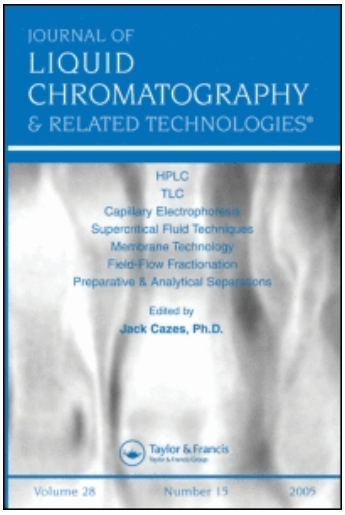

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Rapid Determination of Bupropion in Human Plasma by High Performance Liquid Chromatography

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RAPID DETERMINATION OF BUPROPION IN HUMAN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A high performance liquid chromatographic (HPLC) technique has been developed for the determination of bupropion hydrochloride (Bup) in human plasma, using a reversed-phase method, with UV detection at 250 nm.

The internal standard 5-(P-methylphenyl)-5-phenylhydantoin (MPPH), was used as an aid to quantitation. The plasma was deproteinized with acetonitrile and the clear supernatant was directly injected in the chromatographic system. The lower limit of quantitation was 5.0 ng/ml using only 100 µl of plasma sample.

Linear regression analysis for the calibration plots obtained on five different days over a two-week period for the two ranges used (10-250 ng/ml and 250-2000 ng/ml) in plasma indicated excellent linearity and reproducibility. The mean recovery of spiked Bup in plasma samples over the concentrations studied was found 96.5 ± 3.14%.

The method revealed that more than 30% of Bup was lost when the supernatant was stored at room temperature for 24 hrs.

INTRODUCTION

Bupropion hydrochloride (Bup) [(±)2-tert-butylamino-3'chloropropionphenone hydrochloride] (Fig.I) is a structrally unique anti-depressant with neurochemical different from either commonly used tricyclic antidepressants, monoamino oxidase inhibitors or

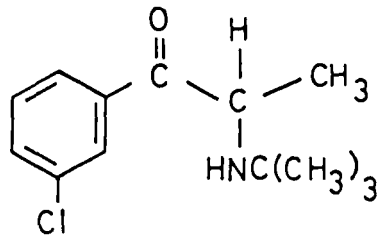

FIGURE 1. Chemical Structure of Bupropion

any of the second generation antidepressants available (1-2). The safety and clinical efficacy of Bup have been shown in open uncontrolled and placebo-controlled studies (3-6).

Limited number of analytical methods have been reported for the assay of Bup in biological fluids. These include radioimmunoassay (RIA) (7) and HPLC (8-9). The RIA is sensitive (<1ng/ml) and specific but needs expensive equipment and well-trained personnel to perform, whereas HPLC method reported by Schroeder et al. (8) is less sensitive (50 ng/ml). Although the method reported by Cooper et al. (9) appears to be sensitive (5 ng/ml), it requires the extraction of drug from plasma samples prior to injection onto the column.

The purpose of this work is to develop a simple, rapid and sensitive HPLC method for the determination of Bup in human plasma within the therapeutic concentrations range taking into account its stability during the time of analysis.

Apparatus:

A Waters HPLC Unit (Waters Associates, Milford, MA, USA) was used. It consisted of an M-45 model pump; an auto-injector (WISP-710B); System Controller Model M-720, UV-detector Model M 481 and a Data Module M 730, cartridge u-Bondapak C_{18} column 10cm length

x 8mm I.D. prepacked with 10-um ultrasphere octadecylsilane (ODS) operated with precolumn (Waters Associates Milford, MA) were used for separation.

Chemicals and Reagents:

All chemicals and reagents were of analytical grade and used as received. Acetonitrile was of HPLC grade. Bup hydrochloride was kindly donated by Burrough's Wellcome Co., (North Carolina, USA) and 5-(P-Methylphenyl)-5-phenylhydantoin (MPPH) (the internal standard) was obtained from Supleco, Inc. (Bellefonte, PA, Swit- zerland).

Chromatographic Conditions:

A mobile phase containing 40% v/v acetonitrile in water, adjusted to a final pH of 3.15 by the addition of perchloric acid was used. The mixture was filtered through a 0.45-um Pore size membrane filter (Millipore Corp. Bedford, MA), and degased before use. The flow rate was 1.4 ml/min. The UV detector was set a 250 nm and 0.002 (AUFS) for sensitivity. The separation was carried out at ambient temperature.

Stock Solutions:

A fresh stock solution of 1.0 mg/ml of Bup was prepared in the mobile phase. A stock solution of 1.0 mg/ml of internal standard (MPPH) was used as received. This solution was diluted to a concentration of 15 ug/ml or 5.0 ug/ml with acetonitrile as needed.

Preparation of Aqueous Standard Solutions:

The stock solution of Bup (1.0 mg/ml in mobile phase) was diluted with water to obtain a working solution containing 10 µg/ml prepared daily. This solution was appropriately diluted with distilled water to obtain the first standard solutions in the range of 10-250 ng/ml. A 100-ul aliquots of these solutions

transferred to 0.5-ml centrifuge tubes and a 100-µl aliquot of the internal solution containing 5.0 µg/ml was added to each tube. The standard solutions used to construct the standard curve in the range of 250-2000 ng/ml were prepared as before except that the internal standard concentration used was 15 µg/ml. A 100-µl aliquot of these solutions was transferred to a 0.5 ml centrifuge tube and 100-µl aliquot of the internal standard containing 15 µg/ml was added. The tubes were vortexed for one minute then centrifuged for five minutes at 4000 rpm (J6B, Beckman, USA) then 20-µl aliquots of the supernatant were injected onto the column within two hours of preparation.

Sample Preparation in Human Plasma:

A 100-µl sample of human plasma was spiked with the appropriate amount of Bup and the internal standard as described above. The tubes were vortexed for one minute and centrifuged at 4000 rpm for five minutes, then a 20-µl aliquot of the supernatant was injected onto the column within two hours of preparation.

Reproducibility:

The reproducibility of the assay was tested by repeating the standard plot over the range mentioned earlier on five different days over a two week-period. Five replicate samples at each concentration were performed for statistical analysis.

Recovery Studies:

The recovery of Bup relative to aqueous standards was assayed by adding equivalent amounts of Bup and the internal standard (MPPH) to plasma and aqueous solution. Five replicate determinations at each concentration were performed, using the same assay procedure described earlier, to assess recovery of Bup from plasma samples. The peak heigh ratio (PHR) (Bup/MPPH) of plasma was compared with the PHR of aqueous solution.

Stability in Human Plasma:

The procedure used in the sample preparation was adopted except the samples of clear supernatant were kept for 24 hours at room temperature after preparation. A 20-µl aliquot of each sample was injected onto the column.

RESULTS AND DISCUSSION

Figure 2 illustrates typical chromatograms obtained in the analysis of Bup (A) and Bup and MPPH (B) in aqueous solution. The retention times were 9.0 and 12.2 minutes respectively. In Figure 3, chromatograms of blank plasma (C),human plasma containing Bup (D) and human plasma containing Bup and MPPH (E);peak (a) and (b) are for Bup and MPPH respectively, with the same retention time as that observed with aqueous solution. Peak shapes and resolution are good and no interfering peaks in the same retention time observed.

A linear relationship between Bup concentration and the PHR (Bup/MPPH) in the concentration range of the two standard curves of plasma was observed (Figure 4). The equations describing the standard curves, determined by linear least-squares regression analysis were: PHR = 0.015 ± 0.00362 (conc.) and PHR = 0.015 ± 0.00124 (conc.) for ranges (10-250 ng/ml) and (250-2000 ng/ml), respectively. The corrleation coefficient (r) of both curves was 0.999.

Reproducibility of the assay was evaluated by comparing the linear regression line of the five standard plots obtained from plasma in the two ranges studied at five different days over the two week period. The results of this evaluation are presented in Table 1. Analysis of variance of these data performed using a computer program (10) showed a statistically insignificant

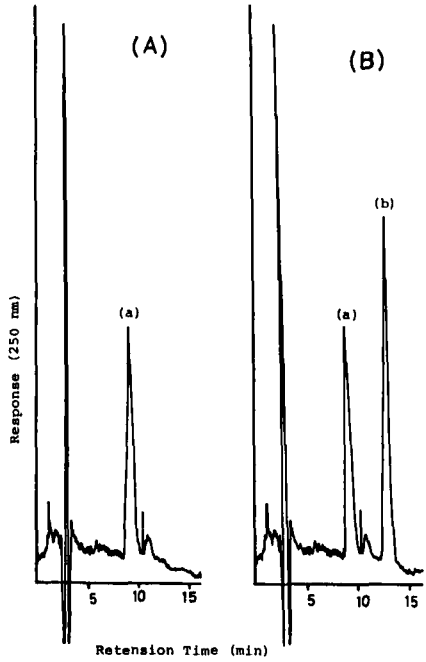

FIGURE 2. Chromatogram of Bupropion (a) and MPPH as Internal/Standard (b) in Aqueous Solution.

A) Aqueous Solution Spiked with Bup, 500 ng/ml.

B) Aqueous Solution Spiked with Bup, 500 ng/ml and MPPH, 15 µg/ml.

difference ($F \blacksquare 4.2 \times 10^{-3}$ at $P = 0.05$) between the five calibration plots obtained on five different days during two week period. These results confirm the linearity of the standard plots and excellent reproducibility of the assay method.

The recovery of Bup was evaluated by comparing the PHR of Bup to the internal standard for concentrations 50, 100, 250, 500, 1000, 1500 and 2000ng/ml in spiked plasma with the same concentration in aqueous solutions assayed within two hours of preparation (Table 2).

TABLE 1

Inter Assay Precision (Reproducibility) in Human Palsma

i) Range 10-250 ng/ml

Amount added (ng/ml)(*)	Peak height ratio (mean ± SD)	CV %
10	0.046 ± 0.003	6.21
25	0.106 ± 0.006	5.80
50	0.202 ± 0.008	4.07
75	0.304 ± 0.009	2.81
100	1.395 ± 0.010	2.46
150	0.595 ± 0.009	1.51
200	0.742 ± 0.009	1.24
250	0.927 ± 0.010	1.03

Linear regression of results yields:

PHR = 0.016 (± 0.0051) ± 0.00369 (± 2.2 x 10^{-5})Conc.
r = 0.999; CV of the slope = 0.6%

ii) Range 250-2000 ng/ml

Amount added (ng/ml)(*)	Peak height ratio (mean ± SD)	CV %
250	0.332 ± 0.006	1.89
500	0.639 ± 0.011	1.80
750	0.938 ± 0.010	1.05
1000	1.269 ± 0.022	1.70
1250	1.525 ± 0.017	1.11
1500	1.905 ± 0.024	1.28
1750	2.167 ± 0.030	1.38
2000	2.529 ± 0.018	0.73

Linear regression of results yields:

PHR = 0.014 (± 0.003) + 0.00125 (± 8.4 x 10^{-5})Conc.
r = 0.999; CV of the slope = 0.68%

(*)Eight plasma samples, each assayed on five days (every other day). Each concentration represents five replicates (n=5).

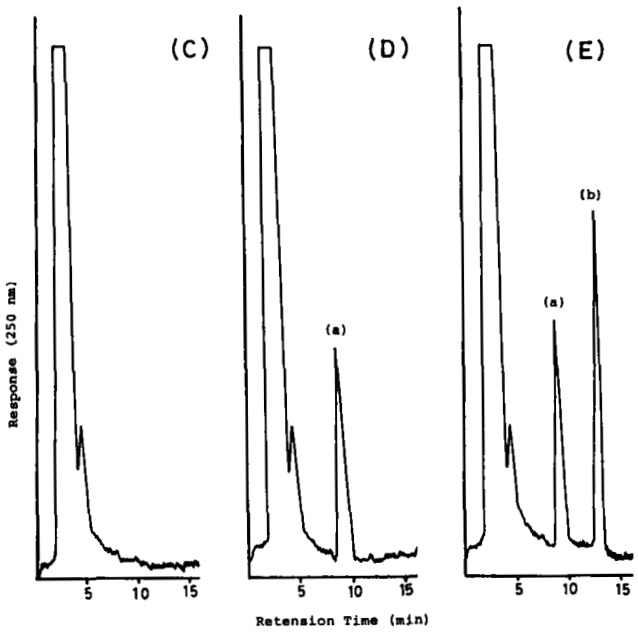

FIGURE 3. Chromatogram of Bup (a) and MPPH as Internal Standard (b) in Human Plasma

c) Blank Human Plasma.

d) Human Plasma Spiked with Bup, 500 ng/ml.

e) Human Plasma Spiked with Bup, 500 ng/ml and MPPH, 15 µg/ml.

TABLE 2

RECOVERY DATA OF BUPROPION FROM SPIKED HUMAN PLASMA (n=5)

Amount added (ng/ml)	Amount found (mean ± SD),ng	Recovery %	CV %
50	45.1 ± 1.9	90.2	4.21
100	95.0 ± 3.9	95.0	4.10
250	241.1 ± 5.4	96.4	2.24
500	490.7 ± 9.1	98.1	1.85
1000	989.9 ± 11.7	98.9	1.18
1500	1466.3 ± 14.6	97.8	1.00
2000	1984.2 ± 18.3	99.2	0.92

Mean recovery ± SD (96.5 ± 3.14), CV = 3.25% (n=35).

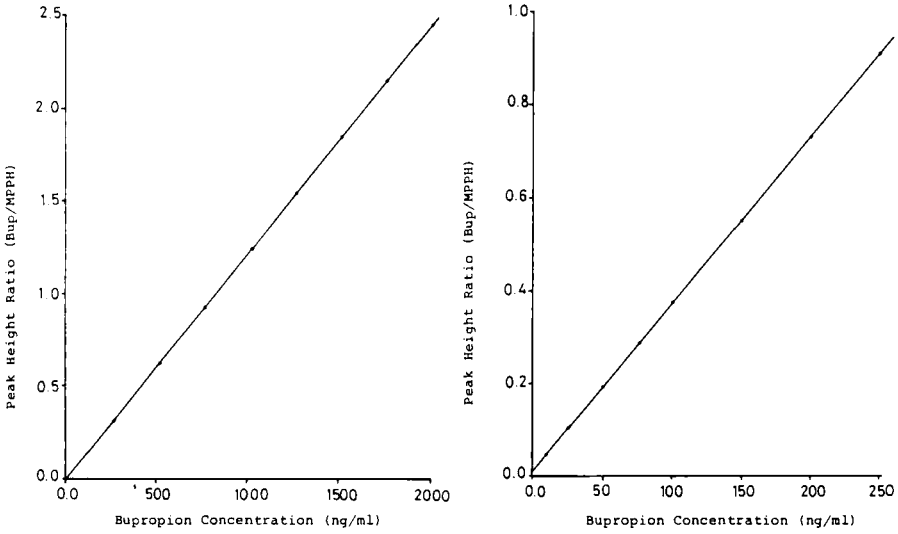

FIGURE 4. Standard Curve of Bup in Human Plasma. The Curve has been Plotted in two Ranges so as to Improve Linearity of Lower Concentrations.

The recovery of Bup was found approximately 96.5 ± 3.14% (CV = 3.25%, n ◘ 5). A decrease in the measured Bup was detected when the supernatant at the same concentration of Bup and internal standard mentioned above were stored for a period of 24 hours at 25°C. The results of these studies are shown in Table 3. The average recovery of Bup was found to be 66.8 ± 2.0% (CV 3%, n=5) which is lower than the value reported (11).

The minimum detectable concentration defined as the concentration in ng/ml that gives PHR equal to twice the background noise was found to be 5 ng/ml of Bup in plasma sample.

The developed HPLC assay method of Bup has sufficient sensitivity for routine analysis of the drug in plasma in the therapeutic ranges.

TABLE 3

STABILITY OF BUPROPION IN HUMAN PLASMA SAMPLES WHEN STORED AT 25°C, pH 3.1 OVER 24 HOURS (n=5)

Amount added (ng/ml)	Amount found (mean ± SD),ng	Recovery %	CV %
50	34.1 ± 1.1	69.1	3.19
100	62.2 ± 1.3	65.1	2.07
250	168.1 ± 5.7	67.3	3.39
500	327.8 ± 7.2	65.6	2.20
1000	644.8 ± 8.7	64.5	1.35
1500	1047.0 ± 9.8	69.8	0.93
2000	1327.8 ± 15.5	66.4	1.17

Mean recovery ± SD (66.8 ± 2.0%), CV = 3.0%, (n = 35)

The method also indicates that caution should be exercized if large number of samples are to be analyzed over a long period of time due to stability problems. In addition, this method has the advantages of speed, sensitivity and small volume needed for analysis over other reported HPLC method.

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