High Performance Liquid Chromatographic Measurement of Bisoprolol in Plasma

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A simple high performance liquid chromatographic method has been devised for the measurement of bisoprolol in plasma or serum. The sample (200 μL) is vortex mixed for 30 s with 2 M Tris solution (50 μL), aqueous internal standard (benzimidazole, 2.0 mg/L, 50 μL) and methyl *t*-butyl ether (200 μL). After centrifugation (9950 × *g*, 2 min), a portion of the resulting extract is analysed on a microparticulate (5 μm) silica column using 1 mM camphorsulphonic acid in methanol as the mobile phase. Detection is by fluorescence at an excitation wavelength of 215 nM. The lower limit of accurate measurement for the assay is 10 μg/L (CV% = 8.9, *n* = 9) with a lower limit of detection of 5 μg/L. There is minimal interference from either commonly prescribed drugs or endogenous compounds.

INTRODUCTION

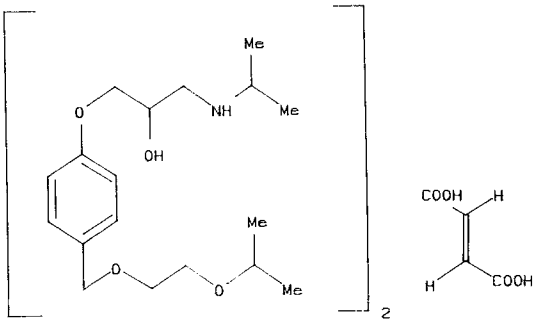
Bisoprolol fumarate **1** is a new β-adrenoreceptor antagonist with no partial agonist or membrane stabilizing activity (Lancaster and Sorkin, 1988). The drug has a high bioavailability (90%) and an elimination half-life which allows once-daily administration in most patients. Approximately 50% of the drug is metabolized by the liver to form three inactive carboxylic acid metabolites, while the remainder of the drug is excreted unchanged in urine (Bühring and Garbe, 1986). Less then 10% of the drug undergoes first-pass metabolism by the liver following oral dosage (Lancaster and Sorkin, 1988).

Two high performance liquid chromatographic methods for the measurement of bisoprolol in biological fluids have been published. Both methods require a relatively large sample volume (1 mL); one method involves acetylation (Bühring and Garbe, 1986) and the other a two-step liquid/liquid extraction, prior to chromatography (Poirier *et al.*, 1988).

The method described here is based on the extraction, at alkaline pH, of a small (200 μL) sample into an organic solvent. This extract is analysed by high performance liquid chromatography (HPLC) with fluorimetric detection. The method is suitable for the measurement of bisoprolol at the concentrations attained following either chronic therapy or single doses.

EXPERIMENTAL

Materials and reagents. Bisoprolol, (±)1-(4-[(2-isopropoxyethoxy)-methyl]-phenoxy)-3-isopropylamino-2-propanol fumarate **1**, was obtained from Cyanamid (Gosport, Hants., UK). The internal standard, benzimidazole, was obtained from BDH Chemicals Ltd. (Poole, Dorset, UK) and was used as a 2.0 mg/L aqueous solution by dilution of a 1 g/L methanolic solution of the compound. Methanol and methyl *t*-butyl ether were HPLC reagent grade (Rathburn, Walkerburn, Peebleshire, UK). Tris was obtained from BDH Chemicals Ltd. (Poole, Dorset, UK). D-10-camphorsulphonic acid monohydrate was obtained from Aldrich Chemical Company Ltd. (Gillingham, Dorset, UK).



Instrumentation. A constant-flow reciprocating pump (Applied Chromatography Systems, Macclesfield, Cheshire, UK, 750/04) was used, with a syringe loading injection valve (Rheodyne, Inc. Cotati, CA, USA, 7125, 100 μL sample loop). The analytical column was a stainless steel tube (125 × 5 mm ID) packed with Spherisorb S5W silica (5 μm average particle size, Hichrom, Woodley, Berks., UK) which was used at ambient temperature. The mobile phase was 1 mM camphorsulphonic acid in methanol, delivered at a flow rate of 2.0 mL/min. The column effluent was monitored using fluorescence detection (Kratos-Schoeffel FS 970, excitation wavelength 225 nm, no emission filter, time constant 0.5 s). Integration of peak areas was performed using a Hewlett Packard 3390A recording integrator.

Sample preparation. The sample (200 μL) was pipetted into a small (Dreyer) test tube, internal standard solution (20 μL), 2 M Tris solution (50 μL) and methyl *t*-butyl ether (200 μL) were added, using Hamilton gas-tight syringes fitted with Hamilton repeating mechanisms. The contents of the tube were vortex-mixed for 30 s, following which the tubes were centrifuged (9950 × *g*, 2 min, Eppendorf 5412). A portion of the extract was taken and used to fill the sample loop of the injection valve. Duplicate analyses were performed and the mean results used.

Instrument calibration. Standard solutions of bisoprolol fumarate at concentrations equivalent to 10, 20, 50, 100, 250 and 500 μg/L analyte free-base were prepared in equine plasma by serial dilution from a methanolic stock solution equivalent to 1.00 g/L bisoprolol free-base. Internal quality control samples containing bisoprolol fumarate at concentrations equivalent to 50, 175 and 400 μg/L, free-base, were prepared

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0269-3879/90/0178-0180 $5.00

in equine plasma by dilution from an independent stock solution of the drug equivalent to 1.00 g/L. These solutions were stable for at least 6 months when stored at −20 °C. On analysis of these standards the ratio of the peak area of bisoprolol, to the peak area of the internal standard, when plotted against bisoprolol concentration, was linear and passed through the origin of the graph, with a mean (±SD) slope, intercept and correlation coefficient (r) of 0.00207 (0.00009), 0.010186 (0.0134) and 0.99 (0.00178), respectively ($n = 10$).

RESULTS AND DISCUSSION

No endogenous sources of interference have been observed. However, flecainide, propranolol and metoprolol co-elute with bisoprolol under the conditions of the assay (Table 1). Interference from either propranolol or metoprolol, both β-blockers, is improbable, as they are unlikely to be co-prescribed with bisoprolol. Flecainide and bisoprolol can be differentiated by means of an emission filter (370 nm), which causes the response to bisoprolol to disappear. Therefore, if co-administration of these two drugs was suspected a separate analysis using this filter would be necessary.

Table 1. Retention times of bisoprolol and some other compounds relative to the internal standard, benzimidazole

Compound	Relative retention time
Desalkylflurazepam	0.55
Nitrazepam[a]	0.61
Mexiletine	0.61
4-Hydroxypropranolol	0.65
Ajmaline	0.68
Nadolol	0.68
Pyrimethamine[a]	0.68
Pindolol	0.69
Triamterene	0.69
Penbutolol	0.69
Propranolol	0.71
Nordextropropoxyphene	0.71
Metoprolol	0.71
Oxprenolol[a]	0.75
Flecainide	0.75
Bisoprolol	0.77
Prajmalium	0.79
Dipyridamole	0.82
Trimipramine	0.88
Desimpramine	0.90
Norverapamil	0.90
Prazosin	0.93
Terazosin	0.94
Timolol	0.95
Protriptyline	0.97
Benzimidazole	1.00
Dextropropoxyphene[a]	1.10
Trazodone	1.16
Orphenadrine	1.47
Butriptyline	1.48
Mianserin	1.60
Verapamil	1·68
Imipramine	1.85

[a] Poor fluorescence under the conditions of this assay.

Table 2. Within and between-assay reproducibility for bisoprolol ($n = 10$ at each concentration)

	Concentration (μg/L)	Coefficient of variation (%)
Within-assay	20	6.7
	50	4·7
	250	2.6
	500	1.6
Between-assay	20	7.0
	50	7.4
	250	5.3
	500	3.1

Silica columns and ionic methanolic eluants have been used successfully to achieve the separation of a number of basic cardioactive drugs (Flanagan and Jane, 1985; Jane *et al.*, 1985). Camphorsulphonic acid is a useful alternative ionic modifier to ammonium perchlorate and is used with fluorescence detection to remove baseline noise and improve peak shape. In this instance, the use of ammonium perchlorate as the ionic modifier did not improve the separation of bisoprolol from other β-blockers.

The within and between-assay coefficients of variation (CV) for replicate analyses of standard solutions of bisoprolol prepared in equine plasma are shown in Table 2. Using a sample size of 200 μL, the lower limit of accurate measurement for bisoprolol in plasma was taken as 10 μg/L (within-assay CV = 8·9%, $n = 9$). The limit of detection, based on a 5:1 signal:noise ratio, was taken as 5 μg/L.

The mean recovery of bisoprolol, from equine plasma, at concentrations between 50 and 500 μg/L was 105.8 ±

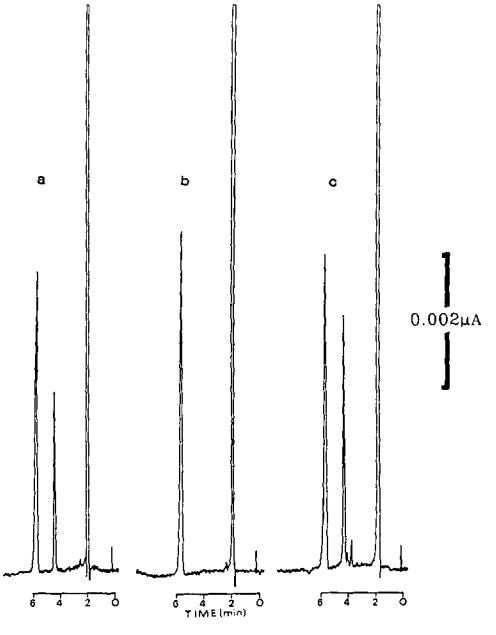

Figure 1. Chromatographs of extracts of (a) a standard solution prepared in equine plasma containing bisoprolol at a concentration of 250 μg/L, (b) bisoprolol-free equine plasma and (c) a plasma sample from a patient receiving bisoprolol; the bisoprolol concentration was 381 μg/L. The original concentration of benzimidazole was 2.0 mg/L in each case.

6.28%, when compared with methanolic solutions of the same concentrations ($n = 10$). The values were greater than 100% due to sample evaporation prior to chromatography of the extract. There was no difference between the recovery of the drug from equine or human plasma.

The chromatography of extracts of an equine plasma standard containing bisoprolol, bisoprolol-free human plasma and a plasma sample obtained from a patient receiving bisoprolol are shown in Fig. 1.

This method has been used successfully to measure bisoprolol in plasma samples from patients receiving the drug for the treatment of hypertension. Daily doses were in the range (0.075–0.150 mg/kg/day). Plasma concentrations in samples collected at random time points after daily dosing ranged from 20 to 380 μg/L. The method has sufficient sensitivity to measure the drug following a single oral dose but, if necessary, the sensitivity could be improved further by increasing the sample size, or altering the attenuation of the fluorimeter.

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Received 29 March 1990; accepted 17 April 1990.