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## Note

# Analysis of buflomedil in mouse, rat and rabbit plasma by reversed-phase ion-pair high-performance liquid chromatography

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Buflomedil hydrochloride [4-(1-pyrolidinyl)-1-(2,4,6-trimethoxyphenyl)-1-butanone hydrochloride] is a vasoactive agent [1—4] marketed as 150-mg tablets (Fonzylane®, Lafon Laboratories and Loftyl®, Abbott Laboratories). We were interested in measuring buflomedil plasma levels in mice, rats and rabbits following oral administration of this drug. Badmin et al. [5] reported an ion-pair reversed-phase high-performance liquid chromatographic (HPLC) method for the determination of buflomedil hydrochloride in tablet formulations. The detection range for the hydrochloride salt in Badmin's method [5] was 100—200 μg/ml. The HPLC method presented here differs in the concentration of the ion-pair reagent, choice of internal standard, and choice of organic modifier for the mobile phase. The extraction and reconstitution procedure developed for this assay increased the plasma drug concentration about ten-fold and thus provided a detection limit of about 0.03 μg/ml.

## EXPERIMENTAL

Nanograde benzene (Mallinckrodt, St. Louis, MO, U.S.A.) and HPLC-grade acetonitrile and ethyl acetate (Burdick and Jackson Labs., Muskegon, MI, U.S.A.) were used. Acetic acid (AR grade) and sodium lauryl sulfate were obtained from Mallinckrodt and Aldrich (Milwaukee, WI, U.S.A.), respectively. Prazepam was obtained from Warner Lambert (Morris Plains, NJ, U.S.A.). All chemicals and reagents were used as received.

Extractions were performed on an Eberbach (Ann Arbor, MI, U.S.A.) reciprocal shaker. A Waters Assoc. (Milford, MA, U.S.A.) pump and automatic injector were used with an LDC (Riviera Beach, FL, U.S.A.) Spectromonitor III variable-wavelength detector and a Varian (Walnut Creek, CA, U.S.A.)

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Model A-25 recorder. The column (30 cm × 3.9 mm I.D.) was μBondapak $C_{18}$ (Waters Assoc.; particle size 10 μm). Peak areas and retention times were determined with a Spectra Physics (Santa Clara, CA, U.S.A.) System I integrator.

The mobile phase was 0.008 *M* sodium lauryl sulfate in water—acetonitrile—glacial acetic acid (52:48:0.1) filtered through a 0.4-μm Nucleopore (Pleasanton, CA, U.S.A.) polycarbonate membrane. Operating conditions were: flow-rate, 2.0 ml/min; column temperature, ambient; detector wavelength, 275 nm; attenuation, 0.005 a.u.f.s.; and chart speed, 0.1 in./min. The injection volume was 50—200 μl depending on the expected drug concentration.

For extraction, 1 ml of the standard or 3—4 ml of the unknown plasma sample were pipetted into 20-ml screw-cap tubes. One ml of 1 *M* sodium carbonate and 10 ml of the organic mixture, benzene—ethyl acetate (7:3, v/v) containing the internal standard, prazepam (0.1 μg/ml), were added to all the tubes. The tubes were tightly capped with Teflon®-lined caps, shaken for 10 min at 180 cycles/min and then centrifuged for 2 min at 400 *g*. Aliquots (8 ml) of the organic layer (upper) were transferred to conical tubes and evaporated to dryness in a heated water bath (55—60°C) under a gentle air stream. The residues were reconstituted in 300 μl of mobile phase and an aliquot injected into the HPLC system. Peak areas for buflomedil and prazepam were measured and the concentration of buflomedil in the unknown determined by the internal standard method.

## RESULTS AND DISCUSSION

Originally the method was developed in human plasma, the standard curve covered the range 0.45—178 μg/ml and nordiazepam was chosen as the internal standard. When this method was applied to animal samples, it was found that many endogenous compounds eluted in the area of nordiazepam. Another benzodiazepine, prazepam, was therefore chosen as the internal standard. The concentration of buflomedil in these animal samples was also much lower than expected so the standard curve range was changed to 0.04—8.94 μg/ml. The final extraction procedure (given above in detail) yielded an 8—10.7 fold concentration of drug from the plasma samples. The same chromatographic conditions, as developed for human plasma samples, were employed to assay for buflomedil in these animal samples.

Chromatograms of blank animal and human plasma samples are shown in Fig. 1. For reference, the retention times for nordiazepam, prazepam and buflomedil are marked on the human sample chromatogram. As expected, the endogenous peaks from these different species produced different background patterns. These differences in background patterns from human to animal samples as analyzed by reversed-phase HPLC with UV detection have been noted before by Assenza and Brown [6]. A point (0.45 μg/ml) on each of the standard curves used for the analysis of buflomedil in rat, mice and rabbit plasma samples is shown in Fig. 2. The retention times were 11 min for buflomedil and 7 min for the internal standard.

Table I lists the parameters for a least-squares linear regression analysis for a typical standard curve for each species. Analysis of 14 standard curves over

a 3-month period indicated that all correlation coefficients from the linear regression analysis were 0.99 or greater. The day-to-day coefficients of variation for the slope of the calibration curve were 10.6%, 8.6% and 11.4% for the mouse, rat, and rabbit samples, respectively. The detection limit was estimated to be about 0.03 μg/ml for all animal samples.

The precision of this HPLC assay for buflomedil in mouse, rat and rabbit plasma samples was determined by calculating a mean concentration ± S.D. for each of the five standards from assays of three or more replicate curves, over a 1—4 week period. The results of this precision study are shown in Table II. The average coefficients of variation (C.V.) in analyzing these different samples were 8.9% for mouse, 8.1% for rat and 9.6% for rabbit samples. The overall, average C.V. for animal plasma samples assayed for buflomedil by this method with a concentration range of 0.04—8.94 μg/ml is 8.9%.

To determine the percent recovery in the extraction procedure, peak areas of an extracted spiked human plasma sample were compared with peak areas of an unextracted mobile phase solution at the same known concentration. The recovery was checked from 0.89—89.40 μg/ml, and the average recovery was 95.1 ±3.2%.

The stability of buflomedil in frozen plasma was studied. Blank human plas-

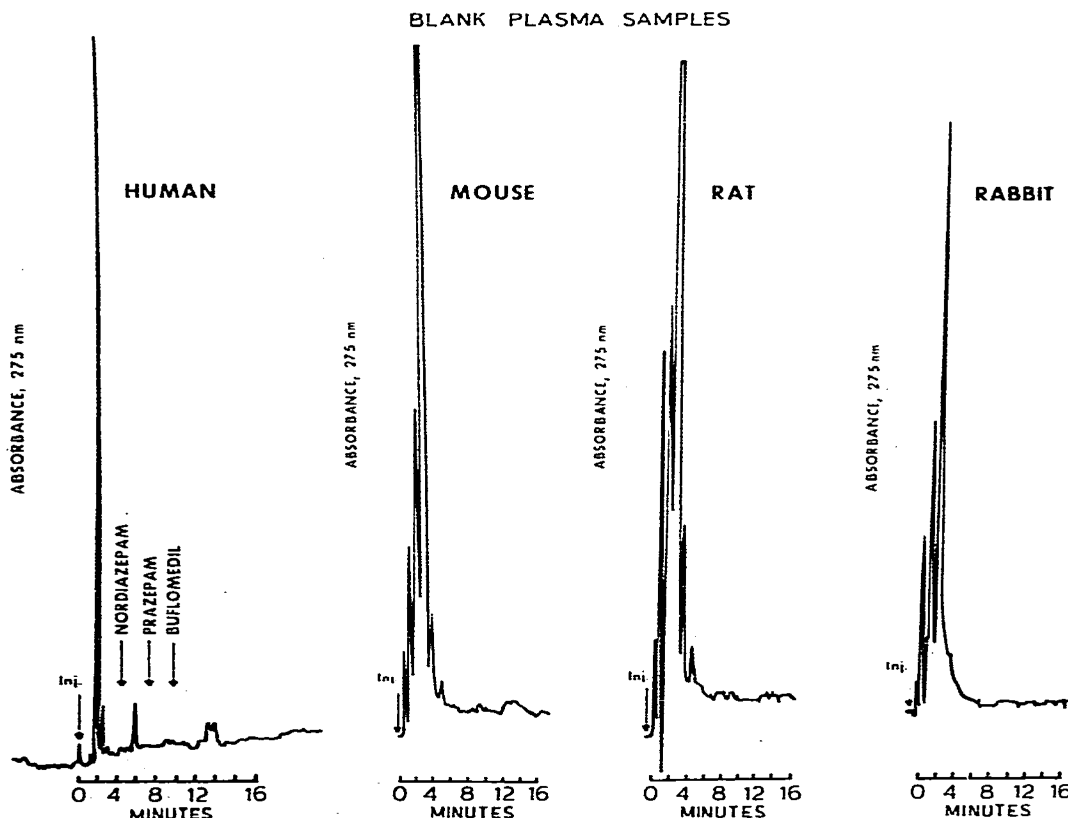


Fig. 1. Chromatograms of extracted blank human, mouse, rat and rabbit plasma as processed by this method.

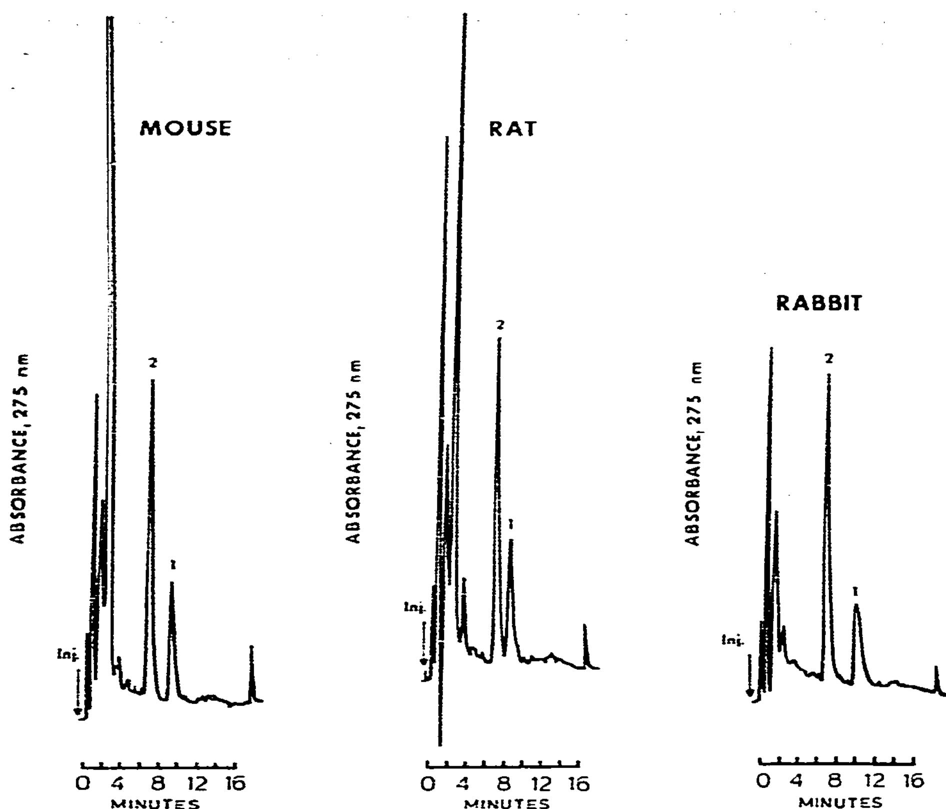


Fig. 2. Chromatograms of extracted mouse, rat and rabbit plasma spiked with buflomedil, 0.45 μg/ml (1) and the internal standard, prazepam (2).

TABLE I

LEAST-SQUARES LINEAR REGRESSION ANALYSIS OF THE CALIBRATION CURVE FOR BUFLOMEDIL BY HPLC

| Animal | y-Intercept | Slope | Correlation coefficient |
|---|---|---|---|
| Mouse | 0.002 | 0.979 | 0.999 |
| Rat | 0.028 | 1.029 | 0.997 |
| Rabbit | 0.005 | 1.056 | 0.997 |

ma was spiked with the drug at the level of 8.94 μg/ml and then stored at −20°C. Four months later, samples were thawed and assayed in triplicate using freshly spiked human plasma sample analyzed in triplicate as the 100% reference. The average assay value was 9.08 μg/ml (102.2% recovery) which shows there is no appreciable degradation on freezer storage for four months.

Six samples of blank human plasma were spiked with buflomedil, 8.94 μg/ml, and processed by this method. The residues were dissolved in the mobile phase and left at room temperature for analysis. They were assayed over a

TABLE II

PRECISION OF THE ANALYTICAL PROCEDURE FOR DETERMINING BUFLOMEDIL IN PLASMA

Values given are mean ±S.D.

| Theory (μg/ml) | Found | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mouse | | | Rat | | | Rabbit | | |
| | μg/ml | C.V. (%) | n | μg/ml | C.V. (%) | n | μg/ml | C.V. (%) | n |
| 8.94 | 9.19 ± 0.51 | 5.5 | 3 | 8.86 ± 0.41 | 4.7 | 8 | 8.70 ± 0.60 | 6.9 | 3 |
| 4.47 | 4.62 ± 0.20 | 4.3 | 3 | 4.45 ± 0.32 | 7.2 | 8 | 4.66 ± 0.31 | 6.7 | 5 |
| 0.45 | 0.44 ± 0.02 | 5.6 | 3 | 0.45 ± 0.03 | 7.7 | 7 | 0.43 ± 0.05 | 11.5 | 5 |
| 0.09 | 0.10 ± 0.02 | 18.0 | 3 | 0.09 ± 0.004 | 4.4 | 7 | 0.10 ± 0.01 | 15.0 | 5 |
| 0.04 | 0.04 ± 0.01 | 11.2 | 3 | 0.05 ± 0.01 | 16.7 | 7 | 0.04 ± 0.003 | 7.8 | 5 |

23-h period and showed no drug or internal standard degradation. The average recovery was 8.37 ±0.26 μg/ml (3.1%). Thus, samples can be processed by this method, loaded onto an automatic sampler and left for an overnight analysis with no detrimental effect on the analytical results.

This method has been used to analyze over 346 plasma samples from mice, rats and rabbits. A typical plot of plasma concentration versus time post dosing (orally) for rats is shown in Fig. 3. With the noted options for the internal standard, it should be applicable to the analysis of buflomedil in other animal and/or human plasma samples.

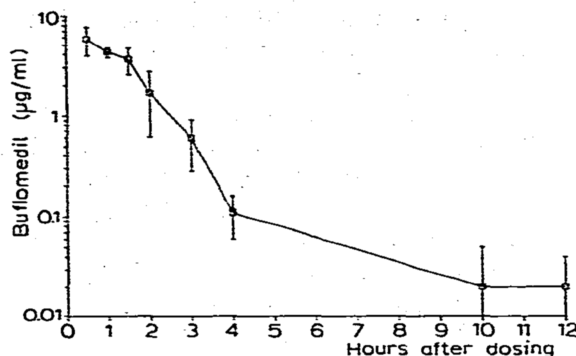


Fig. 3. Mean plasma concentration ±S.D. of buflomedil at selected time intervals following oral administration to five rats.

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