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Notes

HPLC determination of bendrofluazide in capsules

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Summary

A high-performance liquid chromatographic method for the determination of bendrofluazide in extemporaneously prepared 1.25 mg capsules is described. This employs a hexyl reversed-phase column, maintained at ambient temperature. The method is stability-indicating and the mean within-day recovery of bendrofluazide from the capsules was 100.2% (±3.8%, 95% confidence limits).

Bendrofluazide, also known as bendroflumethiazide, is a sulphonylurea diuretic. Its chromatographic behaviour has been studied by high-performance liquid chromatography (HPLC) with ultra-violet detection, using ODS or phenyl columns (Moskalyk et al., 1975; De Croo et al., 1985; Perlman and Kirschbaum, 1986; Smith et al., 1987). Stability-indicating HPLC methods have been developed using an ODS column (Hassan, 1983) and a phenyl column maintained at elevated temperature (Frontini and Mielck, 1992). These latter methods were considered to be suitable for the analysis of solid dosage forms by the authors, but no validation data for this application were presented.

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Stability-indicating HPLC methods for the analysis of bendrofluazide in tablets have employed a phenyl column at an elevated temperature (Perlman et al., 1984; US Pharmacopeia, 1990).

Bendrofluazide capsules, 1.25 mg, may be prepared in hospital pharmacy practice for administration to infants. The dose is administered by sprinkling the capsule contents into a drink or feed. This paper describes a reversed-phase HPLC method for the determination of bendrofluazide in capsules, using a hexyl column, maintained at ambient temperature.

Bendrofluazide powder was from Sigma Chemical Co. (Poole, U.K.). Bendrofluazide tablets, 5 mg, batch BB33, were from Cox Pharmaceuticals (Barnstaple, U.K.). Gelatin capsule shells were size 4 (Farillon, Romford, U.K.). Lactose was of British Pharmacopoeial (1988a) grade (Thornton and Ross, Huddersfield, U.K.).

Methanol was HPLC grade from Rathburn (Livingstone, U.K.). 4-Amino-6-trifluromethyl-1,3-benzenedisulphonamide was synthesized by the method of Hennig et al. (1981). All other chemicals were of analytical grade. Filter paper was Whatman No. 2 grade (Whatman Scientific, Maidstone, U.K.).

The chromatograph consisted of a Cecil CE1100 pump (Cecil Instruments, Cambridge, U.K.), Severn Analytical SA6500 ultraviolet detector (Severn Analytical, Macclesfield, U.K.) and Talbot ASI-4 autosampler equipped with a Rheodyne 7010 injection valve (Talbot Instruments, Alderley Edge, U.K.). Quantitation was by a Shimadzu C-R3A integrator (Dyson Instruments, Houghton-le-Spring, U.K.). The ultrasonic bath was from Langford Electronics (Birmingham, U.K.).

Bendrofluazide capsules, 1.25 mg, were prepared by grinding bendrofluazide tablets, 5 mg, and diluting the powder with lactose. The powder (100 mg) was filled individually into the capsule shells. For recovery studies, preparation was performed using an analytical balance.

Sample preparation was similar to the conditions of the US Pharmacopeia (1990) method for bendroflumethiazide tablets. For individual capsule assays, the contents of each capsule were emptied into a volumetric flask. The shell was rinsed with methanol and the contents and rinsings diluted to 10 ml with the same solvent. The flask was protected from light with aluminium foil, sonicated for 10 min and the mixture was filtered and sealed into autosampler vials.

For the assay of bendrofluazide 5 mg tablets, a sample of 20, were ground to a powder, 250 mg of this was dispersed in methanol, diluted to 100 ml and treated as for the capsules.

The column was a 5 μm Spherisorb hexyl (100 × 4.6 mm) column with a guard column (10 × 4.6 mm) of the same material (Hichrom, Reading, U.K.). The mobile phase was methanol:water (50:50) at a flow rate of 1.5 ml/min. The detection wavelength was 270nm and injection loop volume was 10 μl.

A calibration curve was prepared using methanolic bendrofluazide solutions of 0.06, 0.12, and 0.18 mg/ml.

Samples and standards were injected in duplicate, and quantitation was by measurement of peak area.

Preliminary studies showed that the hexyl column gave a better bendrofluazide peak shape than an octyl or ODS column.

The methanol used for sample preparation must be free from formaldehyde since bendrofluazide reacts with this impurity (Kirschbaum et al., 1982; Perlman et al., 1984). Spiking 25 ml of a methanolic solution of bendrofluazide (0.2 mg/ml) with 100 μl of 35% w/v formaldehyde solution produced an additional peak eluting at 2.2 min. The suitability of a batch of methanol may therefore be confirmed. No additional peak was seen in the absence of formaldehyde spiking.

The stability-indicating capability of the method was confirmed by spiking a standard with the major degradation product of bendrofluazide, 4-amino-6-trifluromethyl-1,3-benzenedisulphonamide (Hennig et al., 1981), and by acid and base degradation. Acid degradation was achieved by adding 2 ml of 0.05 M sulphuric acid to 10 ml of a methanol : water (50 : 50) extract of a bendrofluazide capsule, heating at 60°C for 2 h, cooling, and adjusting the apparent pH to 7.0 with 0.1 M sodium hydroxide. Base degradation was achieved in the same way by using 2 ml 1% w/v aqueous sodium carbonate, and the mixture neutralized with 0.1 M hydrochloric acid. The degraded solutions were then filtered and injected onto the chromatograph (Fig. 1).

Chromatography was linear in the range 0.4–4 μg injected (slope = 1.20×10^7 area units/μg $\pm$ 1.13×10^5, 95% confidence limits; y-intercept = 3.72×10^3 area units $\pm$ 2.69×10^4, 95% confidence limits; $r = 0.99998$). The precision of chromatography, assessed by six replicate injections of a 0.12 mg/ml standard, was 0.22% relative standard deviation.

The within-day accuracy and precision of the whole method was assessed by assaying six capsules (Table 1). The linearity of the whole method was confirmed by assaying capsules prepared with varying amounts of pure bendrofluazide diluted with lactose (Table 1).

Bendrofluazide has been found to be unstable during analysis in solvents containing water. Perl-

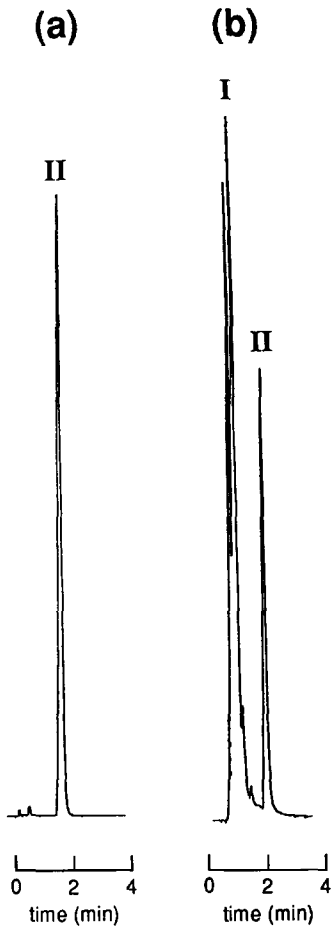

Fig. 1. Chromatogram of (a) capsule extract and (b) alkali-degraded capsule extract. (I) 4-Amino-6-trifluromethyl-1,3-benzenedisulphonamide and (II) bendrofluazide.

TABLE 1

Recovery of bendrofluazide from capsules (% recoveries are calculated with respect to the assay value of the tablets used to prepare the capsules, determined by HPLC)

Range of theoretical capsule weights (mg)	*n*	% recovery	
		Mean	95% confidence limits
Capsules made from crushed tablets			
1.19–1.25	6	100.2	3.8
Capsules made from pure bendrofluazide			
0.90–0.91	3	100.8	–
1.29–1.33	6	103.4	2.7
2.58, 2.60	1 [a]	100.4	–

[a] One anomalous result of 64.3% rejected.

man et al. (1984) avoided this by using a methanolic solvent, whereas Frontini and Mielck (1992) found an acidic aqueous solution was suitable. The methanolic standards and sample extracts used in the present work were stable for up to 5 days at ambient temperature in the dark followed by 1 day in diffuse light in full clear glass autosampler vials. However, with part-filled vials, stability was variable, with corresponding production of 4-amino-6-trifluromethyl-1,3-benzenedisulphonamide. Samples and standards would therefore be stable during automated analysis in diffuse light without any elaborate light protection. Standard solutions were also found to be stable for up to 1 month at −20°C.

The injection of methanolic bendrofluazide solutions has been found to cause peak broadening or splitting, for instance as found by Kirschbaum et al. (1982) or Perlman and Kirschbaum (1986). However, restricting the injected volume of solution to 10 μl produced a bendrofluazide peak with an acceptable shape. The symmetry factor, as defined by the British Pharmacopoeia (1988b), was 1.1. Increasing the injection volume to 20 μl gave an unacceptably broad peak.

The method was compared with the British Pharmacopoeia (1988b) assay method for bendrofluazide tablets. The latter method depends on ultraviolet spectroscopy of an alkali degraded extract. Assay of bendrofluazide tablets with this technique gave a value of 5.20 mg. Determination with the HPLC method gave a value of 5.00 mg.

The HPLC method is suitable for the determination of bendrofluazide in extemporaneously prepared capsules and would also be applicable for the assay of bendrofluazide tablets. Chromatography may be carried out at ambient temperature with acceptable peak shape.

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