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## Note

# Gas chromatographic evaluation of buflomedil in biological samples employing the thermoionic specific detector

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Buflomedil hydrochloride is a vasodilator therapeutically employed both in peripheral and cerebral vascular diseases [1–4]. Although the pharmacokinetics of buflomedil in humans and in animals after oral or intravenous administration are already known [5–8], previous methods using high-performance liquid chromatography (HPLC) with UV detection or gas chromatography (GC) with thermoionic specific detection (TSD) seem to lack sensitivity. They require 2–4 ml of plasma for each analysis [7,8] and the lowest detectable amount is 1 $\mu$g/ml [6] or 30–50 ng/ml [5,7–9]. The extraction recoveries, 55% [7] or 40% [6], are also poor.

This paper describes a highly sensitive, specific and reproducible assay of buflomedil in biological samples employing the GC–TSD technique with a capillary column, which reaches a lowest detectable concentration of 10 ng/ml of plasma.

## EXPERIMENTAL

### *Chemicals*

All reagents and solvents were of analytical grade and were supplied by Merck (Bracco, Milan, Italy). Reagents for GC were supplied by Supelco (Supelchem, Milan, Italy).

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*Instrumentation*

A Varian 3400 gas chromatograph equipped with a thermoionic specific detector was used. Mass spectrometry (MS) was carried out with an MS 70/70 spectrometer from VG instruments, combined with a Varian gas chromatograph (Model 3400).

Data were processed with an Apple MacIntosh personal computer equipped with specific software for statistical and pharmacokinetic calculations.

*Extraction*

In a glass-stoppered test-tube, 1 ml of plasma (or urine) was mixed with 0.2 ml of 1 *M* potassium hydroxide and 5 ml of diethyl ether–*n*-hexane (8:2, v/v). The mixture was stirred for 10 min and then centrifuged at 2400 *g* for 10 min. An aliquot of the supernatant was evaporated to dryness. The residue was redissolved in 300 μl of methanol and evaporated again to dryness. The solution was reconstituted with 20–30 μl of methanol and 0.5–1.0 μl of this solution was injected into the gas chromatograph.

Amitriptyline (internal standard, I.S.) was added just before injection for evaluating buflomedil recovery, and before extraction in routine analysis.

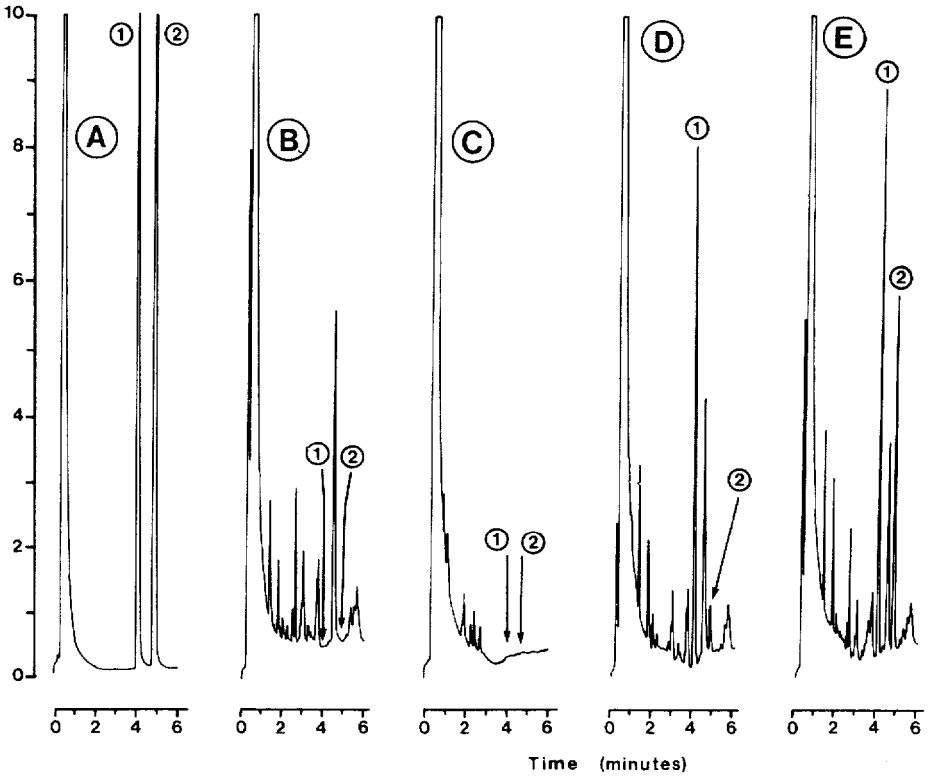


Fig. 1. Typical chromatograms of buflomedil. (A) Buflomedil and amitryptiline (I.S.) as authentic standards; (B) a blank plasma extract; (C) a blank urine extract; (D) a plasma extract spiked with buflomedil (20 ng/ml) and I.S.; (E) an extract of a plasma sample from a volunteer treated with buflomedil pyridoxal phosphate (400 mg per os). Peaks: 1 = I.S.; 2 = buflomedil.

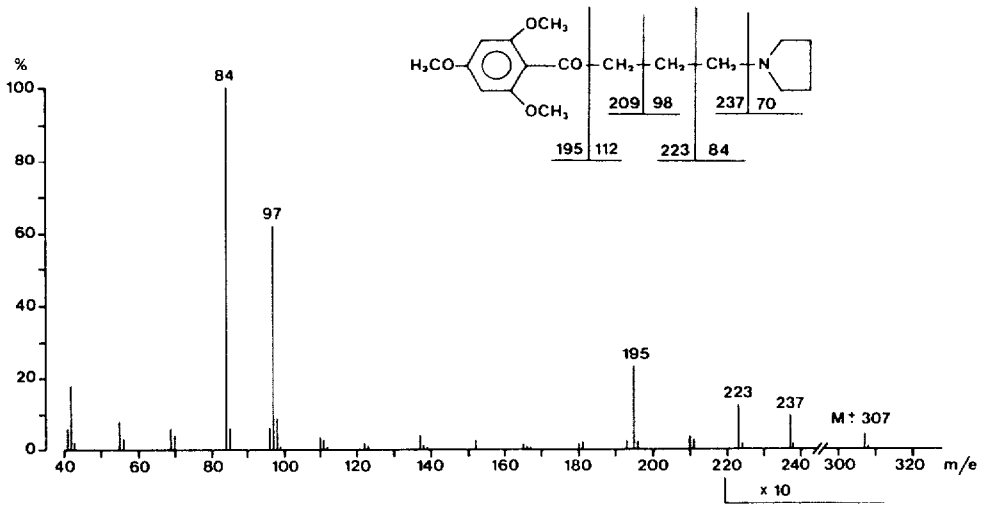


Fig. 2. Mass spectrum of buflomedil.

*Gas chromatographic conditions*

A DB-1 Megabore column (15 m ×0.5 mm I.D.) with a film thickness of 1.5 μm was used. Temperatures were 220°C for the injector, 180°C for 1 min and then 10°C/min up to 250°C for 1 min for the oven and 280°C for the detector. Nitrogen was used as carrier gas (0.6 bar). Bead current was 3.6 A. Quantification of buflomedil was achieved by peak-area measurement carried out with an automatic integrator.

Fig. 1 shows typical GC recordings of buflomedil (retention time 4.76 min) and internal standard (retention time 4.04 min).

In Fig. 2 the mass spectrum of the peak at 4.76 min obtained with the GC–MS system shows the buflomedil fragmentation pattern.

*Application of the method to pharmacokinetic analysis*

Twelve healthy volunteers were treated with buflomedil pyridoxal phosphate salt, two capsules containing 400 mg of the salt, i.e. 222 mg of buflomedil (corresponding to 248 mg of buflomedil hydrochloride). Before and at various times after administration, heparinized blood samples (5 ml) were withdrawn and buflomedil was extracted and analysed as above. Buflomedil was also analysed in the urine collected cumulatively in periods 0–3, 3–6, 6–12 and 12–24 h.

## RESULTS

*Linearity*

Linearity was ascertained with authentic standards by injecting buflomedil and the I.S. in both fixed and variable ratios, with a constant detector response factor (*drf*) taken as an evidence for linearity. At a fixed buflomedil/I.S. ratio (1:1, w/w), linearity was ascertained in the range 0.2–50 ng injected, with $drf = 1.22$ on average and a coefficient of variation in the range 0.37–2.89% (Table I). When the buflomedil/I.S. ratio was varied from 4:1 to 1:4, the *drf* was 1.22 again on

TABLE I

LINEARITY OF DETECTOR RESPONSE TO INCREASING AMOUNTS OF BUFLOMEDIL AND AMITRYPTILINE INJECTED AT CONSTANT 1:1 RATIO

| Buflomedil injected (ng) | Mean *drf* ($n=4$) | Coefficient of variation (%) |
|---|---|---|
| 0.2 | 1.19 | 2.26 |
| 0.5 | 1.21 | 0.37 |
| 1 | 1.22 | 2.89 |
| 2 | 1.24 | 1.33 |
| 5 | 1.23 | 1.21 |
| 10 | 1.22 | 1.66 |
| 20 | 1.23 | 1.85 |
| 50 | 1.22 | 1.52 |
| Mean | 1.22 | |

TABLE II

LINEARITY OF DETECTOR RESPONSE TO BUFLOMEDIL AND I.S. AT VARIABLE RATIOS

| Amount injected (ng) | | Mean *drf* ($n=4$) | Coefficient of variation (%) |
|---|---|---|---|
| Buflomedil | I.S. | | |
| 1 | 4 | 1.22 | 1.41 |
| 2 | 4 | 1.22 | 1.52 |
| 4 | 4 | 1.22 | 2.36 |
| 8 | 4 | 1.22 | 1.21 |
| 16 | 4 | 1.23 | 3.49 |
| Mean | | 1.22 | |

TABLE III

RECOVERY OF BUFLOMEDIL FROM PLASMA

Buflomedil added ($x$) and that found ($y$) were related as follows: $y=-1.56+1.00\,x$ ($r^2=0.99999$).

| Buflomedil added (ng/ml) | Concentration found (mean ± S.D., $n=4$) (ng/ml) | Intra-assay variation (%) | Recovery (%) |
|---|---|---|---|
| 10 | 8.1 ± 4.06 | 5.01 | 81.0 |
| 20 | 19.7 ± 1.72 | 1.75 | 98.5 |
| 50 | 49.2 ± 1.94 | 1.97 | 98.4 |
| 100 | 98.5 ± 2.00 | 2.03 | 98.5 |
| 200 | 200.2 ± 1.68 | 1.68 | 100.1 |
| 500 | 496.0 ± 2.18 | 2.20 | 99.2 |
| 1000 | 995.0 ± 1.84 | 1.84 | 99.5 |
| 2000 | 2000.0 ± 2.29 | 2.29 | 100.0 |
| Mean | | | 96.9 |
| Inter-assay variation | | 6.67 | |

average with a coefficient of variation in the range 1.21–3.49% (Table II). When the same amount of buflomedil (4 ng) was injected ten times into the gas chromatograph, a *drf* of 1.25 on average and a coefficient of variation of 1.42% were obtained.

*Extraction recovery*

In the range 10–2000 ng/ml, the recovery was 96.9% with a linear regression coefficient ($r^2$) of 0.99999. The intra-assay variation was 1.68–5.01% (2.35 on average); the inter-assay variation was 6.67% (Table III). The long-term reproducibility was similar to the inter-assay variability. The recovery of amitriptyline was 97.5% (S.D. = 1.32, four replications).

*Specificity*

The separation between the analytical peaks and endogenous peaks was good both in plasma and urine (Fig. 1). The limit of detection was 10 ng/ml, with a coefficient of variation of 5.01%.

*Pharmacokinetic results on volunteers*

Fig. 3 shows the mean plasma concentrations of buflomedil in twelve healthy volunteers treated orally with 400 mg of buflomedil pyridoxal phosphate salt (222 mg of buflomedil base). Fig 4 shows the cumulative urinary excretion of the parent drug in the same volunteers. The pharmacokinetic parameters were as follows: peak time, $t_{max} = 1.9$ h; peak concentration, $C_{max} = 1153$ ng/ml; area under

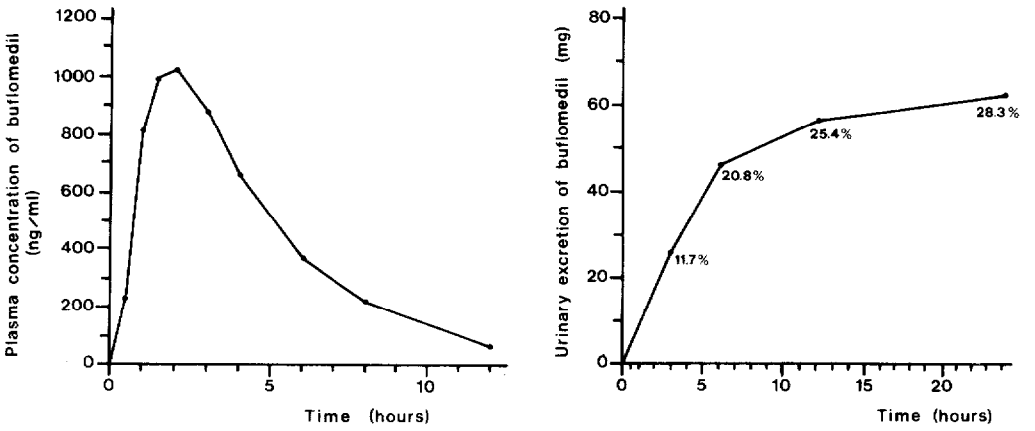


Fig. 3. Mean plasma concentration-time curve of buflomedil in twelve volunteers treated orally with 400 mg of buflomedil pyridoxal phosphate (222 mg of buflomedil).

Fig. 4. Mean cumulative urinary excretion of buflomedil in twelve healthy volunteers treated orally with 400 mg of buflomedil pyridoxal phosphate (222 mg of buflomedil).

the curve, $AUC_{0-\infty}=5474$ ng/ml h; apparent renal clearance = 0.18 l/h/kg; apparent volume of distribution = 2.18 l/kg; terminal half-life, $t_{1/2\beta}=2.5$ h.

## DISCUSSION

This method was validated for the study of the bioavailability and pharmacokinetics of buflomedil. Analytical parameters were optimized to reach a recovery close to 100% and a lowest detectable concentration of 10 ng/ml. In this respect this method is preferable to previously published ones, in that it allows the $\beta$-phase to be carefully evaluated.

The method requires chromatographic equipment commonly available in a pharmacokinetic laboratory. A skillful operator can process more than 40 samples a day by manual injection and using an automatic integrator.

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