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Note

Separation and simultaneous determination of bamipine and salbutamol in dosage forms by high-performance liquid chromatography

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Antihistamines, available by prescription and over-the-counter as single-entity preparations and in mixtures, are among the most widely used drugs. Bamipine [N-benzyl-N-(1-methyl-4-piperidyl)aniline] is an antihistamine and also has sedative effects. As it is well known that antihistamines offer weak protection against bronchospasm, bamipine can be combined with salbutamol {α^1-[(*tert.*-butylamino)methyl]-4-hydroxy-*m*-xylene-α,α'-diol}, which has a selective action on β_2-receptors. Both are administered in tablet form, whereas salbutamol is also available in aerosol spray and as an injection.

Although several methods have been described for the quantification of bamipine and salbutamol in pharmaceutical dosage form, none is suitable for the simultaneous determination of both drugs[1–7]. In this paper, we describe a high-performance liquid chromatographic (HPLC) procedure in which both compounds can be quantified simultaneously under the same chromatographic conditions.

EXPERIMENTAL

Chemicals

Analytical-reagent grade acetonitrile and methanol (Ferak, Berlin, F.R.G.) were used. Ammonium acetate was of zur Analyse grade from Merck (Darmstadt, F.R.G.). Water was purified with a Millipore filtration unit (deionized, <10 $\mu\Omega$).

Reference standards and standard solutions

Bamipine hydrochloride and salbutamol sulphate were kindly donated by Knoll (Ludwigshafen, F.R.G.) and Glaxo (Krioneri, Athens, Greece), respectively.

Two series of standard solutions, 3.75, 5.62, 7.49, 9.36, 11.23 and 13.11 μg/ml and 2.24, 3.36, 4.48, 5.60, 6.72, 7.84 μg/ml, were prepared for salbutamol sulphate and bamipine hydrochloride, respectively, using the mobile phase as a solvent.

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Apparatus

A Perkin-Elmer Series 3B high-performance liquid chromatograph, a Rheodyne 7010 20-μl loop injector valve and an LC wavelength system was used. The spectrophotometer was operated at 0.04 a.u.f.s. (1-cm path length). The wavelength was set at 264 nm. The chromatographic peaks were recorded by employing an LKB (Bromma, Sweden) 2210 potentiometric recorder connected to the spectrophotometer, with an operating voltage of 10 mV and chart speed of 2 mm/min. A 250 × 2.1 mm I.D. stainless-steel column containing Polygosil®-60, C_{18}, 10 μm (Macherey-Nagel, Düren, F.R.G.) was employed. A flow-rate of 0.6 ml/min eluted salbutamol and bamipine in 4.00 and 5.42 min, respectively. All analyses were performed at room temperature.

Mobile phase and stability of chromatographic system

The mobile phase was acetonitrile–methanol–0.015 *M* aqueous ammonium acetate (85:10:5, v/v/v), degassed by vacuum filtration through a 0.2-μm Sartorius S 11807 PTFE membrane filter while the container (flask) was in an ultrasonic bath. To the mobile phase *ca.* 0.6% acetic acid was added to adjust the pH to *ca.* 5.72, the optimum for the chromatographic system. The column was equilibrated with mobile phase at a flow-rate of 0.6 ml/min. The relative standard deviation (R.S.D.) of six replicate injections of a standard was not more than 2.0%, as defined in the USP XXI under 'System suitability for HPLC'.

Sample preparation

For tablets, not less than ten were weighed and the average weight determined. The tablets were finely powdered and a portion of powder equivalent to one average tablet weight was weighed and transferred quantitatively into a 50-ml volumetric flask. A 25-ml volume of mobile phase was added and the dispersion was shaken for 40 min on a mechanical shaker. After ultrasonication for 20 min, the solution was diluted to volume with mobile phase and left to precipitate. Appropriate dilutions were made 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 μm or greater.

For injections, a 10-ml volume containing 0.5 mg/ml was carefully transferred into a 25-ml volumetric flask, which was then placed in a desiccator under vacuum for 24 h. Mobile phase was added to the dried formulation, then shaken and made up to volume. Appropriate dilutions with mobile phase similar to those of the standard solutions were made.

For oral inhalation aerosol, using the manufacturer's directions, twenty metered doses (each containing 0.1 mg of salbutamol) were collected in a 250-ml conical flask. Portions of mobile phase were added and transferred into a 50-ml volumetric flask, then appropriate dilutions were made.

RESULTS AND DISCUSSION

Fig. 1 shows typical standard and sample chromatograms obtained using the above procedure. Although other HPLC methods with biomedical applications have

been described for the quantification of salbutamol (with electrochemical[8], amperometric[9] and fluorimetric[10] detection), the method proposed here proved suitable for the simultaneous determination of salbutamol and bamipine. Salbutamol sulphate and bamipine hydrochloride were eluted in 4.00 and 5.42 min, respectively, from standard and sample solutions.

The relatively small amount of buffered aqueous solution in the mobile phase and the easy adjustment of the pH to *ca.* 5.72 were necessary in order to achieve the optimum chromatographic conditions. The separation and elution were also affected by the increase in methanol concentration in the mobile phase. Therefore, a combination of both pH and polarity was critical for the development of the chromatographic system.

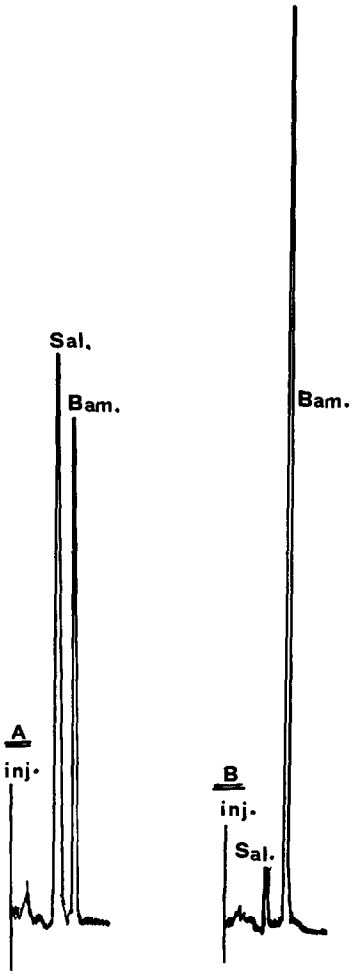

Fig. 1. Typical chromatograms of the separation of salbutamol (Sal.) from bamipine (Bam.) at 264 nm. Left: standard, Sal. 10.61 μg/ml, Bam. 4.34 μg/ml; right: tablet extract, Sal. 0.64 μg/ml, Bam. 8.00 μg/ml. Retention times: 4.00 min (Sal.) and 5.42 min (Bam.).

TABLE I

PRECISION OF METHOD IN ANALYSIS OF PHARMACEUTICAL FORMULATIONS FOR SALBUTAMOL AND BAMIPINE

Samples were purchased in different European countries.

Commercial dosage form	*Active ingredients*	*Labelled amount*[a]	*HPLC results*[b]	*Coefficient of variation (%)*	*Found (%)*
Tablets	Salbutamol sulphate	2	1.97	0.97	98.4
Tablets	Salbutamol sulphate	4	3.95	0.46	98.8
Injection	Salbutamol sulphate	0.5	0.50	0.24	100.4
Injection	Salbutamol sulphate	0.5	0.50	0.32	100.5
Aerosol	Salbutamol	0.1	0.102	0.42	102.4
Film-coated tablets	Bamipine hydrochloride	50.0	49.15	1.04	98.3
Sugar-coated tablets	Bamipine hydrochloride	25.0	24.83	0.96	99.3
	Propylhexedrine	–	–	–	–
Tablet placebo I	Salbutamol sulphate	2	1.97	0.92	98.5
	Bamipine hydrochloride	50	49.05	1.11	98.1
Tablet placebo II	Salbutamol sulphate	4	3.95	0.54	98.7
	Bamipine hydrochloride	50	49.10	1.02	98.2

[a] Tablets, mg per tablet; injection, mg/ml; aerosol, mg per metered dose.
[b] Means of four replicates.

Calibration graphs were constructed of peak height *versus* concentration. Linear regression and correlation showed that the correlation coefficient, intercept and slope were 0.9999, 0.22 and 9.60, respectively, for salbutamol sulphate and 0.9995, 0.23 and 17.48, respectively, for bamipine hydrochloride.

The results of the quantification of salbutamol and bamipine in different pharmaceutical formulations are shown in Table I. These were in agreement with the labelled amounts. No interference from the excipients was observed in the chromatograms. The coefficient of variation for both compounds was in the range 0.24–1.11%.

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