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Short Communication

High-performance liquid chromatographic method for the determination of bumetanide in pharmaceutical preparations*

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Introduction

Bumetanide (3-butylamino-4-phenoxy-5-sulphamoylbenzoic acid) is a potent diuretic which is used mainly in the form of tablets or injections. Analytical methods described for the quantitative analysis of bumetanide have involved spectrophotometry [1–3] and gas–liquid chromatography (GLC) [4] with pre-column derivatization. In recent years, high-performance liquid chromatography (HPLC) [5, 6] with fluorescence detection has been applied extensively for the determination of bumetanide in biological fluids.

This paper presents a simple and rapid method for determination of bumetanide in tablets and ampoules using HPLC with UV-detection and salicylic acid as internal standard.

Experimental

Materials

Acetone, methanol and acetic acid were obtained from Merck (Darmstadt, FRG). Bumetanide was obtained from Leo (Copenhagen, Denmark). "Yurinex" tablets and "Yurinex" ampoules were obtained from Hemofarm (Vršac, Yugoslavia).

Instrumentation and chromatographic analysis

Samples were analysed using Perkin–Elmer Series 3B (Norwalk, NJ, USA) high-performance liquid chromatograph with Perkin–Elmer UV detector and Rheodyne injector (10-μl sample loop). A reversed-phase column RP-8 (Varian) 250 × 4.6 mm,

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10 μm was fitted to the instrument and a dual pen recorder was used at a chart speed 30 cm h^{-1}. The sample, dissolved in 10 μl of eluent, was injected onto the column. The column was eluted at 25°C with methanol–water–acetic acid (60:40:0.5, v/v/v) at a flow rate of 1 ml min^{-1}. The absorbance of bumetanide and internal standard salicylic acid was monitored at 231 nm.

Preparation of solutions

The following solutions were prepared in a mixture of eluent and acetone (9.5:0.5, v/v), 0.1 mg ml^{-1} of bumetanide bulk drug; 0.1 mg ml^{-1} of salicylic acid; 0.1 mg ml^{-1} of bumetanide extract from one tablet and 0.1 mg ml^{-1} of bumetanide diluted one ampoule. For the standardization of the method, eight standard solutions of bumetanide concentrations from 0.04 to 0.18 mg ml^{-1}, and a fixed concentration of 0.1 mg ml^{-1} of salicylic acid were prepared; 10 μl of each solution was injected.

Results and Discussion

Figure 1 shows a chromatogram obtained after injection of 10 μl (1 μg) of bumetanide (peak 2) and 10 μl (1 μg) of internal standard, salicylic acid (peak 1). The peaks were well separated at the analytical wavelength of 231 nm. The retention time of salicylic acid was 4.49 min and that of bumetanide was 9.26 min. The retention of bumetanide relative to that of the internal standard was 2.06 min.

The HPLC–UV spectra of bumetanide (curve 2) and salicylic acid (curve 1) in spectral region from 200–370 nm are shown in Fig. 2. Salicylic acid has three absorbance maxima at 233, 265 and 338 nm, whilst bumetanide has two maxima at 227 and 305 nm. Figure 2

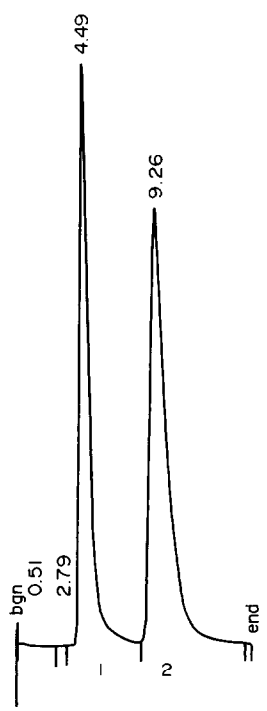

Figure 1
HPLC chromatogram: 1, Salicylic acid; 2, bumetanide. Column RP-8 250 × 4.6 mm, 10 μm. Eluent: methanol–water–acetic acid (60:40:0.5). Flow rate 1 ml min^{-1}. Detector wavelength 231 nm.

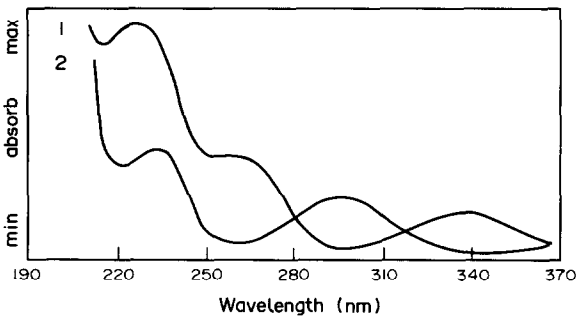

Figure 2
The UV spectra obtained by HPLC: 1, salicylic acid; 2, bumetanide. Wavelength range 200–370 nm.

shows that both spectra have one maximum absorbance around the wavelength range 227–234 nm.

After the chromatographic study, the quantitative application of the method was investigated.

The reproducibility of the method was investigated using three solutions of different concentrations of bumetanide. Each solution was injected five times. The results presented in Table 1 show that maximum value of relative standard deviation (RSD) is satisfactory.

A linear relationship between the peak area and injected quantity of bumetanide was established over the range 0.4–1.8 μg. The lower limit of sensitivity of the method was found to be 20 ng. The regression equation was $y = 0.0561x - 0.255$ ($r = 0.998$).

The applicability of the method for the assay of sample dosage forms was examined using tablets and ampoules. The assay results are summarized in Table 2. The RSDs were <0.88%.

Table 1
Reproducibility of the HPLC method

Quantity of bumetanide injected (μg)	Mean peak area (cm^2)	SD (μg)	RSD (%; $n = 10$)
1.0	0.85	0.005	0.58
1.2	1.09	0.008	0.73
1.6	1.44	0.012	0.83

Mean peak area for 10 determinations.

Table 2
Assay of bumetanide

Sample	Stated amount (μg)	Found* (μg)	Recovery (%)	RSD (%; $n = 10$)
"Yurinex" tablets	1	0.98	98.0	0.88
"Yurinex" ampoules	0.5	0.51	102.0	0.63

*Average of 10 determinations.

Conclusion

A RP–HPLC method has been developed for the determination of bumetanide in pharmaceutical dosage forms. The excellent recovery (98.0–102.0%) and precision (RSD = 0.63–0.83%) obtained indicate the method to be suitable for routine analysis.

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