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Note

Simultaneous determination of bromochlorosalicylanilide and bamipine in pharmaceutical formulations by high-performance liquid chromatography

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Bamipine [N-benzyl-N-(1-methyl-4-piperidyl)aniline] has similar general properties and uses to the antihistamines. It has pronounced sedative effects and also local anaesthetic properties[1]. Bromochlorosalicylanilide (5-bromo-4'-chlorosalicylanilide) is an antifungal agent applied topically. It is available in the form of a pharmaceutical powder or combined with bamipine as a solution or ointment for the prophylaxis and treatment of skin infections[1].

Few methods are available for the determination of bamipine itself in pharmaceutical preparations, whereas no analytical methods have been reported for the quantification of bromochlorosalicylanilide[2–4]. Because there is no published work referring to the simultaneous determination of both drugs, in this paper we present an high-performance liquid chromatographic (HPLC) method suitable for estimating these compounds in various commercial products. This procedure separates bromochlorosalicylanilide from bamipine (soventol) and from chlorpromazine. The latter was employed as an internal standard.

EXPERIMENTAL

Chemicals and samples

The pure pharmacologically active compounds were kindly donated by Knoll (Ludwigshafen, F.R.G.), Germany, whereas the pharmaceutical formulations (commercial samples) such as tablets, ointments, creams, powders and solutions were purchased from different European countries. Chlorpromazine hydrochloride, employed as the internal standard, was obtained from Sigma.

Analytical grade (Ferak, Berlin, F.R.G.) dichloromethane, methanol and 25% ammonium hydroxide were used in the mobile phase.

Mobile phase and stability of chromatographic system

The mobile phase was methanol–dichloromethane (20:80), the methanol containing 0.02% ammonium hydroxide, which was necessary for the development of the chromatographic system. It is accepted that small quantities of water in methanol or dichloromethane could considerably affect the separation of peaks or lead to ir-

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reproducible chromatograms. Therefore the solvents were dried by keeping them for more than 48 h over 3- and 4-Å molecular sieves in storage vessels, with occasional swirling. Each of the solvents was degassed separately by vacuum filtration through a 0.2-μm Sartorius S 11807 polytetrafluoroethylene membrane filter. Ammonium hydroxide was added with a microsyringe to the degassed methanol. The number of theoretical plates of the column was unchanged after completion of the experiment.

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 wavelength system was used. The spectrophotometer was operated at 0.02 absorbance units full scale (a.u.f.s.) (1 cm path length). The use of a higher sensitivity produced an unacceptable level of baseline noise. 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 Bromme potentiometric recorder, connected to the spectrophotometer, with an operating voltage of 10 mV and chart speed of 2 mm/min. A 25 cm × 2.6 mm I.D. stainless-steel column containing 10-μm Porasil silica gel (Perkin-Elmer) was employed.

A flow-rate of 1 ml/min eluted the compounds and the internal standard in less than 4 min. The wavelength was set at 258 nm. The system was left to equilibrate for at least 2 h. The coefficient of variation of the response ratios for both peaks (derived from bromochlorosalicylanilide or bamipine) relative to the internal standard peak for six consecutive injections of any concentration was less than 1%.

Standard solutions

A bromochlorosalicylanilide stock solution was prepared at a concentration of 1.02 mg/ml. A portion of this was diluted by a factor of 50 in mobile phase and an intermediate solution containing 20.4 μg/ml was prepared. Aliquots of the latter were transferred to 25-ml flasks, internal standard was added and diluted to volume in mobile phase to yield seven standard solutions in the range 1.63–6.52 μg/ml.

Similarly, a stock solution of bamipine lactate was prepared at a concentration of 0.964 mg/ml. Part of this was diluted by a factor of 100 in mobile phase and an intermediate solution containing 9.64 μg/ml was prepared. Aliquots of the latter were transferred to 25-ml flasks, internal standard was added and dilute to volume in mobile phase to yield seven standards in the range 0.77–3.08 μg/ml. Following the same procedure, standard solutions were also prepared for other bamipine salts.

Sample preparation

Ten tablets were weighed and the average weight was determined. The tablets were finely ground in a mortar and part was weighed in a 50-ml volumetric flask. Mobile phase was added and the dispersion was placed in an ultrasonic bath for 15 min. It was then made up to volume with mobile phase and left to precipitate. Appropriate dilutions were made from the clear supernatant and internal standard was added to the final test solution. A similar procedure was followed for other formulations. The creams were ultrasonicated with methanol and dilutions were made in mobile phase.

It is known that the bases (excipients) of certain pharmaceutical products like creams or ointments are semi-solid greasy formulations, and pharmaceutical solutions are preparations containing a significant amount of water. Because the excipients are fairly soluble in the sample solutions, an internal standard was added in case the reproducibility of the retention values were affected. In each standard or sample solution the internal standard, chlorpromazine hydrochloride, was at a concentration of 1.31 μg/ml.

RESULTS AND DISCUSSION

The results of the quantification of bamipine and bromochlorosalicylanilide in six different pharmaceutical formulations are presented in Table I. These specific formulations are not referred to in any European pharmacopoeia, therefore no comparison can be made between the present and any official method of analysis. The analysed compounds and the internal standard were eluted in less than 4 min (Fig. 1). The coefficient of variation was in the range 0.57–2.10.

TABLE I

PRECISION OF THE HPLC METHOD FOR BROMOCHLOROSALICYLANILIDE AND BAMIPINE

Commercial dosage form	*Active ingredients*	*Labelled amount (mg)**	*HPLC results** (mg)*	*Coefficient of variation*	*Recovery (%)*
Sugar-coated tablets	Bamipine hydrochloride	25	24.80	1.42	99.20
	Propylhexedrin	—			
Film-coated tablets	Bamipine hydrochloride	50	48.72	1.68	97.44
Powder	Bromochlorosalicylanilide	20	20.82	1.64	104.10
Ointment	Bromochlorosalicylanilide	20	20.18	1.37	100.90
	Bamipine salicylate	10	10.04	0.57	100.40
Cream	Bamipine lactate	20	20.22	2.10	101.10
	Hydrocortisone acetate	—			
Solution	Bromochlorosalicylanilide	20	20.32	0.98	101.60
	Bamipine lactate	10	10.02	0.66	100.20

* mg/tablet and mg/g for other dosage forms.
** Results are based on four sample injections.

Calibration curves were drawn of peak height ratio *versus* concentration. Linear regression and correlation showed that the correlation coefficient, intercept and slope were 0.9997, 0.0164, 0.165 and 0.9998, 0.0193, 0.387 for bromochlorosalicylanilide and bamipine lactate respectively.

The presence of a tiny amount of concentrated ammonium hydroxide in the mobile phase is essential, otherwise no separation occurs. The retention time and the peak shape of bromochlorosalicylanilide are not affected by the absence of ammonium hydroxide, whereas the other two compounds are strongly retained.

Although it is well known that high alkalinity reduces the useful life of silica columns, the use of small quantities of ammonium hydroxide in the mobile phase

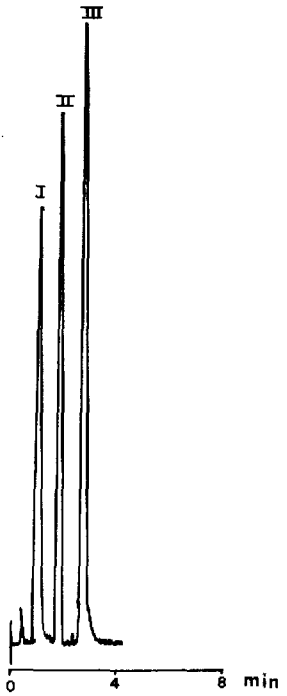

Fig. 1. A typical chromatogram of bromochlorosalicylanilide (I), bamipine (II) and chlorpromazine (III). The latter served as the internal standard. Chart speed: 10 mm/min.

without any noticeable deterioration of the column, has been reported in several papers[5,6]. In this case, after each determination the column was flushed only with methanol. Hence, no changes were observed in the efficiency of the column.

The method described has proved to be suitable for the analysis of ointments, creams and pharmaceutical solutions, as the oily excipients are fairly soluble in the mobile phase and no extraction or other tedious procedure is necessary.

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