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Note

High-performance liquid chromatographic method for the measurement of buflomedil in plasma and serum

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Buflomedil hydrochloride, 2′,4′,6′-trimethoxy-4-(pyrrolidinyl) butyrophenone hydrochloride (Fig. 1), is a vasoactive drug which acts through a variety of pharmacological mechanisms, including smooth muscle α-adrenoceptor blockade, to improve blood flow in ischaemic tissue. It has a broad therapeutic spectrum and has been used successfully in a number of clinical trials to aid the treatment of peripheral arterial vascular disease, cerebrovascular insufficiency and senile dementia, diabetic retinopathy, frostbite, cochlearvestibular disorders, aldodystrophies and Raynaud's phenomenon [1–4].

Buflomedil is extensively metabolised, with approximately 20% of orally absorbed buflomedil undergoing first-pass metabolism by the liver. After oral administration almost 90% of the dose is excreted in the urine; 19% as unchanged drug, the rest as conjugates of buflomedil metabolites [1]. The pharmacokinetic profile of buflomedil has been investigated in patients with renal disease, liver disease, diabetes and in healthy volunteers [5–9]. Typical plasma concentrations, derived from the studies in healthy volunteers, are in the range 0.5–4.5 mg/l [5–9].

The only published high-performance liquid chromatographic (HPLC) method for the measurement of buflomedil has been used for quality control assessment of tablet formulations and has been applied to a number of stability studies [10]. The method described here, however, gives improved peak shape and a shorter retention time.

EXPERIMENTAL

Materials and reagents

Buflomedil hydrochloride was supplied by Abbott Labs. (Queenborough, U.K.). The internal standard, metoclopramide hydrochloride, was obtained from Beecham Research Labs. (Greenford, U.K.) and was used as a 3.0 mg/l aqueous solution. Methanol and methyl *tert.*-butyl ether were HPLC reagent grade (Rathburn, Walkerburn, U.K.). Sodium hydroxide was analytical grade (BDH, Poole,

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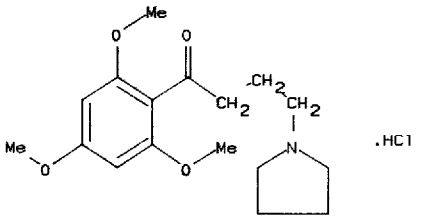

Fig. 1 Structure of buflomedil.

U.K.). D-10-Camphorsulphonic acid monohydrate (reagent grade) was obtained from Aldrich (Gillingham, U.K.).

Instrumentation

A constant-flow reciprocating pump (Knauer Model 64) was used with a syringe-loading valve (Rheodyne 7125, 100-μl sample loop). The analytical column was a stainless-steel tube (125 mm × 5 mm I.D.) packed with Spherisorb S5W silica (5 μm average particle size) (Hichrom, Woodly, U.K.) used at ambient temperature. The mobile phase was 3 m*M* camphorsulphonic acid in methanol delivered at a flow-rate of 2.0 ml/min. The column effluent was monitored by UV absorbance at 215 nm (Jasco Uvidec-100-V, time contant 0.5 s and attenuation 0.02 a.u.f.s.). Peak heights were integrated using a Shimadzu C-R4A recording integrator (Dysan Instruments, Houghton-le-Spring, U.K.).

Sample prepration

The sample (200 μl) and sodium hydroxide (4 *M*, 50 μl) were pipetted into a small glass (Dreyer) test-tube. Internal standard solution (50 μl) and methyl *tert.*-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 tube was centrifuged (9950 *g*, 2 min; Eppendorf 5412). A portion of the extract was used to fill the sample loop of the injection valve.

Instrument calibration

Standard solutions containing buflomedil hydrochloride at concentrations equivalent to 0.2, 0.5, 1.0, 2.0, and 3.0 mg/l bulomedil free-base were prepared in equine serum by serial dilution from an aqueous stock solution containing buflomedil hydrochloride at a concentration equivalent to 1.00 g/l free-base. Internal quality control samples containing buflomedil hydrochloride at concentrations equivalent to 0.75 and 1.5 mg/l buflomedil free-base were prepared in equine serum from an independently prepared stock solution of buflomedil hydrochloride. On analysis of the standards the ratios of the peak height of buflomedil to the peak height of the internal standard, when plotted against the analyte concentration, were linear and passed through the origin of the graph, with a slope of

0.73 ± 0.05, an intercept of 0.05 ± 0.022 and a correlation coefficient of 0.9966 ± 0.004 (mean ± S.D., $n = 10$). These standards were stable for at least six months when stored at −20°C in 0.5-ml aliquots.

RESULTS

No sources of endogenous interference were observed. In general, using this system, there is minimal interference from other commonly prescribed drugs [11] (Table I). Although the retention time for dothiepin is similar to that of buflomedil, it is resolved under the conditions of the assay and does not interfere with the

TABLE I

RETENTION TIMES OF BUFLOMEDIL AND SOME OTHER COMPOUNDS RELATIVE TO THE INTERNAL STANDARD

Compound	Relative retention time	Compound	Relative retention time
Pindolol	0.30	Mepivicaine	0.42
Acebutolol	0.31	Mexiletine	0.44
Doxazosin	0.31	Propafenone	0.45
Nadolol	0.31	Mianserin	0.46
5-Hydroxypropafenone	0.31	Doxepin	0.46
Propranolol	0.31	Amiodarone	0.49
Timolol	0.31	Trimipramine	0.50
Bisoprolol	0.32	Prilocaine	0.50
Flecainide	0.32	Diltiazem	0.51
Atenolol	0.33[a]	Nortriptyline	0.51
Betaxolol	0.33	N-Acetylprocainamide	0.52
Ketanserinol	0.33	Amitriptyline	0.55
Metaprolol	0.33	Phenazone	0.56
Nicardipine	0.33	Tocainide	0.57
Oxprenolol	0.33	Verapamil	0.58
Penbutolol	0.33	Desacetyldiltiazem	0.67
Ajmaline	0.35	Flurazepam	0.71[b]
Cibenzoline	0.35	Buflomedil	0.75
Prazosin	0.35	Dothiepin	0.76
Maprotyline	0.36	Imipramine	0.78
Norverapamil	0.37	Bretylium	0.82[a]
Recainam	0.37	Disopyramide	0.93
Ketanserin	0.38	Metoclopramide	1.00
Terazosin	0.38	Procainamide	1.06
Lignocaine	0.40	Quinine	1.43[b]
Dextropropoxyphene	0.41	Quinidine	1.46[b]
Diazepam	0.41		

[a] Compound does not extract under the conditions of the assay

[b] Tailing peaks.

measurement of buflomedil. Some cardioactive drugs and metabolites, for example lorcainide, desethylamiodarone and nifedipine, were not detected on this system. Conjugated metabolites of buflomedil would not be extracted under the conditions of the assay. The chromatography of extracts of a buflomedil standard prepared in equine serum, of serum from a patient receiving buflomedil and of buflomedil-free human serum are illustrated in Fig. 2a–c, respectively.

The mean recoveries of buflomedil from serum, at concentrations of 0.2, 2.0 and 5.0 mg/l were 76.7, 84.5 and 76.1%, respectively, when compared with a methanolic solution of buflomedil of the same concentration ($n=8$). There was no significant difference between the extraction efficiency of buflomedil from equine serum, human plasma of human serum. A buflomedil standard prepared in analyte-free human plasma at a concentration of 1.0 mg/l gave a mean (± S.D.) measured concentration of 0.98 ± 0.05 mg/l ($n=5$) against standards prepared in equine serum. Similarly, aliquots of human serum spiked to buflomedil concentrations of 0.5, 1.0, and 2.0 mg/l were analysed against standards prepared in equine serum. There was close agreement between the measured and expected values at all three concentrations. The results are shown in Table II.

The within- and between-assay coefficients of variation (C.V.) for replicate

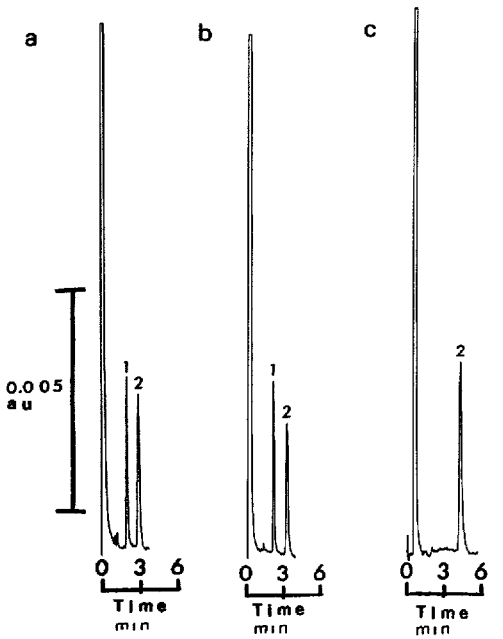

Fig. 2. Chromatograhpy of extracts of (a) an equine serum standard containing 2 0 mg/l buflomedil, (b) a serum sample from a patient receiving buflomedil (the buflomedil concentration was 2 53 mg/l) and (c) buflomedil-free human serum. The initial metoclopramide concentration was 3 mg/l Peaks: 1 = buflomedil; 2 = metoclopramide.

TABLE II

MEASUREMENT OF BUFLOMEDIL IN HUMAN SERUM AGAINST STANDARDS PREPARED IN EQUINE SERUM

Measured concentration (mg/l)			
Added.	0.50 mg/l	Added: 1 00 mg/l	Added: 2.00 mg/l
	0.48	0.90	1.84
	0.51	0.95	1 95
	0.43	0 93	1.91
	0.47	0.93	1.98
	0.51	0.98	1.95
	0 48	0.98	1 99
	0 53	1.03	2.03
	0.51	0.96	2.08
	0.49	1.10	1.93
	0.50	0.98	2.14
Mean	0.49	0.97	1.98
S.D.	0.03	0.06	0.09
C.V (%)	5.72	5.85	4.36

analyses of standard solutions of buflomedil prepared in equine serum are summarised in Table III, together with the minimum and maximum measured concentrations for the spiked standards. Using a sample size of 200 μl, the lower limit of accurate measurement for buflomedil was 0.1 mg/l (C.V. = 10.6%; $n = 10$) with a lower limit of detection, based on a 5:1 signal-to-noise ratio, of 0.05 mg/l. If

TABLE III

SUMMARY OF WITHIN- AND BETWEEN-ASSAY REPRODUCIBILITY FOR BUFLOMEDIL ($n = 10$)

Weighed- in concentration (mg/l)	Concentration found (mg/l)			C.V. (%)
	Mean	Minimum	Maximum	
Within-assay				
0.20	0.21	0.19	0.24	6.58
0.50	0.52	0.51	0 55	2.46
2.20	2.23	2 15	2.28	1.86
Between-assay				
0.75	0.70	0.64	0.79	7 14
1.60	1.66	1.57	1.80	4 12

necessary, the sensitivity of the assay could be improved by either operating the instrument at a more sensitive attenuation, *e.g.* 0.01 a.u.f.s., or by using a larger sample volume.

Silica columns and ionic methanolic eluents have been used successfully for the separation of a number of basic cardioactive drugs. Camphorsulphonic acid provides a useful alternative to ammonium perchlorate when strongly acidic, high-ionic-strength mobile phases are required [11,12]. This method is both sensitive and selective for the measurement of buflomedil in plasma or serum and has been used to measure the drug in samples from patients and volunteers involved in clinical trials and pharmacokinetic studies.

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