazine Hydrochloride	4.20′	1.08	1.50
Trimeprazine Tartrate	3.50′	0.84	2.18
Imipramine Hydrochloride	7.50′	2.75	2.00
Trimipramine Maleate	3.50′	0.84	2.18
Haloperidol	3.10′	0.52	2.90
Chlorprothixene	4.20′	1.08	1.60
Prochlorperazine Edisylate	9.35′	3.60	2.80
Trifluoperazine Hydrochloride	8.40′	3.16	2.60
Thioridazine	8.00′	2.84	2.00

TABLE 2

Concentration ranges and " linear regression and correlation data" of calibration curves for the compounds determined at 251 nm

Compound	Concentration Range (μg/ml)	Peak Heights (mm)	Intercept	Slope	r
Bamipine Hydrochloride	1.44 - 8.68	29 - 182	- 0.71	21.12	0.9999
Promethazine Hydrochloride	0.63 - 3.76	27 - 156	1.68	41.10	0.9999
Trimeprazine Tartrate	0.80 - 2.14	49 - 136	- 2.33	63.03	0.9975
Imipramine Hydrochloride	4.92 -17.20	42 - 145	1.31	8.36	0.9999
Trimipramine Maleate	3.65-12.77	55 - 185	2.49	14.19	0.9998
Haloperidol	5.98 -20.95	45 - 156	0.66	7.43	0.9999
Chlorprothixene	1.59 - 9.56	23 - 135	0.98	14.08	0.9999
Prochlorperazine Edisylate	4.79-16.77	39 - 138	- 0.64	8.25	0.9998
Trifluoperazine Hydrochloride	4.92-17.24	44 - 142	2.05	8.01	0.9990
Thioridazine Hydrochloride	4.37-11.65	31 - 82	0.09	7.04	0.9999

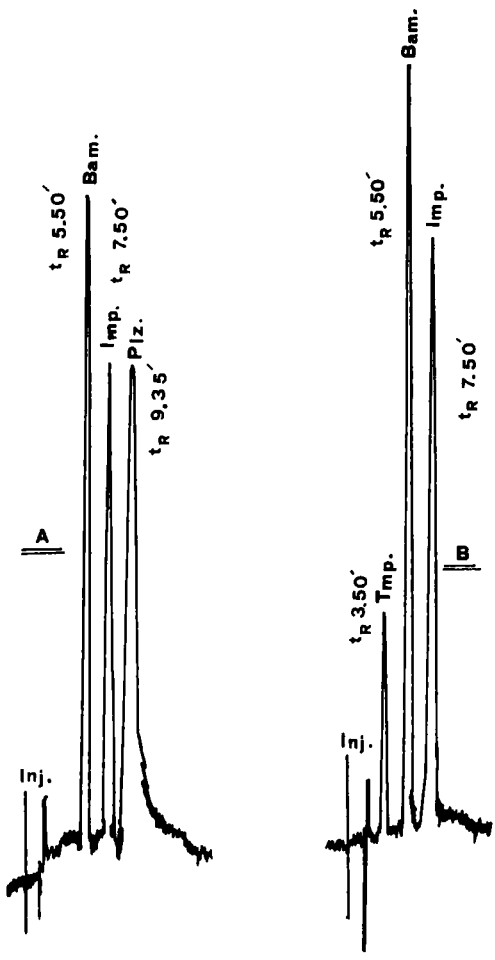

Figure 1. HPLC chromatogram showing resolution of A: Bamipine (Bam) 4.34 μ g/ml, Imipramine (Imp) 9.83 μ g/ml, Prochlorperazine (Plz) 9.58 μ g/ml and B: Trimeprazine (Tmp) 0.67 μ g/ml, Bamipine (Bam) 5.78 μ g/ml, Imipramine (Imp) 12.28 μ g/ml.

TABLE 3

Recovery Study for the Determination of the Following Drugs in Spiked Placebos

Compound	Determined in sample(mg)	Added (mg)	Found (mg)	Material Balance%	Recovery (%)
Bamipine Hydrochloride	28.34	10.14	38.36	99.69	98.82
Imipramine Hydrochloride	32.41	15.64	47.93	99.75	99.23
Trimipramine Maleate	24.66	8.12	32.71	99.79	99.14
Chlorprothixene	42.52	21.16	63.38	99.53	98.58
Prochlorperazine Edisylate	16.72	12.14	28.59	99.06	97.77
Trifluoperazine Hydrochloride	12.40	10.58	22.78	99.13	98.11

Excipients: galactose, gelatine , talk , starch.

TABLE 4

Results of Analysis of six drugs present in pharmaceutical formulations

Pharmaceutical Formulations	Active Ingredients	Labelled Amount*	HPLC Results**	Coefficient of Variation	% Found
Film - coated tablets	Bamipine HCl	50.00	48.92	0.46	97.84
Sugar-coated tablets	Bamipine HCl	25.00	24.76	0.87	99.04
Tablets	Imipramine HCl	25.00	25.38	1.46	101.52
Tablets	Trifluoperazine HCl	5.00	5.08	2.16	101.60
Tablets	Trimipramine Maleate	25.00	25.42	0.41	101.68
Tablets	Chlorprothixene HCl	50.00	48.85	1.14	97.70
Spiked Placebos	Bamipine HCl	20.15	19.84	1.64	98.46
	Imipramine HCl	24.32	24.65	1.18	101.35
	Prochlorperazine Edysilate	8.26	8.16	1.98	98.78

* mg/tablet

** Mean of four replicates

resolution factors, Rs, were calculated between the chromatographic peak of bamipine and each individual peak of the rest of the analysed compounds from the equation $Rs = 2(t_2 - t_1)/ W_1 + W_2$, where t_2 and t_1 are the retention times of the two peaks, W_1 and W_2 are the peak widths at the base of the two respective peaks. The resolution Rs was more than 1.60 signifying complete separation (except in case of promethazine whish is also an antihistamine). These are illustrated in Table 1.The relative standard deviation (% RSD) of six replicate injections of a standard was not more than 2.85% as it is defined in the USP XXI under "System Suitability for HPLC".

Standard Solutions: An appropriate amount of each standard solution was weighed in 25 ml flask and ethanol 95% was added. 5 ml of each was transferred to a 50 ml volumetric flask and diluted to volume again with ethanol 95% thus comprising an intermediate solution. Portions of the latter were transferred to 25 ml volumetric flasks and diluted to volume in mobile phase to yield a series of standard solutions in the ranges illustrated in Table II.

Sample Preparation: Tablets: No less than 15 tablets were weighed and the average tablet weight determined. The tablets were finely powdered and a portion of powder equivalent to one average tablet weight was weighed and quantitatively transferred into a 50 ml volumetric flask. Twenty-five milliliters of ethanol 95% were added and the dispersion was shaken for 40 minutes on a mechanical shaker. Ultrasonication followed for another twenty minutes and then the solution was diluted to volume with ethanol 95 % and left to precipitate. Appropriate dilutions were made with mobile phase from the clear supernatant solution, so that the concentration of each sample solution approached the concentration of that in the middle of the standard solution range. Filtration kits (Millipore) for the sample preparations were used to ultraclean the solutions of particles 0.5 μ m or greater.

RESULTS AND DISCUSSION

The optimum mobile phase was systematically investigated by varying the proportion of the solvents in the mobile phase until good peak shape and acceptable separation was obtained. This separation has proved to be rather difficult using other cromatographic systems, due to a relatively large number of drugs. Two representative chromatograms are illustrated in figure 1, whereas other parameters (t_R, k', R_s) for the drugs analysed are presented in Table 1.

Calibration graphs were constructed of peak height versus concentration. Linear regression and correlation showed that the method is linear. Correlation coefficient, intercept and slope were those presented in Table 2.

A recovery study was also carried out by adding known amounts of drugs in spiked placebos. The parameters "Material Balance" and "Recovery" were calculated by using the formulae: Material Balance = found /added + determined in sample and Recovery = found - determined in sample / added correspondigly,Table 3.

The results of the quantifications of bamipine, imipramine, trifluoperazine, trimipramine, chlorprothixene and prochlorperazine in pharmaceutical formulations and in spiked placebos are shown in Table 4. These are in agreement with the labelled amounts. No noticeable interferance from the excipients was observed in the chromatograms. The coefficient of variation was in the range 0.49 - 2.16 %.

In conclusion , the method presented proved to be rapid and specific for the determination of ten drugs alone or in combination in pharmaceutical formulations.

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