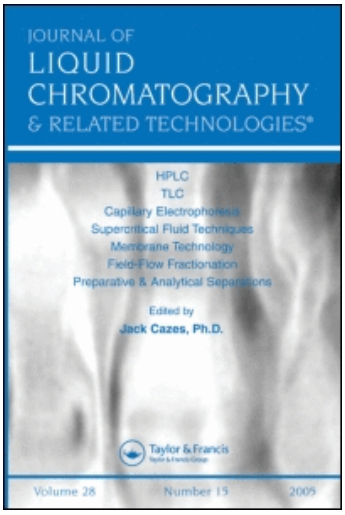

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SEPARATION AND DETERMINATION OF SOME CORTICOSTEROIDS COMBINED WITH BAMIPINE IN PHARMACEUTICAL FORMULATIONS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A simple and reliable h.p.l.c. method for the determination of bamipine, hydrocortisone, dexamethasone, betamethasone and beclomethasone has been developed. The procedure, which simultaneously resolves all five compounds, could be employed for the analysis of each component in different pharmaceutical formulations. Chromatographic separation was accomplished under isocratic conditions using a Bondapak 10 μm, C_{18} column 250x2.1 mm and a mobile phase of acetonitrile: water (48:52) and 0.65% acetic acid pumped at a rate of 1 ml/min. Column effluent was monitored with an U.V. detector at 251 nm. The behaviour (k' values) of the compounds were also investigated under different chromatographic conditions. The compounds were eluted in the range from 2.54 to 8.78 mins. Linearity, reproducibility and recovery (% of labelled amount) were satisfactory for all compounds. The method has been successfully applied to the analysis of ointment, cream, gel and lotion.

INTRODUCTION

Antihistamines are one of the most widely used group of drugs. They are formulated as single-components or combined with other drugs with different pharmacological actions. Bamipine is an antihistamine chemically defined as N-benzyl-N-(1-methyl-4-piperidyl) aniline. It is used topically to treat allergic dermatitis and pruritus caused by insect sting and similar conditions.

On the other hand, corticosteroids which are also applied topically are usually the most helpful in relieving both the inflammation and associated pruritus. Although potent topical corticosteroids may be effective when less potent corticosteroids are inadequate, the risk of adverse reactions is increased. In some cases, it is claimed that combination of a corticosteroid with an antihistamine requires considerably less amount of the former whereas the therapeutic result is the same as when corticosteroid is used as a single therapeutic compound.

There are only a few reports on the determination of hydrocortisone (1-6), dexamethasone (6-7), beclomethasone (9), betamethasone (6.8) and the antihistamine bamipine (10-12) in pharmaceutical formulations or in the bulk drug using HPLC. None of these methods is suitable for the determination of every single compound or their combination with bamipine: In the proposed HPLC method, each drug can be analysed as a single component or in mixtures with bamipine using the same chromatographic system. The method is simple, fast and is applied easily to the determination of the drugs in oitments, creams, gels and lotions.

EXPERIMENTAL

A. Apparatus

A Perkin Elmer Series 3B high performance liquid chromatography 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 wavelength system was used. The spectrophotometer was operated at 0.04 Absorbance Units Full Scale (AUFS). 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 10 mV and chart speed of 1 mm/min.

The analytical column was a Bomdapak C_{18} particle size 10 µm, 250 x 2.1 mm I.D. stainless steel. The column was equilibrated with mobile phase therefore when a stable line was achieved the standard and sample solutions were injected onto the column. A flow rate of 1 ml/min eluted the compounds in the range from 2.54 to 8.78 as illustrated in Table I. The wavelength was set at 251 nm.

B. Mobile phase

The mobile phase consisted of acetonitrile : water, 48:52 and 0.65% CH_3COOH (pH 3.18). It was degassed by vacuum filtration through a 0.2 µm Sartorius S 11 807 polytetrafluoroethylene membrane filter while the flask was in an ultrasonic bath.

C.Chemicals:

HPLC grade acetonitrile, water (acetonitrile Chromasolv^R, wasser G Chromosolv^R Riedel-de Haen) and acetic acid glacial (Merck).

The standards hydrocortisone-21-acetate, betamethasone-17-valerate, beclomethasone dipropionate and dexamethasone were purchased from Sigma Chemical Company. Bamipine lactate was kindly donated by Knoll (Ludwigshafen, W. Germany). The concentrations of the standard solutions are presented in Table II.

D.Sample Preparation

The main source of problems in sample preparations is related to the quantitative recovery of the active ingredients from single or multicomponent preparations.

In pharmaceutical formulations like creams, ointments, gels and lotions, sample preparation by extraction can be a serious problem because of sometimes poor recovery of compounds

TABLE I

High Performance Liquid Chromatographic characteristics of the drug separation

Compound	$t_{R(min)}$	k'*	R_s
Bamipine Lactate	6.83	2.42	-
Hydrocortisone-21-Acetate	2.54	0.29	5.66
Dexamethasone	3.67	0.83	3.45
Betamethasone-17-Valerate	5.17	1.58	1.80
Beclomethasone Dipropionate	8.78	3.39	1.44

t_0=2.00 min

to be analysed. Creams are viscous liquid or semisolid emulsions of oil-in-water type. Similarly ointments are generally more viscous based on either hydrocarbon or water-soluble excipient mixtures. Therefore most methods applied in the analysis of creams and ointments involve liquid-liquid extraction to separate the drug component from fatty-base components. Usually a dispersion of the sample in methanol is extracted with a solvent of very low polarity, such as cyclohexane. Alternatively from the polar constituents, an aqueous suspension of the sample is extracted with mostly chloroform.

Sample also are dissolved in a suitable solvent mixture and portions of the solution are analysed directly. The most convenient solvent with the ability of dissolving the active ingredients is ethanol. However, it is neccessary to combine it with other solvents to achieve complete dissolution of both the excipients and the active compounds, The use of a precolumn provides a good clean up of the multicomponent samples since the non-polar excipients are strongly retained on the pre-column relative to the drug substances.

After each day experiments, only the precolumn was flushed with methanol-tetrahydrofurane (75:25) at 1.0 ml/min flow for at least 30 minutes.

TABLE II

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

Compound	Concentration µg/ml*	Peak Heights mm*	Intercept	Slope	r
Bamipine Lactate	4.65-16.26	32.0-115	0.89	7.07	0.9990
Hydrocortisone-21-Acetate	2.75-9.61	35.0-124	-0.91	12.91	0.9999
Dexamethasone	5.95-20.83	5.95-145	2.93	6.83	0.9998
Betamethasone-17-Valerate	6.76-23.65	6.76-112	0.21	4.74	0.9987
Beclomethasone Dipropionate	14.56-50.96	14.56-126	-1.79	2.51	0.9980

* Mean of four replicates

Ointments:

Ointments are monophasic, semi-solid often anhydrous greasy preparations sometimes consisting mainly of polyethylene glycol. Therefore 1 part of ethanol was combined with 3 parts of pentane to dissolve a suitable amount of ointment in a volumetric flask. The mixture was sonicated in a water bath for 20 minutes. An aliquot of this solution was then transferred into 100 ml flask and made to volume with ethanol. Because some particles were formed from the fatty excipients the solution was left for some time to precipitate. From the clear supernatant solution a portion was certifuged and filtered, then injections were made onto the column.

Creams:

Creams are semi-solid dermatological products consisting of two immiscible phases, one of which is dispersed in the other. Usually the continuous phase is the more polar one, that is, the emulsion is of the type o/w. Therefore a mixture of water: ethanol:tetrahydrofurane 50:25:25 was used for the dispersion of the excipients together with the active ingredients of the formulation. Water and ethanol were used to dissolve the external hydrophylic phase whereas tetrahydrofuran together with ethanol dissolves the internal phase and the active ingredients. Since tetrahydrofurane and water are immiscible, ethanol also helps to mix them fully.

Therefore a certain amount of cream was weighed in a volumetric flask, solvent mixture was added and stirred for 15 minutes at 40°C using magnetic stirrer. The whole mixture was made to volume and cooled in an ice bath where some fatty excipients were precipitated. From the clear supernatant solution, a certain amount was certifuged and filtered. Then appropriate dilutions were made by using certain amounts from the stock solution.

Lotions:

The same process of sample preparation was used for lotions as it is described for creams.

Gels:

The sample was dissolved in ethanol, ultrasonicated in water bath, then filtered and injected directly onto the chromatographic column.

RESULTS AND DISCUSSION

The optimum chromatographic conditions were investigated by varying the tiny amount of acetic acid added in the mobile phase. Figure 1(A) shows that by varying the amount of acetic acid present in the mobile phase, there was a dramatic change in the k' value of bamipine whereas there was no considerable, change in the k' values of the rest of the compounds. However the presence of acetic acid was essential for the elution of bamipine in reasonable time compared with the rest of the corticosteroids analysed. Similarly the amount of water present in the mobile phase was also studied. It was also observed that proportion of water in the mobile phase greater than 45% resulted in longer retention time which also reflected in greater k' values. These are illustrated in Figure 1(B).

The retention time of bamipine together with the rest of the corticosteroids were found to be reproducible under the present chromatographic conditions for a period over twenty days, which makes the method most suitable for screen testing. A typical HPLC chromatogram showing a simultaneous complete separation of the drugs used in different pharmaceutical formulations together in spiked placebos is illustrated in Figure 2.

The resolution factors R_s, were calculated between the chromatographic peak of bamipine and each separate peak of the rest of corticosteroids from the equation $R_s = 2(t_2 - t_1)/W_1 + W_2$, where t_1, t_2 are the retention times of the two peaks and W_1, W_2 are the peak widths at the base of the two respective peaks. The resolution R_s was more than 1.10, signifying complete separation between bamipine and each corticosteroid drug. The numerical values of the parameters R_s, t_R and k' are shown in Table I.

Calibration graphs were constructed of peak height versus concentration. The linear regression equations and correlation coefficients showed that the method is linear. The characteristics of the regression equations are presented in Table II.

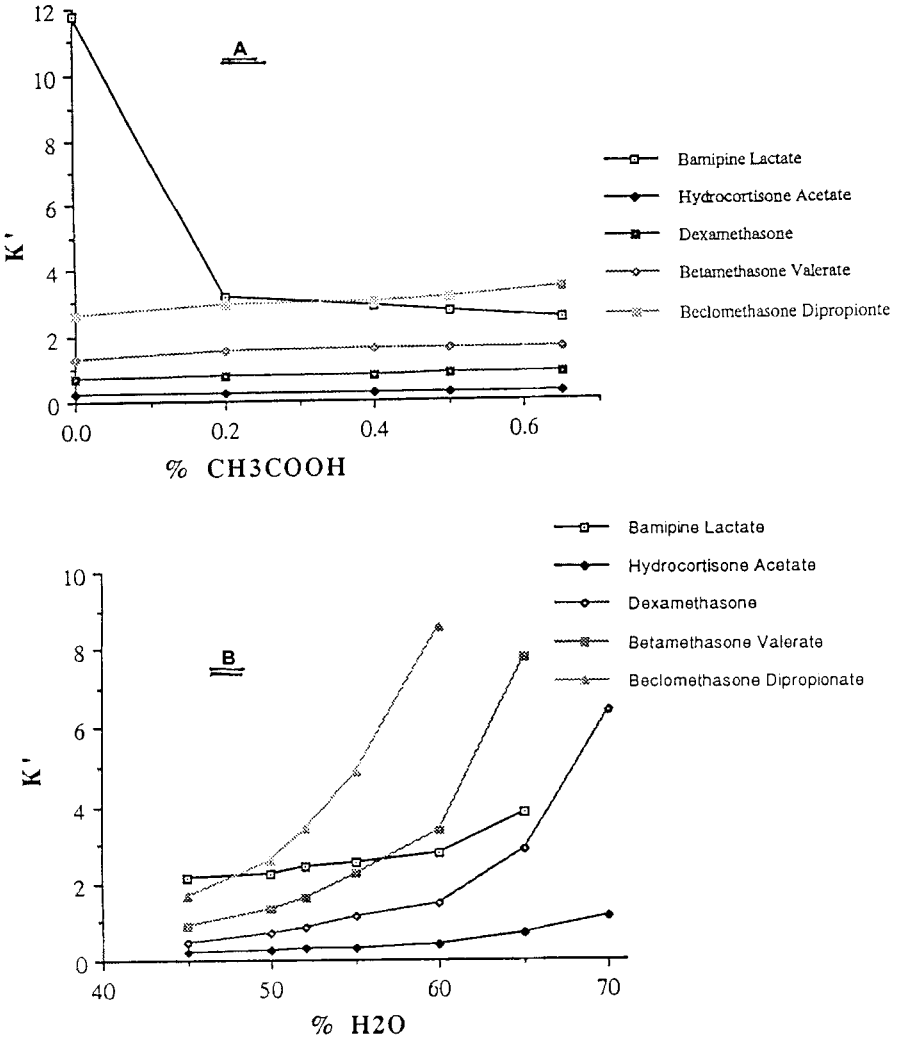

Figure 1. The relationship between (A) the percentage of acetic acid and (B) the water content in the mobile phase and the capacity ratio (k') of the compounds analysed by h.p.l.c.

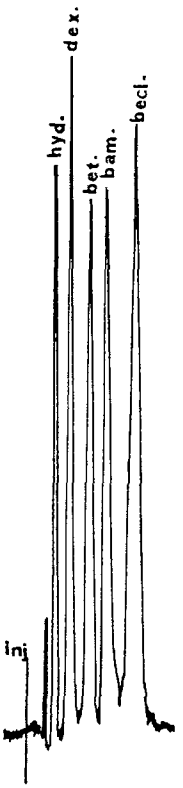

Figure 2. High Performance Liquid Chromatography of the simultaneous separation of hydrocortisons (hyd) 8.24 µg/ml, dexamethasone (dex) 17.86 µg/ml, betamethasone (bet) 20.27 µg/ml, bamipine (bam) 13.94 µg/ml and beclomethasone (becl) 43.68 µg/ml. The retention times are presented in Table I.

The results of the quantitations of bamipine, hydrocortisone, dexamethasone, betamethasone and beclomethasone in pharmaceutical formulations and in spiked placebos are shown in Table III. These are in agreement with the labelled amount. No noticeable interference from the excipients was observed in the chromatograms. The coefficient of variation was in the range 1.21 - 2.45.

TABLE III

Results of analysis of six drugs present in pharaceutical formulations

Pharmaceutical Formulation	Active Ingredients	Labelled Amount*	HPLC Resultes**	Coefficient of Variation % C.V	% Found
Cream	Bamipine Lactate	20.00	20.02	1.24	100.90
	Hydrocortisone-21-Acetate	2.50	2.41	2.08	96.34
Cream	Hydrocortisone-21-Acetate	10.00	9.84	2.36	98.44
Gel	Bamipine Lactate	20.00	20.87	1.48	104.35
Lotion	Dexamethasone	0.25	0.23	2.14	94.16
Ointment	Betamethasone-17-Valerate	10.00	10.35	2.45	103.45
Spiked Placebos	Bamipine Lactate	20.00	19.97	1.65	99.85
	Beclomethasone dipropionate	2.50	2.56	1.21	102.67

* mg/g

** Mean of four replicates

CONCLUSION

In conclusion, the results of the present study show that the proposed HPLC method is an efficient and reliable means of quantitating bamipine and some corticosteroids in pharmaceutical formulations. The active ingredients can be determined either as a single component or in combination with bamipine.

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